U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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U.S. FOOD AND DRUG ADMINISTRATION

WORKSHOP

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APPLICATION OF ADVANCES IN NUCLEIC ACID AND PROTEIN-BASED DETECTION METHODS FOR MULTIPLEX DETECTION OF TRANSFUSION-TRANSMISSIBLE AGENTS AND BLOOD CELL ANTIGENS IN BLOOD DONATIONS

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WEDNESDAY

APRIL 10, 2013

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The workshop was held in the Main Auditorium at the Natcher Conference Center, Building 45, National Institutes of Health main campus, Bethesda, Maryland, at 8:00 a.m.,

Sanjai Kumar, Moderator, presiding.

PANEL FOR SESSIONS I AND II:
LOUIS KATZ, M.D., America's Blood Centers, Session I Moderator
CHARLES CHIU, M.D., Ph.D., University of California San Francisco, Session II Moderator
THOMAS BRIESE, Ph.D., Columbia University/WHO
PHILIP FELGNER, Ph.D., University of California Irvine
INDIRA HEWLETT, Ph.D., CBER/FDA
PETER MARKS, M.D., Ph.D., CBER/FDA
HIRA L. NAKHASI, Ph.D., CBER/FDA
SUSAN L. STRAMER, Ph.D., American Red Cross
NATHAN WOLFE, M.A., D.Sc., Stanford University/Metabiota

PANEL FOR SESSION III:

CONNIE WESTHOFF, Ph.D., New York Blood Center, Session III Moderator
GREGORY DENOMME, Ph.D., BloodCenter of Wisconsin
BILL FLEGEL, M.D., NIH Clinical Center
CHRISTIAN GABRIEL, M.D., Red Cross Transfusion Service of Upper Austria
M. SUE LEFFELL, Ph.D., Johns Hopkins University School of Medicine
GORKA OCHOA, M.D., Ph.D., Progenika, Inc.
ALSO PRESENT:
HARVEY ALTER, M.D., NIH
MICHAEL BUSCH, M.D., Ph.D., Blood Systems Research Institute
ROBERT DUNCAN, CBER/FDA
JAY S. EPSTEIN, M.D., CBER/FDA
ANDREW KASARSKIS, Ph.D., Mount Sinai School of Medicine
MATTHEW KUEHNERT, M.D., CDC
SANJAI KUMAR, Ph.D., OBRR/CBER/FDA

KEVIN McLOUGHLIN, M.S., Lawrence Livermore National Laboratory
SAYAH NEDJAR, Ph.D., CBER/FDA

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NIH Clinical Center

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

2 8:03 a.m.

3 DR. KUMAR: Good morning.

4 My name is Sanjai Kumar, and I

5 work with the Office of Blood Research and

6 Review here at CBER, FDA.

7 The first things first, let's

8 acknowledge the people who helped us. And by

9 the way, I am one of the moderators for this

10 workshop. So, I would like to thank the

11 Scientific Program Committee members, many of

12 you here in this room, which helped us along

13 the way in developing the scientific concept,

14 developing the scientific agenda that you see,

15 and helped pick the speakers and panelists.

16 So, we could not have done it without them.

17 We have a number of the sponsors

18 here. I won't go into details, but many of

19 them helped with some of the travel support.

20 I would like to especially thank

21 Dr. Simone Glynn from NHLBI, who made this

22 beautiful conference facility available to us.

1 We had help from Lydia Kibiuk from
2 NIH Medical Arts and Design, who made these
3 graphics that you see.
4 And the real hero of this
5 workshop, Jennifer Scharpf, who determined
6 this help, and there are many others who
7 worked in the background to make this workshop
8 possible.
9 Just a few -- I will be very quick
10 -- a few housekeeping announcements here.
11 There are ample restrooms here on this floor
12 and many more on the floor above.
13 For lunch, there is a large
14 cafeteria here. If that becomes full, there
15 is another one across the street in the Lister
16 Hill Building.
17 The most important thing I would
18 like to draw your attention to the Session
19 Chairs. Please make sure any speakers, I urge
20 you also, to stay on time because every minute
21 is counted here really. And if you are
22 delayed, then you are cutting into somebody

1 else's time, and that will cut the mission of
2 this workshop. So, please, please, stay on
3 time.
4 What else? The last thing, I
5 would introduce Dr. Peter Marks, who is our
6 Deputy Director for the Center for Biologics
7 Research and Evaluation. He will give the
8 welcome remarks and open this workshop.
9 Dr. Marks?
10 DR. MARKS: Good morning.
11 On behalf of the Center for
12 Biologics Evaluation and Research at FDA, I
13 would like to welcome you all to this workshop
14 on application of advances in nucleic acid and
15 protein-based detection methods for multiplex
16 detection of transfusion-transmissible agents
17 and blood cell antigens in blood donations.
18 The goals of this workshop are:
19 First, to review the technological
20 advances and status of multiplex platforms and
21 recent advances in gene-based and protein-
22 based pathogen and blood cell antigen

1 detection methods.
2 And second, to discuss the
3 scientific pathways to support the development
4 of more efficient, effective, and flexible
5 multiplex assays to screen blood donors for
6 blood-borne pathogens and to perform blood
7 group antigen and HLA typing.
8 Rigorous implementation of donor
9 screening for the commonly-known blood-borne
10 pathogens such as HIV, HTLV, hepatitis B and
11 C, West Nile virus, Chagas, and syphilis,
12 along with the use of the risk-based donor
13 screening questionnaire, have made the blood
14 supply extremely safe. However, there are
15 many challenges to blood safety and
16 availability with regard to emerging and re-
17 emerging blood-borne pathogens. These
18 challenges include genetic variance of
19 existing pathogens.
20 In addition, there are no donor
21 screening tests available for a number of low-
22 prevalence pathogens and for those pathogens

1 transmitted only in specific geographic
2 regions of the United States, such as Babesia,
3 or transmitted seasonally, such as dengue
4 virus. In addition, infectious agents such as
5 variant Creutztfeldt-Jakob disease can be
6 brought into the country through international
7 travel, and immigrants and visitors from
8 endemic countries may bring infection with
9 malaria or leishmania with them.
10 Along with the need for an
11 expanded portfolio of tests for infectious
12 diseases, review of decades-old serology-based
13 red cell antigen typing and current HLA
14 methods reveal the need for further
15 improvement of transfusion compatibility
16 testing in donors.
17 The current paradigm of donor
18 screening is dictated by the limitations of
19 the number of licensed tests that can be
20 performed practically for a variety of agents,
21 as well as by the volume of blood used in the
22 testing and by the cost of testing associated

1 with each additional test implemented.
2 Therefore, cost-effectiveness,
3 risk/benefit, and logistics have combined to
4 produce a challenging situation for blood
5 centers potentially interested in routine
6 testing for more pathogens. Similar reasons
7 may also explain the reluctance of industry to
8 place more resources on product development in
9 this area.
10 In recent years, technological
11 advances, such as next-generation sequencing,
12 microarrays, protein arrays, and
13 bioinformatics tools have revolutionalized the
14 diagnosis and treatment of several forms of
15 cancer and some infectious diseases. Now the
16 sequencing of the entire human genome, along
17 with sequence assembly and annotation, can be
18 accomplished in a matter of days. The
19 sequencing of much smaller genomes of viruses
20 or parasites is much easier to perform, and
21 molecular typing of blood cell antigens is a
22 reality.

1 More recently, researchers in
2 academia and industry have been developing
3 multiplex platforms for simultaneous detection
4 of several pathogens in their variant forms.
5 These technologies are now being harnessed
6 both for diagnosis and donor screening for
7 infectious agents. Additionally, genetic
8 methods for blood cell antigen typing are
9 being further developed.
10 The recent advances in nucleic
11 acid sequencing, genome-wide transcription
12 profiling, and molecular methods and discovery
13 of novel pathogens, when combined with
14 simultaneous detection of several pathogens in
15 high-throughput format, could increase the
16 number of pathogens that can be detected as
17 part of routine donor screening.
18 This workshop is our proactive
19 approach to bring together the basic
20 researchers, the test developers, blood
21 centers, and NIH funding partners, along with
22 FDA, to become familiar with the recent

1 scientific advances, to understand the
2 scientific barriers, and to have discussions
3 on how to facilitate the development of next-
4 generation multiplex technology-based devices
5 that would further improve transfusion safety
6 and enhance protection of the blood supply
7 from rapidly-emerging pathogens and agents of
8 bioterrorism.
9 I hope that you will find the
10 program stimulating and exciting. I want to
11 thank you all for attending today and for
12 taking the time to participate in this
13 workshop. I wish you a lot of success in
14 accomplishing its goals.
15 Thanks very much.
16 (Applause.)
17 DR. EPSTEIN: So, I will just
18 introduce myself. I am Jay Epstein, Director
19 of the Office of Blood Research and Review.
20 And staff in my office have put
21 together today's program with the able
22 assistance of many experts in the field, and

1 we are very grateful.
2 So, let me just add my welcome to
3 you. We have over 300 registrants for this
4 scientific workshop, which is one of the
5 largest that we have ever hosted. And I think
6 that this broad interest is indicative of the
7 excitement that we all feel about the new
8 technologies that may be brought to bear to
9 advance blood transfusion safety.
10 So, what I am going to do in the
11 next few minutes is provide a little bit of an
12 outline to frame the workshop. By way of
13 background, let me first touch on CBER's
14 vision in general, part of our mission
15 statement, which is innovative technology
16 advancing public health. And within this
17 vision, we seek to protect and improve public
18 and individual health in the United States
19 and, where feasible, globally. And perhaps
20 most fundamental to this workshop, to
21 facilitate the development, approval, and
22 access to safe and effective products and

1 promising new technologies and, in so doing,
2 to strengthen CBER as a preeminent regulatory
3 organization for biologics.
4 So, this slide defines the basic
5 challenge that we face in blood safety
6 regulation. Obviously, as I have already
7 explained for blood and for biologics in
8 general, we seek to enhance product safety,
9 purity, and potency consistent with our
10 statutory authority.
11 However, there is an interplay
12 between the implementation of safety
13 technologies and externalities that affect
14 either cost or availability, or both. And so,
15 every time that we consider safety
16 intervention, we have to consider
17 consequences, particularly the avoidance of
18 product shortage and major increased costs.
19 Fortunately, there is a cross-
20 cutting opportunity, which is that
21 technologies often provide answers that can
22 advance simultaneously safety, availability,

1 and perhaps reduce costs at time. And we
2 believe we are at one of those exciting
3 moments where technology opportunities exist
4 that could improve blood product safety,
5 efficacy, and availability, while minimizing
6 or avoiding system disruptions. And our goal,
7 simply stated, is to facilitate their
8 development.
9 Okay. So, this workshop is in two
10 parts: prevention of transfusion-transmitted
11 infectious disease and improved methods for
12 determining blood cell antigens.
13 So, first, talking about
14 transfusion-transmitted infectious diseases,
15 we have been following for several decades a
16 general fivefold strategy which has been very
17 highly effective in reducing risks of
18 transfusion. We call these the five layers of
19 safety.
20 Briefly, donor screening and
21 deferral based on epidemiologically-
22 established risk factors which are geographic,

1 behavioral, and medical, and we approach these
2 through donor education, offering the
3 opportunity for self-deferral, and then, a
4 donor interview, essentially a questionnaire.
5 Second, and most fundamental to
6 today's workshop, we make great use of
7 laboratory testing as a basis for temporary or
8 permanent deferral of donors. Obviously,
9 these are tests for markers of transfusion-
10 transmittal infections associated with disease
11 in recipients.
12 Other related tools include the
13 use of deferral registries to prevent
14 collection and use of blood from donors who
15 have been deferred, quarantine controls to
16 prevent the inappropriate release of
17 unsuitable units, and investigation and
18 correction of deviations in manufacturing.
19 Now, as I said, these strategies,
20 namely, the combination of epidemiologically-
21 based donor selection and laboratory testing,
22 have dramatically reduced the risk of

1 transfusion-transmitted infectious diseases in
2 the United States and, indeed, in almost all
3 parts of the world. This is a logarithmic
4 scale. You can see the dramatic reductions
5 that have taken place in reducing risks of
6 hepatitis B, HIV, and hepatitis C.
7 But, as was alluded to by Dr.
8 Marks, whereas donation testing has
9 significantly reduced these risks, and other
10 risks, HTLV, CMV, West Nile, syphilis, T.
11 cruzi, there are remaining risks, and I am
12 going to address those shortly.
13 So, perhaps the Holy Grail of
14 infectious safety for blood transfusion is
15 pathogen-reduction technologies. These are
16 under study for blood components in the United
17 States. They have been implemented generally
18 on a small scale in various parts of the
19 world, although there are some whole
20 countries, like Switzerland, that are now
21 doing this for platelets or for plasma. But
22 in the U.S. they are available only for

1 pathogen-reduction-treated pooled plasma and
2 for plasma derivatives.
3 Generally speaking, they involve
4 two things, either killing viruses, so-called
5 virus inactivation, by manufacturing
6 procedures like solvent/detergent, incubation,
7 pasteurization, et cetera, or virus removal or
8 partitioning during the processing of
9 generally plasma derivatives, such as
10 nanofiltration, chromatographic procedures, et
11 cetera.
12 But the point is that, except for
13 pooled plasma, these technologies are not
14 available presently, at least in the U.S., for
15 transfusable products. And so, donation
16 testing remains an essential safeguard to
17 prevent transfusion-transmitted infectious
18 diseases. And indeed, testing is likely to
19 remain in place, even when we have pathogen
20 reduction for various agents, for the simple
21 reason that some of these agents presented
22 very high titers, and we would always want to

1 ensure a safety margin. So, those are
2 discussions that we will have to have in the
3 future, as those methods reach the stage of
4 approval, hopefully in the not-too-distant
5 future.
6 So, as I said, we have these
7 ongoing challenges for infectious diseases,
8 despite the very dramatic successes that have
9 been achieved in the last three decades. And
10 just to briefly give you a survey, these
11 challenges include risks from known and
12 emerging pathogens, like dengue viruses;
13 Babesia; TSE agents, for which there is no
14 test technology; Chikungunya virus, which
15 emerged on the islands and the mainland, the
16 Indian Ocean, and where we have potential for
17 spread in the U.S., and also, not to forget
18 potential bioterrorism agents. Of course, we
19 had shocks with anthrax, and there are many
20 other agents of concern.
21 But, then, there are also
22 reemerging pathogens that could threaten blood

1 safety. For example, we know that there are
2 drug-resistant and circulating recombinant
3 forms of HIV variants. There are hepatitis B
4 mutants which potentially could produce false-
5 negative HBsAg or NAT tests.
6 Also, we are concerned about the
7 significant donor losses that we currently
8 have when we use deferral criteria based on
9 risk in the absence of tests. This is
10 particularly true for certain parasitic
11 diseases like malaria or leishmania, where
12 there are some voluntary deferrals. You have
13 a very high cost in donor loss because you
14 can't directly screen.
15 Additionally, we have a problem of
16 inability to re-enter uninfected donors who
17 have false-positive screening tests because of
18 the general lack of availability across the
19 board of needed supplemental assays.
20 So, the promise here is for
21 technology solutions. And what are we looking
22 for these technologies to do? We would like

1 better tools to simultaneously detect these
2 infectious disease markers in single blood
3 samples in an efficient and cost-effective
4 way. We would like these formats to involve
5 not just multiplexing, that is to say, many
6 simultaneous anolytes, but also flexibility,
7 the more convenient and easily regulatory,
8 approvable methods to swap in and out the
9 target anolytes. We would like these tests
10 also to be rapid with high throughput because
11 that is the fact of life. Close to 40,000
12 donations processed every single day.
13 And we recognize that, given the
14 technology complexity of some of the gene-
15 based methods, that there is also a dimension
16 of bioinformatics, but that also provides the
17 option for increased extraction of
18 information, based on the use of these tools.
19 So, that is the promise.
20 Now CBER is involved in wet lab
21 research to try to advance the underpinning
22 regulatory science that enables us to

1 efficiently assess and, ultimately, hopefully,
2 approve new technologies across the board.
3 And in this area of robust detection and
4 multiplex detection of blood-borne pathogens,
5 we have activity looking at open array
6 systems, looking at resequencing microarrays,
7 looking at nanoparticle microarray-based
8 assays.
9 I am not going to discuss these in
10 any detail. I just wanted to illustrate the
11 point that we have an active research program
12 in Center for Biologics. It is mission-
13 related, and a core issue is to resolve or
14 remove barriers to effective regulation
15 through informing FDA about the issues with
16 the technologies and enabling us to move
17 toward review standards.
18 So, now I am going to turn to the
19 second part of my introduction and, of course,
20 also, the workshop, which is to prevent
21 antigenic incompatibility of red blood cell
22 and platelet transfusions. And the theme here

1 is that gene-based methods offer tremendous
2 promise for advancements in this field. I am
3 going to comment on what some of the
4 limitations and some of the prospects are.
5 So, as many in the audience
6 already know, there has been astounding
7 progress in the last 50 years in the
8 identification of the 30 known blood group
9 systems -- actually, one more was just
10 recently defined -- with over 300 allo-
11 antigens expressed by a variety of human
12 genetic polymorphisms.
13 In contrast, our serologic tools
14 have been somewhat limited compared to our
15 genetic tools. We have used serology, which
16 harkens back to the early days of the 20th
17 century, shortly after the discovery in 1899
18 by Karl Landsteiner of the blood groups and,
19 then, the introduction by around, I think,
20 1907 of serologic tools to actually match
21 compatibility. And these include the tube
22 method, the solid phase method, a column

1 agglutination method.
2 Of course, these methods have
3 produced tremendous success in avoiding
4 transfusion incompatibility. However,
5 incompatibility still occurs with significant
6 hemolysis and death every single year.
7 And so, we are very focused on
8 ways that we can move beyond the limitations
9 of these methods. So, these methods have
10 difficulties both of sensitivity and
11 specificity. They include issues related to
12 recency of transfusion, the presence of warm
13 auto-antibodies, the limited availability of
14 antisera for some specificities, the quality
15 problem which is inherent with some reagents;
16 for example, weak reactivity with RHD and weak
17 expression of some red cell antigens.
18 Additionally, with the available
19 tools, screening large numbers of donor units
20 for negativity for potential antigens is
21 essentially impractical. The typing for rare
22 antigens is often not performed, leading to

1 risks for alloimmunization and hemolytic
2 reactions. And also, just the methods can be
3 cumbersome and typing of the red cell antigens
4 and, correspondingly, platelet antigens can be
5 very time-consuming, unfortunately, on a
6 different timescale compared to patient need.
7 So, where do we go from here?
8 Well, we believe that the gene-based methods,
9 particularly for red cell antigenic typing,
10 have key advantages that, in fact, address
11 these shortcomings. This is because the
12 molecular basis, that is to say, the gene
13 sequence and the polymorphisms, are, in fact,
14 known for the vast majority of blood group
15 antigens. And therefore, they provide the
16 basis for predicting the antigenic phenotype
17 from the genotype. And then, that can, then,
18 be used to make clinical decisions.
19 So, there are a number of
20 technology advantages as well. First of all,
21 that these are synthesized reagents, primers,
22 and probes, and that lessens the concerns

1 about limited availability of serologic
2 reagents. Clearly, they can be designed to
3 target virtually any genetic variant as soon
4 as the variant is known. They permit typing
5 of the recipient phenotype when the available
6 antibodies are weakly reactive or are in
7 limited supply. They can be adapted to high-
8 throughput platforms, and they can be put into
9 multiplex platforms.
10 So, once again, as in the area of
11 transfusion-transmitted infectious disease,
12 these technologies offer a very clear promise
13 of advancement to overcome the shortcomings
14 and, in fact, may replace serologic methods.
15 So, clinical treaters are already taking
16 advantage of these tests. And some of the
17 applications include the following: the
18 determination of the blood type in patients
19 with recent transfusion, in patients who
20 develop warm auto-antibodies, or in the case
21 of positive direct antiglobulin tests, the
22 resolution of phenotype discrepancies

1 determined serologically, the detection of
2 rare, but significant antigens, and the typing
3 of antigens where anti-sera are not available.
4 But there are still problems in
5 trying to move this forward in the regulatory
6 domain. One of the key issues is a worldwide
7 paucity of DNA reference materials, especially
8 for red cell genotypes. Once again, we are
9 taking a wet lab approach to try to advance
10 the underlying science in this area.
11 We have established a molecular
12 testing laboratory, and the goal is to create
13 DNA reference panels to cover genetic variance
14 for the 17 most common blood group systems,
15 consistent with recommendations of the AABB.
16 And Dr. Jason Lu is spearheading this
17 laboratory, which we are still in the process
18 of booting up, along with collaborations that
19 have been established.
20 So, this is just to remark on the
21 almost exponential discovery of HLA loci.
22 Well, I am shifting here focus, just to

1 comment that, just as we have been engaged in
2 red cell antigenic determination through
3 genetic tools, we are doing the same thing
4 also with HLA. So, similar to red cells, but
5 on a much more compressed timeframe, perhaps
6 reflecting all the advances in genetic
7 technologies, we are seeing an exponential
8 expansion in the characterization of the
9 genetic underpinnings of HLA antigens.
10 And so, we have already approved a
11 number of gene-based technologies for HLA
12 typing using three different technology
13 approaches, sequence-specific primers,
14 sequence-specific oligonucleotide probes, and
15 Sanger sequence-based typing. So, whereas we
16 have already cleared or approved a number of
17 such systems, we are also looking forward to
18 the possibility that so-called next-generation
19 sequencing might further advance this
20 technology area. And the reason this is still
21 needed is the incredible complexity of the
22 HLA. For perhaps transfusion, we don't

1 necessarily need the so-called high-resolution
2 testing, but there are many applications
3 where, in fact, higher-resolution HLA
4 phenotyping is needed.
5 One of the issues is that only
6 exons encoding the antigen-recognition domains
7 are currently routinely assessed, that many
8 devices provide only partial sequences, that
9 the current technologies cannot always resolve
10 so-called phase ambiguity. And so, the hope
11 is that the next-generation sequence may
12 reduce these ambiguities by sequencing large
13 or entire genomic regions and through
14 technologies that can overcome the problem of
15 a phase inconsistency.
16 So, in summary, now speaking of
17 both domains, we believe that new technologies
18 and their applications have tremendous promise
19 to improve transfusion safety, both in regard
20 to infections and antigenic compatibility,
21 both of red blood cells and platelets.
22 We are going to be talking

1 specifically about a number of these
2 technologies, next-generation sequencing, DNA
3 microarrays, real-time nucleic acid
4 amplification testing, nanofluidics,
5 nanoparticle-based technologies, proteomic
6 arrays, and perhaps things that we haven't
7 thought of.
8 So, in conclusion, and sort of
9 getting down to the real work, I just want to
10 reiterate objectives of the workshop, which,
11 of course, were put forward in the very
12 comprehensive summary that you should have all
13 received. And these are the following:
14 To review the technological status
15 of multiplex platforms that could be applied
16 to the detection of pathogens in blood and the
17 determination of red blood cell and platelet
18 antigens to assure compatible.
19 To discuss advances in nucleic
20 acid and protein-based detection methods that
21 could be applied on multiplex platforms.
22 And to discuss the science-based

1 regulatory pathways that would support the
2 development of sensitive, specific, and
3 flexible multiplex assays.
4 And hopefully, part of why we are
5 here is to find the venues to leverage with
6 you yourselves, our stakeholders, to bring
7 these multiplex platforms to market.
8 So, I thank you for your attention
9 and look forward to a very exciting workshop
10 over the next two days.
11 (Applause.)
12 DR. KATZ: Thanks, Jay, for
13 context.
14 My name is Dr. Louie Katz. I am
15 chairing the first session, which is entitled,
16 "Blood Safety from Infectious Disease:
17 Present and Future". I think it is actually
18 past, present, and future, reviewing the
19 slides from the speakers.
20 The first speaker is going to be
21 Dr. Nathan Wolfe, who is the Lorry I. Lokey
22 Business Wire Consulting Professor of Human

1 Biology at Stanford University, one of the
2 longer-endowed Chairs that I have read in my
3 career.
4 He is the founder and CEO of
5 Metabiota, which is a for-profit specializing
6 in microbiologic research; also, the Chairman
7 of Global Viral, a nonprofit promoting
8 understanding, exploration, and stewardship in
9 the microbial world.
10 He got his doctorate in immunology
11 and infectious diseases at Harvard in 1998,
12 and was a Fulbright Scholar, NIH Director's
13 Pioneer Award recipient, and this is the one
14 I like the best, National Geographic Emerging
15 Explorer in 2009.
16 Over eight years in Africa,
17 Southeast Asia, doing research on the ground
18 in the area that we are interested in,
19 emerging infectious diseases; $60 million in
20 grant support -- I'm jealous -- mostly from
21 the usual suspects that you can read in his
22 bio in the handout.

1 So, Emerging Infectious Diseases:
2 Global Hotspots, Risk to Blood Safety," Nathan
3 Wolfe from Stanford University.
4 DR. WOLFE: Thank you very much.
5 It is really a pleasure to be here.
6 As you will see from my
7 presentation, I think the timing for this
8 workshop is really just about ideal. And it
9 is exciting to see the group of folks that
10 have been brought together for this meeting.
11 I am looking forward to a lot of the
12 discussion.
13 So, I have been sort of working
14 with the FDA on these kinds of topics for just
15 about 10 years, when we started this work,
16 started work with Indira Hewlett and her
17 group. And sort of my job here is really to
18 present sort of an overview of emerging
19 infectious diseases and put blood safety in
20 that context, and talk a little bit about sort
21 of field work and biosurveillance, which is my
22 sort of area of specialty.

1 But, as I was putting the
2 presentation together, I just got so excited
3 on what has gone on in terms of the methods
4 that we have and that have been developed over
5 the last 10 years, that I sort of took the
6 speaker's prerogative to sort of change my
7 title a little bit, and not just provide that,
8 but also just to talk about how I believe the
9 timing is just about perfect to do
10 international biosurveillance to address some
11 of these emerging infectious disease issues
12 and blood safety.
13 I think you can't have this
14 conversation without talking, first and
15 foremost, about the global AIDS pandemic. I
16 think the reason I show this slide is just,
17 while it is not the case in this audience,
18 most people in the world, when they think
19 about sort of the beginnings of the AIDS
20 pandemic, will think back to sort of the
21 1980s. Maybe they will think about the point
22 at which Magic Johnson first announced that he

1 was HIV-positive.
2 But, of course, as we all know,
3 HIV as a virus, as a human virus, is now over
4 100 years old. And so, by the time this
5 particular photograph was taken in Brazzaville
6 before the Great Depression, HIV had already
7 crossed from chimpanzees into human
8 populations. It had already spread, almost
9 certainly, to city centers, probably places
10 like Brazzaville here.
11 And so, there is this, I think,
12 really sort of basic question that all of us
13 in this field have to address, which is, if
14 this is a virus that had entered into human
15 populations before the Great Depression, then
16 why did it take us until 1981 to see the first
17 real sort of reports about the symptoms of
18 AIDS? Why did it take us until 1983 to
19 identify the virus that was the cause of AIDS?
20 Why was it not until sort of 1986 or 1987
21 before senior political leaders in our own
22 country here in the United States began to use

1 the word "AIDS" in public addresses?
2 And the point is we need to do a
3 lot better with regards to these sorts of
4 things. I think that the costs with regards
5 to human lives, you know, HIV, probably the
6 most dramatic example, but the sorts of
7 emerging infectious diseases that we have had
8 have exerted a massive, massive cost in terms
9 of, of course, morbidity and mortality, but
10 also in terms of economic cost to governments
11 and losses to corporate industry.
12 These are phenomena which are
13 increasing dramatically over time. So, there
14 is a range of factors associated with
15 contemporary society that have dramatically
16 increased the rate at which novel infectious
17 diseases are spreading in human populations
18 and entering into human populations.
19 This is just sort of a list of
20 some of the factors. But what I would like to
21 sort of spend a little bit more time on is the
22 one that I think that is, arguably, the most

1 important, which is connectivity between human
2 populations and human and animal populations.
3 I think, arguably, this slide sort
4 of gives the impression, I think more
5 dramatically, than any one that sort of I have
6 seen, which is we live in a profoundly
7 different place. Now, when we go through sort
8 of our human lives, things are clicking
9 through sort of very slowly, so it is hard to
10 remember how dramatically different this
11 moment of history is than life was even like
12 30, 40, 50 years ago, and certainly how
13 different it was from life 100 years ago.
14 We live in a world where pathogens
15 that enter into human populations virtually
16 anywhere in the world have the potential to
17 move anywhere else in the world. And what
18 this creates is sort of a microbial mixing
19 pot, if you will, this incredible capacity for
20 these agents to move from one location to
21 another. And then, obviously, critically for
22 those of us who study viruses, there is a

1 tremendous capacity for viruses that wouldn't
2 normally have interacted with each other to
3 come into contact, to go through recombination
4 or reassortment. And so, there is going to be
5 the generation of an incredible amount of
6 novel viruses. This is going to continue to
7 be a part of our world. This is just sort of
8 the world that we live in.
9 And we have also experienced major
10 changes in technology over the last really
11 hundreds of years that have changed this in
12 ways that I think are sort of very important.
13 Now, obviously, these technologies have led to
14 the sorts of medical advances and health which
15 are pivotal and important. I don't want to
16 diminish that. But, if we think about
17 connectivity between human populations, the
18 advent of the needle, when we think of blood
19 transfusion, these are all things that connect
20 human populations, obviously, injection drug
21 use. These provide new sort of modalities and
22 mechanisms and bridges for microbes to cross

1 boundaries between individuals. So, there is
2 sort of layer upon layer of interconnectivity,
3 which is really facilitating the sort of
4 connectivity of microbes on the planet and
5 really putting us all in a situation where we
6 have a tremendous amount of risk.
7 And I think, again, look, it is
8 very important to think about our history.
9 You know, there was obviously a lot of concern
10 about the origins of HIV. Fortunately, it was
11 not the case that HIV originated through use
12 of unsterilized needles, but I think it is the
13 case that, if we are all honest with
14 ourselves, that we sort of dodged a bullet on
15 that one because, certainly, treatment
16 campaigns and vaccination campaigns with
17 unsterilized needles were associated with the
18 transmission of viruses in the human
19 population.
20 I think this is sort of most
21 critically seen in hepatitis C that was
22 transmitted very extensively in the

1 schistosomiasis treatment campaigns in North
2 Africa and sub-Saharan Africa. These were
3 campaigns that really led to, arguably, the
4 hepatitis C pandemic as we currently see it.
5 And I think that there is a whole
6 range of other risks that have been sort of
7 alluded to. Bioterror is another one, you
8 know, the potential for blood supplies
9 globally to be contaminated associated with
10 either purposely bioterror or accidentally as
11 the results of the introduction of agents
12 associated with bioterror.
13 So, this is how we might
14 conceptualize our current way of responding to
15 epidemics. Okay? So, if you actually think
16 about the emergence of a particular epidemic,
17 it takes us quite a bit of time to respond to
18 it, to catch that first case, to detect the
19 spread of a virus within a population, and to
20 respond to it.
21 Now, really, the ideal, though, of
22 course, is for us to move to a situation which

1 is much more like this. Okay? And I think
2 this is really one of the primary reasons for
3 convening a workshop like this, is we now have
4 the capacity and technology to dramatically
5 increase the speed at which we can identify a
6 new epidemic which is spreading through the
7 human population.
8 And, of course, the benefits of
9 that aren't even really fully sort of
10 demonstrated by this slide. It is sort of the
11 way that I like to think of it is sort of how
12 you would think about compounding interest.
13 For every sort of year or month early that you
14 catch one of these outbreaks, you can
15 potentially save massive amounts of life and
16 cost in future out-years, not just sort of in
17 a temporary way.
18 The good news is that sort of
19 compared to, again, 10-12 years ago, when I
20 think back about starting sort of
21 collaboration with the FDA, our knowledge of
22 the nature of how emerging infectious diseases

1 actually enter into the human population and
2 spread has increased dramatically. This is
3 just sort of one way of envisioning this and
4 conceptualizing sort of the transmission of
5 the viruses from animal populations.
6 We know a lot about these. We
7 know hotspots around the world that are
8 critically important for the emergence of
9 infectious diseases. We understand that the
10 vast majority of novel pandemics emerge from
11 wild animal populations.
12 And critically, we have started to
13 sort of focus on this level, what we see here
14 referred to as viral chatter. And that is a
15 term that comes from my post-doctoral mentor,
16 Don Burke. We are really starting to sort of
17 watch the interfaces where these viruses are
18 pinging human populations and entering into
19 human populations.
20 And I think that the capacity now,
21 the sort of international surveillance
22 efforts, the increase in our capacity to

1 detect novel agents, have really come together
2 at a time where it is sort of a lot of bad
3 news, but also a little bit of good news,
4 right? We are hyperconnected as a global
5 population. We are going to experience a lot
6 more of these things. But we increasingly
7 have the sorts of knowledge of how to set up
8 surveillance systems and how to detect
9 viruses, which potentially will give us the
10 capacity to control these sorts of things.
11 When we think about this, we
12 really have over the last 15 years or so moved
13 to really sort of think specifically in a
14 targeted and strategic way about where do we
15 want to be monitoring in order to catch this
16 viral chatter, in order to catch the
17 transmission of viruses moving from animal
18 populations into human populations.
19 And so, this just gives you an
20 idea of some of the sort of strategic
21 surveillance approaches that we use along with
22 a whole range of different partners on

1 different projects that we are involved in:
2 Identifying regions of the world
3 which are vitally important or that have a
4 history of the emergence of novel infectious
5 diseases, and where modeling sort of
6 demonstrates that the combination of
7 biodiversity and contact with animal species
8 is likely to facilitate the origins and sort
9 of transmission of new viruses.
10 To focus on particular animal
11 species and to understand the underlying viral
12 repertoire and viral diversity of species that
13 can contribute their viruses to human
14 populations.
15 And then, to sort of look at
16 sentinel human populations. I think this is
17 really critical, right? Surveillance, we are
18 going to have to be very focused on sentinel
19 populations. We are not going to be able to
20 monitor everyone around the world constantly.
21 But, certainly, there is going to
22 be a range of sort of digital approaches. It

1 was exciting to see the new Chief Technical
2 Officer of the FDA, who obviously has a lot of
3 experience with digital epidemiology, Taha
4 Kass Hout, who I think will help us to sort of
5 address the sort of things in the context of
6 blood safety as well.
7 So, our own approach -- and this
8 is just to show you some of the sort of major
9 sites -- so, we now work in about 20 sites
10 around the world with a whole range of
11 colleagues, focused on early detection of
12 emerging infectious diseases. The idea is to
13 put in place selective biosurveillance
14 activities, to focus on increasing laboratory
15 capacity in all the countries that we work
16 with, engaging scientists, and really working
17 to identify these novel viruses as they move
18 into human populations.
19 I thought it would be useful to
20 just go through some of the work that has come
21 out of my own group over the last 10 or 15
22 years, of course, along with a whole range of

1 collaborators and colleagues. And the point
2 is, if you put these surveillance systems into
3 place, you can certainly identify large
4 numbers of new microbes. You can collect
5 massive amounts of collections, and these
6 become hugely valuable for a range of
7 different exercises. Critically, you can
8 actually document that some of these viruses
9 jump the species boundary from animals into
10 human populations.
11 I am going to go into a little bit
12 of detail on some of these viruses, but this
13 is just to give you an overview of some of the
14 novel agents that we have alone been able to
15 detect in some of the work that we have done
16 around the world. And when you add this to
17 the other efforts that have really been
18 emerging over the last 10 years in this space,
19 we really are starting to radically increase
20 the number of viruses that we are aware of.
21 I think you will be hearing about
22 this over the next couple of days. A lot of

1 the sort of expertise in the field is
2 represented in this room.
3 But the idea is there is a
4 tremendous diversity of these viruses out
5 there that have the capacity to enter into
6 human populations, and they are constantly
7 entering into us. We increasingly have the
8 capacity to detect them.
9 I will just go through a few of
10 them. When we sort of started this work in
11 1998 in central Africa, one of the major sort
12 of points of interest was, look, HIV has
13 crossed into human populations. What is the
14 chance that other retroviruses have the
15 capacity to cross over into human populations?
16 And sort of what we started off to
17 do is really go out, and I sort of had a beer
18 bet with Don. I don't know; some of you
19 probably know Don Burke. He is inclined to
20 have these sorts of beer bets.
21 It was clear that these
22 retroviruses were crossing into human

1 populations. Don said, within a few years, we
2 are actually going to find novel retroviruses
3 crossing into humans. As I normally do with
4 Don, I lost that beer bet because I knew that
5 we would eventually find it; I didn't
6 anticipate that it would be so rapidly.
7 The first sort of clear evidence
8 that we saw was of a retrovirus simian foamy
9 virus. Not only did we see the transmission
10 of one of these viruses, but we saw three
11 individual hunters, people who had been
12 exposed to a tremendous amount of blood and
13 body fluids from these animals, who were
14 infected with foamy virus.
15 Now this was, of course, sort of
16 interesting. It was the first sort of
17 evidence of natural transmission of
18 retroviruses into human populations that was
19 sort of seen in real-time. But, of course,
20 simian foamy virus, there wasn't the history
21 of spread and disease associated with this
22 particular virus. So, it was interesting as

1 a model, and, obviously, we have continued to
2 follow simian foamy virus over time. And
3 there is a number of additional cases.
4 But the next virus that we really
5 looked at was HTLV. And again, a lot of folks
6 in the sort of general population don't have
7 a sense of HTLV; I think many of you will.
8 This is an important pandemic virus. It is
9 infecting on the order of 20 million
10 individuals globally and causes severe illness
11 in somewhere around the range of 5 to 10
12 percent of the individuals that it has an
13 impact on.
14 Now HTLV, just like HIV and a
15 number of human retroviruses, has origins
16 among non-human primates. When we started
17 this work, there was sort of HTLV-1 and HTLV-2
18 were the two primary viruses that we were
19 aware of. These both had clearly come from
20 non-human primate reservoirs.
21 There was also a third primate
22 reservoir, STLV-3, that had not yet been sort

1 of seen. There was no sort of homologue of
2 that virus that had been identified in human
3 populations.
4 So, what we set out to do was to
5 really determine whether or not STLV-3 had
6 crossed into human populations. What we were
7 able to demonstrate fairly rapidly was that,
8 in fact, yes, we could find individuals that
9 were infected with STLV-3. We now, along with
10 a number of different colleagues now -- there
11 are tens of these viruses that have been
12 identified in human populations.
13 As interesting for us, perhaps
14 even a bit more interesting, was that we
15 identified a completely novel retrovirus,
16 HTLV-4. This was a virus that was not -- at
17 the time, there was no clarity on what non-
18 human primate reservoir it had emerged from.
19 We still continued to look for which reservoir
20 HTLV-4 emerged from.
21 But what it meant to us was that
22 there was a tremendous amount of retroviral

1 diversity out there, that we were just sort of
2 scratching the surface, and that none of us
3 could take for granted that we were accurately
4 sort of detecting the viruses that were
5 flowing into human populations, obviously
6 critical if one of the major sort of health
7 events of the 20th century was the cross-
8 species transmission of a retrovirus from
9 primates into human populations.
10 And so, one of the things we did
11 was to really have a look at whether or not
12 these viruses would be detected in the blood
13 supply. And so, we looked at existing assays
14 that were used to screen in blood centers to
15 attempt to identify HTLV as a way of
16 decreasing its spread in the blood supply.
17 And what we found, there was,
18 again, sort of a little bit of good news, a
19 little bit of bad news in this situation. The
20 good news is that the general assays would, in
21 fact, by and large, detect HTLV-3 and HTLV-4.
22 The bad news is that they would not identify

1 it as distinct from HTLV-1 and HTVL-2. So, if
2 HTLV-3 and HTLV-4 are spreading within our
3 blood supply -- and it is certainly very
4 possible that they are -- they would probably
5 be stopped in the countries where we do have
6 screening. And I think it is important to
7 point out here that is still a minority of
8 countries in the world. But we would not know
9 that it was HTLV-3 and HTLV-4.
10 And so, novel retroviruses or
11 other agents that are spreading in the human
12 population that are similar to existing agents
13 might be stopped, but it might also be the
14 case that they may have different virulence
15 profiles, and we might not be identifying them
16 as particular agents.
17 This is just to sort of point out
18 that the sort of strength of these metagenomic
19 techniques and the sort of other assays and
20 viral discovery that are coming online, I
21 think that there are other folks that are
22 likely to talk to you a little bit more about

1 this.
2 This was a finding that we had
3 last year. This is something that we couldn't
4 have done 10 years ago because, basically, it
5 was a virus that was so distinct that standard
6 conserved primer strategy would never have
7 identified this particular virus. This is
8 something that I think Charles Chiu or Mike
9 Busch will probably chat with you folks about
10 over the next day or so.
11 So, what we are going to be
12 talking about over the next day or so is
13 really sort of the amazing new technologies
14 that we have out there. But, before we do
15 that, I sort of want to really focus for a
16 moment on biosurveillance and to think about
17 it.
18 The reality is I think we all
19 agree that this is what we want and this is,
20 frankly, what we need. But, by the time these
21 viruses have really entered into human
22 populations and spread in multiple countries,

1 our capacity to really stop that spread is
2 hugely diminished. We really need to detect
3 these things early if we are going to have any
4 chance of stopping these epidemics from really
5 bubbling up and spreading substantively.
6 But, if we want this, we are going
7 to need the sort of technology we are talking
8 about over the next couple of days, but we are
9 also going to need a couple of other things.
10 One of the things we are going to need -- and
11 this has been something which has been sort of
12 a pivotal part of the philosophy of my own
13 group -- is the development of capacity in
14 hotspots around the world where these viruses
15 are crossing into human populations.
16 This is Cyrille Djoko. We met
17 about 11 years ago. He was a young doctoral
18 student at the time. He basically was someone
19 who was going to end up in an academic
20 institution in central Africa and was going to
21 do wonderful work teaching a next generation
22 of scientists.

1 Having said that, Cyrille has
2 ridiculously good hands, and he belongs in a
3 laboratory. But, at the time we arrived,
4 there wasn't the sort of laboratory capacity
5 and diversity in central Africa that could
6 really accommodate his skills. Now that has
7 been changing substantively over the last 10
8 years, but developing that kind of capacity
9 and engaging scientists is a fundamental part
10 of this process.
11 One of the things that is really
12 pivotal to us is not just sort of developing
13 capacity and, when necessary, training
14 scientists, but it is also linking up
15 networks. Increasingly, there are different
16 skills in different countries around the
17 world, and what we really need is tremendous
18 sort of linking of these networks and
19 scientists to be mentored in various different
20 places around the world. And so, that is a
21 fundamental feature of what we need.
22 But we are also going to need to

1 advance biosurveillance, and this is something
2 that we have been thinking a lot about, right?
3 So, what these approaches, the ones that you
4 see on the right side here, really are doing
5 are detecting viruses as they cross over from
6 animal populations into humans. And that is
7 a very important and critical element of this
8 process.
9 But there is another element to
10 this process that I think is very relevant to
11 the workshop that we are having right now,
12 which is it is not just the cross-species
13 transmission and early spread of these
14 viruses; it is also the points at which these
15 viruses start to spread more widely.
16 And I will tell you just a little
17 bit about how we have been approaching this,
18 and we have been doing this work really over
19 the last five to ten years, but we are really
20 just starting to expand it. Because we need
21 to look not only at the interface between
22 human and animal populations, but also

1 critically at the nodes where we can really
2 detect the viruses that are spreading in human
3 populations.
4 And I think blood banks, blood
5 centers, blood research institutes are the
6 kinds of places where we really can do this.
7 So, one of the things that we have been doing,
8 at least in a select sort of handful of
9 hotspots that we work in, is doing donor
10 recipient studies and looking at multiply-
11 transfused individuals.
12 These individuals have tremendous
13 capacity, right? When we think about it, of
14 course, monitoring these individuals is going
15 to be critical for blood safety, but this also
16 has the potential for early detection of
17 viruses that are spreading in the human
18 population.
19 Again, I would like to just ask
20 you to think back on the first couple of
21 slides that I showed you. Had we been doing
22 monitoring of the sort that we are going to be

1 talking about in central African blood banks
2 during the sixties and seventies, it is almost
3 certainly the case we would have seen evidence
4 of HIV being transmitted at that point.
5 So, in addition to sort of
6 domestic blood safety and biosurveillance, I
7 think thinking very selectively about sentinel
8 populations at sort of blood banks and blood
9 transfusion and multiply-transfused
10 individuals in hotspots around the world will
11 be sort of a critical set of specimens that
12 really permit us to, No. 1, understand the
13 diversity of viruses not only that have jumped
14 into human populations, but are starting to
15 spread in human populations. No. 2, it will
16 help us to improve blood safety in those
17 regions of the world where there is a
18 tremendous amount of need for even sort of
19 basic blood safety.
20 But, also, critically, again, the
21 connectivity that you saw on those maps is so
22 substantive that what is happening in these

1 sort of blood banks anywhere in hotspots
2 around the world is only one or two steps
3 removed from the blood banks we have here in
4 the United States.
5 And I will just sort of leave you
6 with a slide that this is just for me to
7 remind myself to say that sort of us, for us,
8 we are primarily a field-based
9 epidemiologically-oriented organization. We
10 focus on biosurveillance. And we really focus
11 on pulling together collections, the right
12 kind of collections and the right kind of
13 sentinel populations.
14 For us, our repositories are
15 living repositories. Any sort of specimen
16 that is just sitting there is not of us for
17 anyone. And so, we have a tremendous amount
18 of interest in engaging scientists throughout
19 the community to really think about how do we
20 expand the kind of biosurveillance we do, how
21 do we increase the collections in critical
22 places, but also to provide these sorts of

1 specimens to really groups that have a
2 particular interest in looking at these
3 specimens to try to identify these phenomena
4 early.
5 Sadly, this is the slide which is
6 difficult to read. I get the sort of good
7 fortune to talk about this work, but there is
8 a tremendous amount of collaborators within
9 the U.S. Government and outside of it,
10 laboratory collaborators, sponsors of the
11 research, international research
12 organizations, that really sort of have all
13 played a fundamental role in the results that
14 I have discussed.
15 And then, they put this at the end
16 of my presentation. So, just contact
17 information.
18 But, certainly, again, we are
19 happy to talk to folks that are interested in
20 this. I think I am certainly excited about
21 the discussion we are going to have over the
22 coming days.

1 We are at a really critical and
2 interesting point here where we have the
3 techniques available to help us to address
4 this. We know the kind of surveillance which
5 needs to be done, and it is really just a
6 matter of, will there be the will and will
7 there be the capacity to go out there and do
8 this in the way that needs to happen?
9 So, thank you very much.
10 (Applause.)
11 DR. KATZ: Those of you of a
12 certain age in the audience might contrast Dr.
13 Wolfe's vision with how we responded and how
14 we thought about Legionnaires' disease when I
15 was on the faculty of the University of Iowa
16 in the mid-1970s. It is a very jarring
17 contrast, I think.
18 Our next speaker is Dr. Hira
19 Nakhasi, who needs no introduction to most of
20 the people in this room. He is the Director
21 of the Division of Emerging And Transfusion-
22 Transmitted Diseases at CBER in the Food and

1 Drug Administration. They are responsible for
2 licensure of donor screening assays for blood-
3 borne pathogens that assist in protecting the
4 nation's blood safety and availability.
5 His scientific interests, he is
6 what David Leiby and I call a Leishmaniac. He
7 has studied Leishmania donovani in the context
8 primarily of vaccines and has been an
9 excellent resource to the blood community in
10 a variety of venues.
11 Dr. Nakhasi is going to address
12 the FDA's approach to maintaining blood safety
13 from infectious agents in a changing
14 landscape.
15 Hira?
16 DR. NAKHASI: Thank you very much.
17 Thank you.
18 And I want to add my thanks to all
19 the speakers who have come today, as well as
20 the audience, from the FDA perspective.
21 So, my job today is really to tell
22 you what has been FDA's approach in

1 maintaining the blood safety over the years
2 and, as you heard, in the ever-changing
3 landscape because every time we have been
4 faced with many different issues and new
5 pathogens. My job is really to give you sort
6 of a bird's eye view of where we are and at
7 every time point when we had challenges how we
8 responded to it. And now, we are at a new
9 challenge, which you heard today from both Dr.
10 Peter Marks and Dr. Jay Epstein, that we need
11 to go ahead now with new challenges; i.e.,
12 when we have more pathogens coming into the
13 blood supply, how do we maintain the blood
14 safety?
15 So, as you heard from Dr. Epstein,
16 the major challenge we always have in the
17 blood safety arena is how do we prevent the
18 transmission of existing, emerging, and re-
19 emerging agents through transfusion versus how
20 do we maintain the adequate supply of blood.
21 So, FDA's response over the years
22 -- and many of you in the audience must have

1 heard over the period of time because we had
2 a lot of discussions, both in the scientific
3 as well as in the Advisory Committee meetings
4 -- that we basically have three approaches to
5 respond to any pathogen threat to blood
6 safety.
7 One is, first of all, first and
8 foremost, is the identification of that agent
9 or the threat. Then, also, we keep on
10 monitoring those threats to the blood supply.
11 And also, not just willy-nilly, we recommend
12 tests, but there is a risk-based approach to
13 this blood safety.
14 So, for the identification of the
15 transfusion-transmittal risk, obviously, we
16 need to, first of all, identify whether a
17 pathogen is blood-borne, is transmitted
18 through blood, transmitted through
19 transfusion, obviously, to blood, and is it
20 able to survive in blood bank processes, such
21 as including refrigeration. And then, the
22 next question is, is there a suitable assay

1 available and is there an asymptomatic period?
2 Because there may be a person who is infected
3 and doesn't show any symptoms and, then, goes
4 on to donate blood, and that can be
5 transmitted.
6 But there are some infections
7 which you don't have that much of an
8 asymptomatic period. And therefore, you are
9 not able to donate blood. So, that is a very
10 important point to remember here, the
11 asymptomatic period.
12 Obviously, we have been monitoring
13 risk. Then, the second is, how do we monitor
14 the risk? Obviously, we have been doing many
15 things in that arena. Always we have horizon
16 scanning and, also, communicating with the
17 world and our institutions where this can come
18 from. And you heard an excellent talk by Dr.
19 Nathan Wolfe this morning, how surveillance is
20 part of it and an important issue in the blood
21 safety.
22 Obviously, we will look at the

1 disease databases and maps through internet
2 search and literature reviews. And in
3 addition to that, the communication piece is
4 through internal discussions, obviously, in
5 CBER itself. We have now for the last several
6 years a CBER-wide, which is the different-
7 offices-wide, with not only blood safety, but
8 the vaccine, tissue safety, and others, and
9 including the Office of Biostatistics and
10 Epidemiology group, working together to really
11 discuss these pathogens, what is the risk, and
12 how we can monitor those risks.
13 In addition to that, at the higher
14 level, at the Department level, also, we have
15 a subgroup called PHS EDI subgroup, which is
16 part of the other PHS agencies, which keep on
17 periodic discussions of these pathogens where
18 there is an blood safety issue in that.
19 Then, obviously, the ones we have
20 the identification, we do always monitoring.
21 And then, the last thing is, before we go to
22 implementation, risk assessment. So, the risk

1 assessment is usually done through the
2 critical impact on the donor, critical impact
3 on the recipient, and the prevalence of
4 infection in the donor population.
5 Obviously, this has tremendously
6 been helped by our counterparts, the group in
7 the Office of Biostatistics and Epidemiology.
8 There is a risk assessment group and, in
9 collaboration with other outside groups, it
10 has been very, very helpful in developing this
11 risk assessment.
12 And many of you must have heard at
13 different Blood Product Advisory Committee
14 discussions we always have risk assessment and
15 discussion.
16 Then, once we have these
17 identified, we have looked at the horizon,
18 what the problems are, we also do the risk
19 assessment. Then, how do we initiate the
20 implementation of the intervention? This
21 implementation of the intervention depends on,
22 obviously, recognition of the risk to the

1 blood supply, and then, the development of
2 these interventions through many ways.
3 First of all, recommendations
4 through guidance for industry; obviously,
5 donor screening questions; test licensure and
6 implementation and optimizing the testing
7 strategy. Obviously, the story goes that, you
8 know, it can be many times in testing pools
9 and, then, in the situation of West Nile what
10 we initiated initially was the testing of the
11 pool. And soon, we became aware that in the
12 highly-endemic areas, where there is a high
13 endemicity of this West Nile activity going
14 on, West Nile pool testing is not sufficient.
15 And so, we had to trigger the ID-NAT.
16 So, obviously, for detection, it
17 is important, as Dr. Nathan Wolfe suggested,
18 that the earlier we can detect the possible
19 infection, the better we are. So, in order to
20 do that, we need to understand the infectious
21 agents and, obviously, pathogenesis of that
22 agent. We need to understand the markers

1 which can be used for direct detections,
2 whether it is a protein, whether it is a
3 nucleic acid, or an antibody as far as any
4 host response to those pathogens, such as
5 serological markers.
6 So, just to give you an example
7 how the evolution of testing has been over a
8 period of time, for example, in the HIV
9 infection, initially, first, the test
10 introduced, the first-generation test was the
11 EIA. The second was the p24 antigen. And
12 soon, it became clear that we cannot reduce
13 the risk -- we can only reduce the risk to a
14 certain extent. That had to be nucleic acid
15 testing, and that is the third generation.
16 And is a slide borrowed from Dr.
17 Busch's slides, basically, showing you how
18 these markers can change in, you know, early
19 detection versus late detection.
20 So, with that, I think that blood
21 safety at this time is safe and sound. And
22 also, it is a big impact on the public health.

1 You can see from this slide that 18 million
2 units were transfused in 2011. Risk of
3 transmission through transfusion has been
4 significantly reduced with the introduction of
5 many tests. And currently, you can see by
6 yourself the number of tests which have been
7 already mentioned also that we have there.
8 And then, there are some which are under
9 development.
10 And with that, the risk has been
11 significantly reduced, especially for HIV,
12 HCV, and HBV. And even though the HBV risk is
13 the earlier data before the NAT was
14 implemented, but, then, in relation to that,
15 now you have heard that we have instituted
16 West Nile, Chagas, syphilis, and others, which
17 has further reduced the general risk of
18 transfusion, of transmitted diseases known so
19 far. And this is a list of the FDA-
20 recommended and the voluntary tests.
21 In addition to that, not only did
22 we approve the tests to maintain the blood

1 safety, we in-house have also been developing
2 theses reference panels, which is an important
3 component of the blood safety because these
4 are the panels, reference panels, used for
5 validating the assays, to make sure these
6 tests perform the way it should be performing.
7 And I have listed some of these
8 things here, some of this panel development,
9 which we are proactively developing in-house
10 for future use. So, obviously, when the tests
11 are available, those tests can be validated
12 against some of these reference panels. And
13 there is a tremendous amount of work that goes
14 into the development of these.
15 Not only is this in-house
16 development, but this development also is in
17 collaboration with the international
18 collaborating centers. For example, CBER
19 being part of the WHO Collaborating Center, we
20 have been involved in developing international
21 standards in the world arena. So, obviously,
22 it is not just the U.S., but also the other

1 parts of the world.
2 And obviously, I think going back
3 to this, this is a very important issue here
4 because, as Dr. Nathan Wolfe suggested, that
5 having those repositories which could be
6 important for developing these panels is an
7 important component. We welcome his offer for
8 those kinds of samples.
9 Now, just to give a quick -- this
10 is some of the stories I just told you
11 initially how we have maintained so far the
12 blood safety. But, then, there is ongoing
13 initiatives on emerging diseases, which starts
14 with -- one of the success stories is the West
15 Nile story. I call it the West Nile virus
16 story.
17 You will hear from many people in
18 the audience today and tomorrow, but I just
19 wanted to give you that the theme of the next
20 two slides is it is very important, the
21 cooperation between all the stakeholders.
22 That is the FDA, the PHS agencies, the blood

1 establishments, and test kit manufacturers.
2 This West Nile, as many of you
3 know, it started in the summer of 1999, was
4 the first outbreak. In August 2002, the first
5 theoretical potential for transmission of West
6 Nile by blood transfusion was raised in a
7 publication, and that turned out to be true;
8 in September 2002, through just transmission
9 of West Nile by transplantation and followed
10 by confirmation by blood.
11 AABB sponsored the meeting of
12 these decisionmakers. And right after that,
13 we had an FDA workshop which basically was to
14 support test development and facilitate
15 communication between test manufacturers and
16 users.
17 And in July of 2003, in the span
18 of less than nine months, there was a test on
19 the market, thanks to the industry, that they
20 responded to that emergency, and we had a test
21 on the market to screen blood on a nationwide
22 screening of blood, first for mini-pool NAT,

1 and, eventually, then, yes, ID-NAT.
2 Now, in addition to that, we have
3 been holding periodically these workshops to
4 really monitor the risk to blood safety and
5 what could be done to ensure that safety. You
6 know, there is a list of workshops here.
7 We discussed the Babesiosis. We
8 have been talking about the emerging
9 arboviruses, emerging infectious diseases, and
10 not only just understanding the scientific
11 nature of these, then, pathogens and how it
12 impacts the blood safety, but also we have
13 been dealing with the risk assessment. And
14 there were several workshops by the Office of
15 Biostatistics and Epidemiology, talking about
16 the interaction with the blood establishments
17 to how we can get this data collected and what
18 type of samples need to be tested for
19 assessing the risk, and, then, obviously, risk
20 management.
21 So, the outcome of these workshops
22 was that West Nile should be a good model for

1 mitigating the risk to blood safety. There is
2 an important need for cooperation and
3 communication among the stakeholders, which
4 resulted in the blood-screening assay, as I
5 said.
6 In connection to that, too, we
7 need to have a very sound risk identification,
8 risk assessment, and risk management. On our
9 Committee, there were several unanswered
10 issues. There is still this paucity of having
11 these tests available in small markets and the
12 profitability, lack of new funding, and lack
13 of new technologies.
14 However, in connection to that, as
15 you heard from Dr. Epstein's talk, we always
16 have challenges. Challenges is the re-
17 emerging pathogens, which you heard from both
18 Dr. Nathan Wolfe's thought that now we have
19 HTLV-3 and HTLV-4. Luckily, thank God that
20 they are detected by the current HTLV-1
21 assays, but there could be some pathogens
22 which may not be, whether these tests which

1 are currently licensed, can they detect those
2 pathogen variants and mutants.
3 Then, we have always these new
4 emerging pathogens and tropical diseases. We
5 have a significant donor loss with many of
6 these pathogens where we don't have tests and
7 the donors are deferred because of potential
8 exposure. And then, obviously, the false-
9 positive screening test. How can we re-enter
10 the donors in the absence of having
11 supplemental assays?
12 So, the next step in blood safety
13 is, obviously, the current paradigm we have is
14 the limitation. The limitation is how many
15 tests versus how many agents. So, we cannot
16 have each test for each individual agent. By
17 the time you start testing, there won't be any
18 blood to transfuse. So, obviously, there is
19 an important issue there, how we can maximize
20 the effort here and minimize the use.
21 Obviously, the blood volume and
22 the logistics of testing, you know, in the

1 blood center setting, how many tests can be
2 done individually? Luckily, there is some
3 hope out there that these new technologies
4 that you will hear in the next two days can
5 help in the question. Therefore, the
6 challenge to us is, how do we harness those
7 technologies?
8 And you heard we will have
9 discussions about various detection platforms,
10 multiplex detection platforms. I don't want
11 to go into really a litany of all these
12 assays, but you will hear these things.
13 But the important issue here, and,
14 obviously, as Dr. Epstein mentioned, we in
15 CBER are proactively working on some of these
16 platforms. A few years back, because of the
17 issues of emerging pathogens, there was a
18 laboratory created in our Division, the
19 Division of Emerging and Transfusion-
20 Transmitted Diseases, called the Laboratory of
21 Emerging Pathogens, which basically the task
22 was to keep on developing, you know,

1 proactively developing proof-of-concept assays
2 and work on those issues, what kind of
3 validations that may be needed, what kind of
4 sensitivities/specificities are needed. So,
5 there are some sera technologies being tested,
6 and you will hear more details in the
7 presentations today.
8 And this is another technology
9 called nanomicroarray technology, where we can
10 differentiate different influenza viruses, and
11 studies are done by Dr. Indira Hewlett's
12 group. The previous study was done by Dr.
13 Robert Duncan's group in collaboration with
14 the lab technologies.
15 So, that brings us to this point,
16 today's point. Now what we need to do is, for
17 the next two days, we need to focus on these
18 following things, at least from my point of
19 view:
20 What are the technological
21 challenges and opportunities it provides to
22 us? Obviously, we need to have simultaneous

1 detection. Do we need the genomic and
2 proteomic risk testing? What is needed for
3 validation? And what is the sensitivity and
4 specificity in a multiplex format used by
5 informatics tests, and how those technologies
6 can be used to interchange new emerging
7 pathogens. And, more importantly, how can
8 they be adapted in a donor setting/testing
9 environment turnaround, which is very
10 important in high throughput?
11 In relation to that, there are
12 issues of cost-effectiveness. How can these
13 new technologies -- they may be very good and
14 very flashy -- but can they be cost-effective
15 in the blood donor setting?
16 And obviously, you heard that the
17 funding is an important issue. And the more
18 important issue really is the regulatory
19 issues. That is, what kind of clinical trials
20 we need to design this thing. Can we use
21 foreign study data? What kind of pre-clinical
22 studies we may need. What type of samples we

1 need. And I think you heard this morning that
2 there is an opportunity that we may have some
3 samples. And what kind of good manufacturing
4 practices we have to establish for this.
5 Many of these pathogens are not
6 universally -- West Nile was a good example to
7 begin with, but now there are several
8 pathogens which are only in certain
9 geographical regions or seasonal. So, how do
10 we tackle that issue?
11 So, in conclusion, basically, I
12 want to thank you all of you.
13 And then, the question really is,
14 where do we go from what we have now and what
15 we are anticipating in the future? And
16 therefore, the challenge for us for the next
17 two days is to really come up with some
18 criteria or discussion points where really, as
19 FDA, we take those discussion points forward
20 and, then, have an interim discussion about
21 what next steps we need to take.
22 Thank you very much for your

1 attention.
2 (Applause.)
3 DR. KATZ: So, from global to the
4 FDA's world view, and now, down onto the
5 ground, actually in the blood centers, blood
6 collection facilities and laboratories in the
7 U.S.
8 Sue Stramer from the American Red
9 Cross is going to discuss advances in
10 pathogen-testing technology and transfusion
11 safety.
12 Sue trained at the University of
13 Northern Illinois and, subsequently, at the
14 University of Wisconsin, moved to the CDC,
15 then to industry at Abbott Labs, and almost 20
16 years ago -- I can't believe it -- to the
17 American Red Cross, where she remains the
18 Executive Scientific Officer.
19 And a subject of great envy on my
20 part, the Red Cross is kind of a top-down
21 organization in a certain sense. And the
22 people that work with Sue assure me that she

1 has a stranglehold on what happens to donor
2 samples. I, on the other hand, have an
3 association of 70-odd members, and it is,
4 indeed, herding kittens.
5 Sue has been active across the
6 spectrum of opportunities in the blood
7 community, and particularly at AABB, where she
8 has been a valued colleague of mine on the TTD
9 Committee for some long period of time, has
10 been on the Board of Directors, and is the
11 current President of the Association.
12 She has won lots of awards and
13 publishes lots of papers that really have been
14 at the core of our understanding of
15 transfusion safety in the United States.
16 Sue?
17 DR. STRAMER: Thanks, Lou.
18 Thanks, all.
19 I am the last speaker before you
20 get to go to the bathroom. So, let me get
21 started as soon as I figure out the control
22 panel here. Am I supposed to use this thing

1 to advance? All right.
2 So, it is unfortunate that I am
3 going to talk about history, unfortunate
4 because it means I am getting older. But, in
5 the days back when, we did agar gel diffusion
6 as one of the earliest methods to screen blood
7 donors for safety. And the first marker we
8 did this for was hepatitis B surface antigen.
9 We now, for HBsAg and for most
10 serological markers or all serological
11 markers, have the opportunity to use fully
12 CGNP automated instruments, high-throughput,
13 high-quality. So, if you look at what was
14 important then versus now, and you consider
15 one agent or one marker, HBsAg, in converting
16 these technologies, we have seen a 5-log
17 reduction in the detection of HBsAg. And that
18 is quite significant from 10 micrograms per mL
19 to less than .1 nanograms per mL. So, that is
20 quite an amazing accomplishment and likely
21 something that we won't see comparably moving
22 forward.

1 So, I just remind you all, I was
2 in the United Red Carpet Club, as I spend a
3 lot of my time, looking through magazines. I
4 came across this image and related articles in
5 Spectrum. It is in this month, if you get to
6 the Red Carpet Club, courtesy of United.
7 This marks the 60th anniversary of
8 a lot of things, including the discovery or
9 publication of DNA in three papers in Nature.
10 So, April 1953 marks that point. We are now
11 in 2013, at the 60th anniversary. The
12 photograph I show you there or the
13 photomicrograph of the x-ray diffraction of
14 Rosalind Franklin was the key to uncovering
15 the fact that DNA was a double helical
16 molecule.
17 So, we know now the testing for
18 nucleic acid is routine. We do that every day
19 in blood centers and in diagnostic
20 laboratories.
21 To remind you or to note to you
22 that the first sequencing of the human genome

1 took 13 years and cost $3 billion. Now
2 desktop analyzers are available that can do
3 the same thing in one day for about $1,000.
4 So, technology marches on.
5 Dr. Nakhasi went through a lot of
6 the tests, and you will probably hear this
7 reiterated, but, again, this is what we do
8 today. Starting in 1938 with syphilis, now we
9 do IND testing for novel agents such as dengue
10 and Babesia. The routine tests that we do
11 perform are antibody and NAT tests for HIV-1
12 and -2, including Group O; HCV, again,
13 antibody and NAT. HBV, we have the luxury of
14 doing three tests, a test for HBsAg, as I
15 mentioned, anti-core antibody, and mini-pool
16 NAT.
17 Most laboratories, if not all
18 laboratories, in the United States do NAT for
19 HIV, HCV, and HBV in a pooled, a mini-pool
20 format using multiplexed assays, one of two
21 manufacturers.
22 We screen for antibodies to HTLV-1

1 and -2. As already referenced, we screen for
2 West Nile in mini-pool and, then, due to low
3 viral loads during epidemic periods, we
4 convert to single-unit testing. We do
5 selective donor testing for T. cruzi. We do
6 selective product testing for anti-CMV.
7 We also screen markers and plasma
8 fractionation. So, plasma companies also look
9 for parvovirus B19, HAV, and, most recently,
10 HEV, and this is because these are pathogen-
11 reduction-resistant agents, non-enveloped, and
12 difficult to inactivate.
13 There are two multiplex mini-pool
14 NAT platforms available for parvovirus and
15 HAV, and those are used widely.
16 There are other transfusion-
17 transmittal agents for which we do not test
18 because there are not available tests. So, we
19 screen donors for health history or travel,
20 including malaria, variant Creutzfeldt-Jakob
21 disease, and Leishmania.
22 There are many emerging infectious

1 agents of concern. I just listed a few. HEV
2 is one of particular heightened interest right
3 now because of known transfusion-transmitted
4 cases in Japan and in Europe.
5 There have been recent
6 publications about transfusion transmission of
7 the two tick-borne bacteria, Anaplasma,
8 Ehrlichia. There are concerns about other
9 arboviruses. I mention Chikungunya, although
10 there is no documentation that that is
11 transfusion-transmitted, but really how could
12 it not be, judging from the prior outbreaks?
13 We do screen for bacteria. And
14 relative to the technologies that we use for
15 the agents that I mention in the first slide,
16 we are in the infancy for bacterial testing,
17 for tests and systems that are only about 50
18 to 75 percent sensitive. They detect multiple
19 bacterial organisms, mainly skin flora.
20 And then, we do yet nothing about
21 red cell contamination, for example, with
22 bacterium Yersinia, which has been involved in

1 many red cell transfusion transmissions.
2 Looking at technology advances
3 over the years, starting at the top row, we
4 can see manual, semi-automated, or automated.
5 The techniques are, then, listed, whether we
6 started with tube agglutinations, gel
7 precipitations, have gone on to RIAs and EIAs,
8 various generations. We have introduced NAT
9 testing in a semi-automated fashion, and I
10 have listed the two technologies that were
11 used.
12 And automated testing really began
13 with the Olympus in 1988 when they launched
14 their first instrument for blood group
15 serology. But now we use PRISM for our
16 routine serology, TIGRIS or the cobas s201 for
17 NAT.
18 So, the years, again, are listed
19 in the third row, and, then, the markers I
20 have listed. So, we have everything now in an
21 automated fashion, either on serology, on
22 PRISM, or comparable technology by Ortho, and

1 NAT, using either TIGRIS or the s201.
2 So, here you can see someone
3 loading a PRISM. This is in the Charlotte
4 NTL, one of our labs. And again, the PRISM
5 may not be or the technologies used for
6 serology may not be multiplex per se, that is,
7 all the reactions occurring in one tube, but
8 it doesn't really matter. You put the samples
9 in and, then, you come back later and you get
10 the results for all markers on six channels.
11 So, for serology, it has been as multiplexed
12 as we can get.
13 The situation is a little bit
14 different for NAT, where we do have all the
15 reactions occurring in one tube through using
16 high-throughput instruments. Both of these
17 instruments are relatively-comparable as far
18 as high throughput and CGNP.
19 It is not just about screening
20 tests. It has already been alluded to, I
21 believe, by Dr. Epstein that we also do
22 supplemental testing or have the need to do

1 supplemental testing when we have reactive
2 results for donors and we need to counsel
3 them, or we need to look back on products and
4 let hospitals and consignees know what to tell
5 recipients.
6 So, for serology, I won't go
7 through this painful list, but it does include
8 a variety of technologies. And we do AIDS
9 screening tests and, to confirm for serology
10 those AIDS screening tests, we use 16
11 supplemental tests. So, we want to make sure
12 we get the answer rights before we counsel the
13 donor and before we send them off into the
14 healthcare system.
15 For NAT, we have a triplex test
16 for HIV, HCV, and HBV, as I mentioned. We do
17 PCR for confirmation. West Nile, also a
18 different NAT test. It is not multiplexed
19 with the others. We do confirmation by PCR
20 and antibody.
21 And to remind you that, again, we
22 do other testing for parvovirus HAV in plasma,

1 HEV in some, and we have INDs that are active
2 for dengue viruses and for Babesia.
3 So, going back to note another
4 significant date, this is the 30-year
5 anniversary of the discovery of HIV and, also,
6 the 30-year anniversary of when AIDS
7 interventions were introduced to prevent
8 transfusion-associated AIDS and HIV
9 transmission.
10 And if you want a good history on
11 what has happened in the last decades in blood
12 donor screening, I urge you to read the two
13 reviews that I put in bold. One is Simone
14 Glynn and colleagues. We had a workshop at
15 the NHLBI. And even though it is titled
16 "Emerging Infectious Agents," et cetera, most
17 of the manuscript focuses on how we got to
18 where we are today. And then, an excellent
19 editorial by Herb Perkins and Mike Busch.
20 But I urge you to read what is in
21 quotes: "...the blood bank in San Francisco
22 developed a process whereby donors could

1 discreetly exclude themselves by answering
2 `yes' or `no' to a block of questions about
3 known AIDS risk factors. By mid-1983 many
4 blood collection organizations, particularly
5 those in cities at high risk, had adopted a
6 donor qualification process known as self-
7 deferral by which presenting donors were
8 provided with information about AIDS risk
9 factors and where those with such risk factors
10 were asked not to donate."
11 And to show the significance of
12 that, this is a graph in the same publication,
13 Mike's graph from the early days of HIV in San
14 Francisco. If you look on the Y-axis, you can
15 see risk for HIV was in excess of 1.5 percent.
16 In 1983, HIV was discovered. Risk factor
17 education and self deferral and other
18 processes were put into place, confidential
19 unit exclusion. So, donors at risk could
20 defer, be deferred, or self-defer.
21 And you can see that that dropped.
22 Then came HIV screening which was implemented.

1 And the impact of that, actually, if you look
2 at this graph, and the data are real,
3 actually, the health history and the early way
4 we tried to combat HIV was highly effective,
5 and some could argue more effective than
6 screening.
7 Looking at screening -- and this
8 is a slide from Abbott Diagnostics showing the
9 evolution of the various screening tests -- I
10 bolded on the bottom "Available outside of the
11 United States," because although we believe
12 the processes that we use in the United States
13 are very effective in ensuring donor health
14 and recipient safety, we sometimes have been
15 decades behind the rest of the world in
16 implementing new technology, such as
17 recombinant assays for HIV detection or
18 automated platforms.
19 This slide has already been shown
20 before. But what this translates to in window
21 period reduction, in yellow here is antibody
22 testing, first gen, second gen, which is

1 really purified antigens as opposed to a viral
2 lysate, and third gen, which included the
3 ability to detect IGM when we did antibody
4 testing. And each of those, sort of
5 progressive improvement in reducing window
6 period risk.
7 We implemented p24 antigen, very
8 controversial, to get about a five-day closure
9 on the window period; implemented NAT, even in
10 mini-pools, which added another five days.
11 And you can see that the pink line -- and I
12 will show you this in another slide -- is a
13 direct subset of the black line. So, we could
14 drop p24 antigen, which has really been the
15 only FDA-licensed test that we have ever
16 dropped.
17 Looking at HIV, our
18 accomplishments with HIV in another way, is to
19 look at the number of transfusion-associated
20 HIV transmissions since blood donor screening.
21 There have been 57 since the implementation of
22 NAT. On this slide, it shows four, but

1 actually there have been five, at least five
2 cases. And I show you those just to
3 familiarize yourself with when they occurred,
4 where they occurred, and the components which
5 transmitted.
6 But a very important point are the
7 two cases that are bolded, the Maryland and
8 Georgia cases. Those are Red Cross cases in
9 which the FFP transmitted, but the red cell
10 components did not. And it has been estimated
11 that there is about 20 mLs of plasma in a red
12 cell component versus 200 mLs or so in an FFP
13 component.
14 So, if you see that the larger-
15 volume components transmitted but the smaller
16 plasma component did not, even with mini-pool
17 NAT, one could say that we are at the edge of
18 infectivity for what we are doing, and pretty
19 successful in combating HIV. Considering that
20 since 1999 these are the only documented cases
21 -- certainly, there have been more; probably
22 not-recognized recipients have died -- but

1 these are the ones that are known. And again,
2 we are probably reaching the edge of
3 technology related to infectivity.
4 This slide now shows you similarly
5 for hepatitis B from Dr. Alter, although I got
6 this actually from Rick Davy. We all work in
7 strange ways to get other people's data. But,
8 anyway, thank you, Harvey, indirectly.
9 This shows you the reduction, the
10 same way as I showed you for transfusion-
11 associated AIDS for hepatitis. If you
12 consider the 25 percent or more in the late
13 sixties/early seventies could get hepatitis
14 infection from a transfusion of blood, these
15 are observational studies of the NIH. And you
16 can see, with each progressive screening test
17 that was introduced, especially the impact of
18 HBsAg introduction, even at the levels of 10
19 microgram-per-mL sensitivity, what a profound
20 impact that had on risk reduction.
21 The other one was the introduction
22 of HCV 1.0, perhaps not the best screening

1 test, only one putative antigen, but, again,
2 lots to improve safety.
3 If you look back on what screening
4 tests we used in the early days and what the
5 residual risk was from those tests, I have
6 listed them here from the Red Cross experience
7 for HBsAg, anti-HIV, and anti-HCV. The anti-
8 HIV data are system data. But if you look at
9 just the Washington, D.C. area, as noted in
10 the review by Perkins and Busch, certainly,
11 the highest risks occurred in the urban
12 centers. And in D.C., we saw a residual risk
13 of about 1 in 300.
14 Looking at what they are today,
15 those are the numbers in red. You don't have
16 to take in a lot of the data on this slide,
17 although I will torture you through it. The
18 bottom line is residual risk is HIV, HCV, and
19 now HBV is about 1 in a million. And the
20 residual risk reduction for HBV is really a
21 combination of sensitive HBsAg testing,
22 sensitive nucleic acid testing, and a very

1 effective HBV vaccine that has allowed us to
2 reduce incidence by about 50 percent, looking
3 at the two reporting periods I have there,
4 2006 to 2008 and 2009 to 2011.
5 Now I am just going to show you
6 three slides giving you each virus in a little
7 detail. I have already walked you through
8 HIV, but this actually gives you data on a
9 real donor, a plasmapheresis donor. The
10 purple line, again, starting with antibody;
11 the blue being p24 antigen; the red being
12 quantitative nucleic acid testing, and, again,
13 the blue being a subset of red. And then, the
14 green lines, the open bars show you pooled
15 NAT, and the solid green triangles show you
16 individual unit NAT. So, clearly, again, with
17 individual unit NAT, it increased safety,
18 reduced the residual risk, not by much, but it
19 certainly can occur.
20 The driver for the implementation
21 of NAT worldwide was hepatitis C virus. And
22 this slide clearly can show you why, the blue

1 being antibody testing, even using 2.0 or
2 multi-antigen tests, relative to any
3 technology for NAT. We see about in this
4 example 46 days, but, generally, about a 50-
5 to-60 day window period reduction. So, that
6 is quite remarkable.
7 For hepatitis B, the plasma donors
8 that were studied early on didn't go out far
9 enough so we could see the early events that
10 occur post-infection. But, from slides like
11 this, you really can see that HBsAg in orange
12 and HBV DNA -- this is a quantitative HV DNA
13 test -- in pink basically parallel, which
14 brings us to the question, if we have a good
15 NAT assay, really do we still need HBsAg
16 tests?
17 Putting this all together from a
18 review article that was published by Steve
19 Kleinman and colleagues, you can see all the
20 window period reductions that were implemented
21 for each of the tests, anti-HIV, leaving what
22 residual risk we have in the dashed line post-

1 NAT. And this is theoretical infectivity. As
2 I showed you from the cases of HIV
3 transmission, if we are in pooled NAT now and
4 we see differential transmissions, one can
5 argue, even when we go to individual unit NAT
6 or beyond that, how much HIV will we actually
7 see or how much will there be to prevent.
8 HCV, a few days prior to what we
9 are doing with pooled testing. HBV is a
10 different story, and it is a slow ramp-up
11 period, early in infection, low viral loads.
12 So, it has been quite a challenge to reduce
13 the residual risk for HBV, requiring quite
14 sensitive HBV DNA assays.
15 The United States is not alone in
16 doing this. I mentioned that this occurs
17 worldwide. This review in VoxSanguinis from
18 the ISBT working party just shows the number
19 of countries that have implemented NAT, when
20 they have implemented them, and for what agent
21 it has been implemented.
22 Now, moving to what is unknown or

1 what we do when something is unknown, we can
2 look at West Nile as an example of our ability
3 to respond to the unexpected. We had a NAT
4 platform in use which allowed additional
5 targets to be implemented rapidly. West Nile
6 was identified as transfusion-transmitted in
7 the late summer of 2002. And within eight to
8 nine months, we not only had an
9 investigational test, but that test was
10 implemented and in practice.
11 And I will contrast the situation
12 with another vector-borne agent, Babesia, that
13 hasn't seen the same level of traction, but
14 perhaps recently we are moving forward. Just
15 giving you a flavor for West Nile and the Red
16 Cross experience, we have seen nearly 1600
17 West-Nile-positive donors, the states in red
18 with the highest level of activity.
19 But, again, the challenge for West
20 Nile is really the viral loads that we see
21 with this agent. This is a box-and-whisker
22 plot. And the four boxes and whiskers who you

1 the level of viral RNA in different phases of
2 infection. And Hira already mentioned that we
3 do triggering, and we do triggering to pick up
4 the viruses in the first box and the last box.
5 Our 95-percent confidence limit for the
6 detection by pool testing is about 190 or 200
7 copies per mL. So, in order to reliably
8 detect low levels during West Nile epidemics,
9 it is really critical to trigger or we will
10 miss those.
11 Looking at the level of
12 sensitivity of triggering, we had done a
13 retrospective analysis in 2002. Looking at
14 the number of samples we detected in a
15 repository that were West-Nile-positive for
16 RNA, over 60 percent of them could not be
17 detected in pools.
18 So, once we started screening and
19 we started to do triggering, which we did at
20 the Red Cross in 2003, the first year we
21 started to see testing, as we noted in
22 Nebraska, in particular, that in certain blood

1 drives we were seeing 1 in 40 donors being
2 positive for West Nile RNA. And we knew they
3 were low viral load. So, we had to do
4 something different. So, over time, as we
5 were refining our triggering criteria, the
6 percent of donations that we detected, the
7 total that are only detected by an individual
8 test strategy increased.
9 Going to Babesia, this is a
10 neglected pathogen. But now there are INDs in
11 place for which we can do testing, but not
12 from the typical players that we get our NAT
13 tests or our antibody tests from, but from
14 smaller or niche companies.
15 This review by Barbara Herwaldt
16 shows you the problem with Barbesia. It is
17 certainly exploding, that last bar, 2005 to
18 2009. It only gives you really the tip of the
19 iceberg about what we are seeing: 162 cases
20 published here, primarily in seven endemic
21 states.
22 So, we have introduced IND testing

1 last year. We do an antibody test and a PCR
2 test. The challenge with Babesia being an
3 intraerythrocytic parasite is it is not just,
4 you know, you put serum in a tube and go test.
5 It is a little bit more complicated because
6 you need red cells to PCR.
7 We also do a high number of tests
8 for confirmation, including serology,
9 parasitologic tests, and infectivity assays.
10 We screen predominantly for our prospective
11 screening in Massachusetts and Connecticut.
12 I will just show you two maps, even with IND
13 testing, about how many yield samples we have
14 been seeing.
15 The green dots show you antibody-
16 only positives. These can be low-titer
17 antibody positives. The orange are antibody
18 plus DNA-positive samples. And the three on
19 this map that are in red -- note this was only
20 last year's data -- show you PCR window period
21 cases. So, just like other markers, HIV, HBV,
22 HCV, for something like Babesia, in order to

1 be effective, we are going to need both
2 technologies, NAT and antibody testing.
3 Looking at the same for
4 Massachusetts, here, again, you see an array
5 of either antibody-positive, a mixture of
6 antibody plus NAT, and NAT-only, the two in
7 red. This is eastern Massachusetts.
8 Looking at the positive predictive
9 value of what we do by phases in Babesia, I
10 show this slide for the reason that these IND
11 tests are remarkably reliable. In fact, as
12 far as positive predictive value, Babesia has
13 a higher PPV than any test we do routinely in
14 blood center testing, even though those tests
15 have been around for a long, long time.
16 So, with the exception of the
17 bottom row, late-resolving, compared to window
18 period, acute, where both markers were
19 positive, early-resolving, if you look at
20 late-resolving, that is the only category that
21 we have seen donors who haven't confirmed.
22 Perhaps they are further-resolved positives or

1 perhaps they are false-positives.
2 So, if you put all of this
3 together for what we are doing in blood
4 centers, actually, Babesia microti, if you
5 look at prevalence, takes the cake as the No.
6 1 with the rate of 1 in 150 in endemic areas,
7 followed similarly by dengue, and then, the
8 rest of the agents. T. cruzi there has a U.S.
9 in parentheses after it because that
10 represents when we were doing nationwide
11 screening. As I mentioned, we do selective
12 screening now.
13 Lastly, I just want to highlight
14 emerging infectious disease agents. As was
15 highlighted by Dr. Nakhasi, in addition to the
16 U.S. Public Health Service, which has work
17 groups to which the industry as a whole is not
18 privy, we have developed our own working group
19 through the AABB, through the Transfusion-
20 Transmitted Diseases Committee. We have put
21 together a supplement to list and prioritize
22 EID agents.

1 Lacking a current and effective
2 intervention, we identified 68 at the time
3 such agents, with the highest priority going
4 to dengue, Babesia, and variant CJD because we
5 know they are transfusion-transmitted; we know
6 they cause dire diseases with high fatality
7 rates, and because there is no intervention.
8 We have been busy updating fact
9 sheets, adding fact sheets. So, the first
10 five on the top are our new fact sheets. The
11 bottom 11 are fact sheets that have either
12 been released or Anaplasma and Ehrlichia,
13 which are in process.
14 We have also spent a lot of time
15 as an industry chasing agents that turned out
16 not to be transfusion-transmitted. So, I show
17 you this slide to remind us all of XMRV, and
18 perhaps we don't want to be reminded of that.
19 As far as what we are doing now,
20 we are building a toolkit, that is, where do
21 we start, how do we do what we have been
22 doing, how do we evaluate what we have been

1 doing, and how do we pass it on to the next
2 generation.
3 So, we have written a mission
4 statement. So, I will read part of it.
5 "To develop a systematic approach
6 to risk assessment and intervention
7 development for the impact of emerging
8 infections upon blood safety in North
9 America."
10 So, in order to do that, we are
11 starting with a process map, and our process
12 map, of course, looks at threat inputs, which
13 has already been highlighted today with
14 horizon scan, perceived threats the community,
15 outbreaks that have been recognized, cases
16 recognized by clinicians, whether they are
17 sentinel in transplant or transfusion, et
18 cetera.
19 We ask the question, is the agent
20 identified? If so, we continue. If not, we
21 continue further assessment. Is the agent or
22 condition a threat to recipient safety? Is

1 the agent or disease threat severe? And is an
2 intervention needed with, then,
3 recommendations of implementation, a
4 validation, and assessment?
5 So, in closing, if answers in
6 technology have allowed the implementation in
7 routine screening for multiple agents using
8 high-throughput, automated, and fully CGNP
9 platforms, threats remain for which solutions
10 will be identified and adopted, some faster
11 than others.
12 To combat the threat of
13 potentially-increasing numbers of EID agents
14 that may be transfusion-transmissible, our
15 options are: we can continue with the same
16 paradigm, which I just reviewed. We can
17 further improve throughput and capacity, which
18 is the theme of this workshop.
19 And I do want to comment, as we
20 will hear about sequencing, where the role of
21 sequencing is, at least today, and then,
22 pathogen reduction, which will, hopefully,

1 take us to testing simplicity.
2 So, I will just end showing you
3 that we have had, since NAT for HCV, although
4 these have not been published, we have had
5 transfusion-transmissions. And the only way
6 we know that is through sequence analysis.
7 So, be it for dengue or Babesia or for HCV,
8 the importance of sequencing is great. And I
9 don't want to get into the details of these
10 cases. They will be published.
11 So, I want to end by saying thank
12 you.
13 (Applause.)
14 DR. KATZ: All right. You have a
15 15-minute bio and coffee break. The Q&A will
16 be after Session II.
17 (Whereupon, the foregoing matter
18 went off the record at 9:50 a.m. and went back
19 on the record at 10:13 a.m.)
20 DR. CHIU: Okay. Why don't we got
21 ahead and get started, because we are running
22 on a quick time schedule here?

1 I will be chairing the second
2 session, the Session No. 2, which we titled
3 "Advances in Blood-Borne Pathogen Detection".
4 I am Charles Chiu. I am faculty
5 at the University of California, San
6 Francisco. I am also the Director of the UCSF
7 Abbott Viral Diagnostics Laboratory.
8 So, the first talk will be Dr. Tom
9 Briese at the Center for Infection and
10 Immunity at Columbia University. Dr. Briese
11 is Associate Director of the Center for
12 Infection and Immunity and is also the
13 Associate Professor of Clinical Epidemiology
14 at the Mailman School of Public Health. He
15 also serves as the Associate Director of the
16 World Health Organization's Collaborating
17 Center on Diagnostics, Surveillance, and
18 Immunotherapeutics for Emerging Infectious and
19 Zoonotic Disease".
20 He is internationally recognized
21 for characterizing originally the Borna
22 disease virus. Also, he has made major

1 contributions to identifying West Nile virus,
2 of particular relevance here as well, given
3 its concern as a transfusion-transmissible
4 agent; Lujo virus; the first hemorrhagic
5 fever-associated arenavirus from Africa
6 identified in the past three decades, and also
7 discovery of a third previously-unrecognized
8 species of rhinovirus, human rhinovirus C.
9 His research focuses on the
10 molecular epidemiology of emerging viral
11 diseases, the identification of potential
12 biothreat agents, and innovative approaches
13 towards pathogen discovery.
14 So, he will be talking today to
15 you about some of these new technologies and
16 their potential applications for blood-borne
17 pathogen screening.
18 Tom?
19 DR. BRIESE: Hello. Is the
20 microphone on? Hello. Yes.
21 Good morning, and thanks to Sanjai
22 and the organizers for their timely and

1 wonderful meeting and, also, for the
2 invitation to present to you some of the work
3 that we are doing at the Center for Infection
4 and Immunity.
5 Now pathogen detection or
6 identification, there are a limited number of
7 approaches that one can take. One would be an
8 indirect approach, that you look for secondary
9 responses of the host to the infection; for
10 example, changes in messenger RNA or protein
11 expression, which is not highly specific for
12 an individual agent. Alternatively, one can
13 use antibodies or the human immune response,
14 which is very specific and a good target. But
15 inherent to all of these techniques, there is
16 a gap in detection between the actual
17 infection and the manifestation of responses.
18 Immediate detection is permitted
19 by direct identification and detection of the
20 agent itself, methods like isolation and
21 growth in cultural or visualization work, but
22 they are limited in sensitivity and the

1 property of the agent to grow in culture. So,
2 if it is fastidious requirements, it doesn't
3 work. If it is low-titer, it is not very
4 useful.
5 Then, one can go for the actual
6 components that make up the microorganism,
7 lipids. Lipids are also not specific for
8 individual agents. Proteins, it is a feasible
9 target to detect. However, compared to
10 nucleic acid detection, it doesn't provide the
11 tremendous amplification of target that we can
12 achieve in PCR-based methods, for example.
13 So, over the past 10-15 years,
14 nucleic acid protection, specific PCR-based
15 methods have become very popular in diagnosis,
16 surveillance, and pathogen identification.
17 Around the time 15 years ago or so, Ian
18 Lipkin, Director of our Center, and myself
19 started to think about a comprehensive toolkit
20 that one would want to have to identify agents
21 known, unknown, emerging, maybe even
22 synthetic, to get a rapid identification.

1 So, what we are currently using is
2 a front-end multiplex PCR film that can detect
3 20 to 30 agents in a single reaction, a single
4 tube. And we built panels of primers that
5 target syndromic entities. For example,
6 respiratory infection, you can think about 20-
7 30 agents that come to mind or other
8 conditions, gastroenteritis, and so on,
9 sepsis, that are characterized by the number
10 of agents that you want to test for.
11 If samples come up negative, then
12 you have to do a more elaborate evaluation.
13 At the time, microarrays were a very cost-
14 efficient and promising tool. So, we
15 developed the GreeneChip, which comes in
16 different flavors, just detecting all viruses
17 or panmicrobial, where you also have detection
18 of bacteria, parasites, or fungi, and one can
19 break down these arrays in smaller separates
20 and target specifically, for example,
21 respiratory agents, subtyping influenza, and
22 so on.

1 But if the agent is really novel,
2 it may not be detected just on the basis of
3 homology to known organisms. So, sequencing
4 provided a method to address really novel
5 agents. You don't have to make any
6 assumptions or have to have any knowledge
7 about the nature. You just sequence all the
8 nucleic acid, subtract the host background,
9 and then have candidates that might represent
10 your pathogen.
11 So, keep in mind with all these
12 sequence-based assays, you get sequence
13 information. So, you characterize the agent
14 by its sequence. You gain no knowledge about
15 the biology, about pathogenicity, or any
16 phenotypic features. So, that is downstream
17 work that comes after the characterization and
18 identification of an agent. Some of that can
19 feed back into the loop, but it is additional
20 work.
21 Nevertheless, high-throughput
22 sequencing or next-generation sequencing has

1 become a very powerful tool to characterize
2 and detect novel agents. And that is part of
3 the list that represents agents that we
4 detected in the past three or four years,
5 covering not only human pathogens, but also a
6 number of animal pathogens.
7 So, that leads a little bit back
8 to Nathan's talk, that is, emerging diseases.
9 There are cyanotic agents. The majority of
10 the agents are cyanotic. And we like to use
11 the sequencing approach, also, in a project
12 called the PREDICT project, which involves a
13 number of collaborators and institutions, also
14 the Global Viral Forecasting Initiative.
15 USAID PREDICT has the mission to
16 detect or identify pathogens of pandemic
17 potential that have not yet emerged. So, that
18 poses certain challenges on the design of the
19 study and how to do testing. We cannot go
20 into detail in the timeframe of the talk here.
21 But, as an example, I want to go
22 to a study that actually precedes the program

1 and triggered some of the concepts in the
2 program. It was a study that we did with the
3 EcoHealth Alliance, Jon Epstein, in
4 Bangladesh, where they were collecting samples
5 from bats, the large fruit bats that transmit
6 Nipah virus through secretor or saliva or
7 feces that they contaminate with the palm sap
8 collections that the people do there for human
9 consumption.
10 So, what we were asking was the
11 question there may be other viruses or
12 pathogens in those bats that might be
13 transmitted to human via the same mechanism or
14 route. And when we analyzed our sequenced bat
15 samples from a certain area in Bangladesh, we
16 did find another virus, a GB-like agent, in
17 the bats. Now a GB-like agent, it is a
18 flavivirus that has been now classified into
19 the genus pegivirus, distantly related to the
20 genus hepacivirus, with hepatitis C as the
21 most prominent member. So, this was a virus
22 that is distantly related to hepatitis C.

1 The next question, then, was can
2 it also transmit or transfer into the human
3 population. So, we collected blood samples
4 from people in the area and wanted to test for
5 immune reactivity to the agent, which would
6 confirm infection. And then, one can ask the
7 next question: is infection related with any
8 disease or adverse effects?
9 To that end, a number of studies
10 have been performed since, one by Amit Kapoor
11 in the Center, where we looked into dogs with
12 respiratory symptoms. And strange enough, we
13 found a canine hepacivirus, a homologue of
14 HCV, in those dogs.
15 And then, the story went on with a
16 serologic assay, LIPS assay, where we use
17 recombinant protein expressed from a sequence
18 we obtained from those agents. The
19 recombinant protein is linked to a Luciferase
20 reporter sequence. And then, we basically do
21 a classical immune precipitation assay with
22 the test sera and ask, do we bring down any

1 Luciferase labeled protein? So, it is the
2 antibody to the agent.
3 And that showed, in the case of
4 the hepaciviruses that these canine
5 hepacivirus-related agents are way more
6 prominent in horses than in dogs. And then,
7 the story went on. Just recently, Amit found
8 rodent hepaci- and pegivirus in a large
9 quantity or very diverse.
10 Now, meanwhile, parallel studies
11 in bats were going on. And it is a just-
12 submitted or accepted paper where we show
13 hepaci- and pegiviruses in multiple bat
14 species, actually comprising Micro- and
15 Macrochiroptera, basically, in many species in
16 countries around the globe. So, it is a
17 worldwide distribution of these multiple
18 lineages of hepaci- and pegivirus, suggesting
19 that bats are a natural reservoir for those
20 viruses.
21 So, this shows that there is an
22 ample opportunity for emergence or maybe

1 species transfer of these hepatitis C virus-
2 related agents. But does that pose any risk?
3 So, how about pathogenicity? How
4 do you make a link between presence of an
5 agent and the causal relationship to disease?
6 First of all, this is done by Koch's
7 postulates, fulfilling Koch's postulates, so
8 show that the agent is specific for a disease,
9 isolate the agent, throw it in culture, put it
10 back into an organism, and recreate the
11 disease.
12 Now there are many problems with
13 the concept and the molecular error. There
14 might be no model system in which you can
15 recreate the disease. The agent may not grow
16 in culture. And there are many examples where
17 environmental factors, genetic background, or
18 even co-infection that we find very frequently
19 now in this multiplex testing, modulate
20 disease symptoms and outcome.
21 So, modifications to the concept
22 have been made y Fredericks and Reiman

1 introducing molecular markers as a synonym for
2 the agent itself. That is good, but it
3 doesn't solve really the problem.
4 Rivers introduced the presence or
5 establishment of an adaptive immune response.
6 That is a very attractive concept, at least to
7 confirm infection. And so, we have begun to
8 build serologic tools. As we heard in the
9 previous presentations, there are no really
10 21st century serologic assays out there. It
11 is pretty basic singleplex assay or testing.
12 So, what we do here is we print 12
13 nanopeptides, shift it by one amino acid, to
14 cross three antigenetic targets of epivirus,
15 vertebrate virus that is known or
16 characterized.
17 This is an example showing proof
18 of the concept where we used small, 100,000
19 feature arrays with Pteropus virus and
20 flavivirus peptides to test previously-
21 characterized sera. As you see, you can get
22 specific recognition of flavivirus, dengue,

1 West Nile, and flavivirus peptides that not
2 only show immunoarray activity, but in one
3 step, also, determine, map the actual epitope
4 that is recognized by the antibodies that are
5 present in the serum. And that is
6 characterized by multiple neighboring peptides
7 giving a signal as opposed to spurious single
8 peptide signals that are not specific.
9 So, using these tools, we think we
10 can improve undercharacterization of agents
11 and build causal relationships, although, as
12 summarized by Ian in a recent publication, we
13 don't think of it as an one-off system or
14 yes/no. There is a continuum of confidence
15 that you can build.
16 So, presence of an agent makes a
17 good candidate. And it is possible that it is
18 related to disease. Then, additional features
19 as antibody reactivity presence in the site of
20 the actual lesion or homology analogy to other
21 characterized agents or diseases can build
22 additional corroborating evidence and, then,

1 fulfillment of postulates, or reaction
2 treatment with specific lots can actually
3 prove that the causal relationship is there.
4 Now that is certainly one aspect
5 of the work, to look for novel, exotic
6 emerging agents, but in the context of the
7 blood donor testing, it might be a more
8 tangible goal to just test a larger proportion
9 of known agents that might be present.
10 And we already heard about the
11 West Nile story. In 1999, a new agent
12 occurred on our shores and, then, multiplied
13 and spread along the northeastern seaboard for
14 two years. But, then, in 2002, one of the
15 biggest West Nile virus epidemics ever
16 recorded occurred in the U.S. And in the
17 context, there was transmission of the agent
18 by transfusion.
19 And you heard just in the previous
20 talk. What I want to use that slide also for
21 is to highlight the point that a single assay
22 platform or method might not solve all your

1 questions or be applicable in all cases.
2 So, although we were able to show
3 early on in the outbreak that there is a viral
4 agent, West Nile virus nucleic acid, in blood
5 samples of infected people, these were the
6 initial patients or cases in New York City, at
7 the time that finding was not considered very
8 relevant with regard to diagnostics because in
9 flavivirus infection the viremia recedes very
10 rapidly after symptoms occur. So, when people
11 show up for testing, there is usually little
12 nucleic acid present.
13 So, antibody testing was
14 considered far more important. But, then, in
15 the context of the blood transmission in
16 transplantations and other cases, donor
17 testing by nucleic acid testing, real-time
18 PCR, technique PCR, or transcript-mediated
19 amplification was instituted.
20 So, adding another assay is good
21 and certainly improves the situation, but it
22 is not really where you want to go. With the

1 limited amount of sample that is available,
2 what you want to do is parallel testing of all
3 the agents you are interested in in a single
4 sample that may not consume more material,
5 more time, or more cost.
6 In addition, what you want to have
7 modeled in assays is a digital readout that
8 can be easily distributed digitally, and you
9 also have to keep in mind always that there
10 are new agents or unknowns you should have
11 covered or taken into account.
12 But primarily aiming at parallel
13 testing of multiple agents, what we use is a
14 multiplex PCR system, in our case a MassTag
15 PCr. What is MassTag PCR? A regular PCR
16 where you have two primers that amplify a
17 piece of your target nucleic acid. Each
18 primer is labeled with a small molecule attack
19 that is different for each primer. A and B
20 might be influenza virus; C and D might be
21 metanuma virus, and so on.
22 We can have up to 20-25 different

1 primer pairs in one tube, in one reaction.
2 And depending on the target present, you will
3 get amplification. Purify the amplification
4 product on a fertile plate to remove
5 unincorporated primer that would disturb
6 analysis later on. And then, inject it into
7 a mass spectrometer.
8 The linkage of these tachs to the
9 primers is via photoachievable linkage. So,
10 pausing your rebar, the tachs are removed from
11 the long fragile DNA fragments, which we don't
12 analyze. We only analyze the small molecule
13 attacks. We also know their mass, so it is
14 not an elaborate mass spectroscopy analysis.
15 We just ask, is the mass that we know present
16 in the product? And by that, you get a binary
17 readout, the two primers that identify the
18 target that was there or the targets. You
19 will also get multiple detection by those
20 assays for co-infection.
21 Specificity is introduced by the
22 matching pairs. So, if you get non-matching

1 signals, it is spurious and not of relevance.
2 Now mass spectrometers are not
3 very popular in diagnostic labs. So, we built
4 some interface, user interface, that you don't
5 deal with the actual hard data, but an Excel
6 readout across minors or a bar graph of the
7 result, which agents are present.
8 So, the benefits of the system are
9 it is highly multiplex. It is not limited to
10 fluorescent dyes that are using four- or
11 eight-plex assays. With the 20-plex assay and
12 the 96, the aforementioned do about 2,000
13 assays, individual assays, in just one run.
14 For discovery and screening
15 purposes, we can use the general primers. So,
16 we can do genus-wide or family-wide testing,
17 and it is reasonably economic and open source.
18 In addition, in comparison to some
19 other assay platforms where you target common,
20 stable genetic regions with variable regions
21 in between, like our RNA testing for bacteria
22 identification, we use specific genes of

1 interest. So, in case of bacteria here, toxin
2 genes, or pathogenicity markers, and not
3 identifying just the species E. coli, but is
4 it a toxin-producing E. coli strain relevant
5 to pathogenesis?
6 And the same you can envision for
7 blood marker testing. You can target specific
8 genes or loci.
9 In one example, we developed a
10 panel for hemorrhagic fever agents that have
11 a good sensitivity. Comparative testing with
12 previously-analyzed sera, sera analyzed by
13 real-time PCR show comparable performance of
14 the multiplex assay with blood serum samples
15 and, also, with samples from an acute outbreak
16 in Angola.
17 In 2004-5, there was the Marburg
18 virus outbreak in Angola. That showed the
19 limitation of the system also. So, here we
20 got a sample from a person that was a
21 healthcare worker that was deployed to Angola
22 and, then, fell sick, and one week after

1 arrival died from acute disease.
2 Testing in the field by the
3 Cangene mobile lab showed that there was no
4 Marburg virus. Sample was then forwarded to
5 us for extended testing. So, we used the
6 MassTag multiplex panel. It was negative.
7 We used the GreeneChip, the viral
8 chip, testing for all characterized viruses.
9 It was negative. But, then, on the
10 panmicrobial chip, it was positive for
11 Plasmodium falciparum. So, a very sad case,
12 could have been prevented if detected in time.
13 But it shows kind of that staging of the
14 testing and why you want multiple levels of
15 detection.
16 For the oligonucleotide array that
17 was used, we did a lot of bioinformatic
18 analysis of database information that is
19 available. So, we made sure that all the
20 sequences in the database are represented on
21 these arrays, not just full genomes or certain
22 genes, but that all the information that is

1 relevant is represented in the arrays.
2 To do that, we also found a
3 partner for the manufacturing of the arrays.
4 So, we worked with Roche/NimbleGen. They
5 currently have a platform to print 3 million
6 features on one slide that can be split in
7 different setups from 1 to 24 individual
8 arrays on one slide. And they can print
9 oligonucleotide as well as peptides. So, they
10 are synthesized in situ on the glass surface
11 and, by that, it is essentially the same
12 setup. You can change sequences or probes on
13 the fly. It is no pre-manufacturing. It is
14 synthesis in situ, and you just provide the
15 sequences.
16 Now Roche also has a nice and
17 efficient method to release those features
18 from the array. Here is shown for the
19 oligonucleotide array. So, you synthesize 3
20 million features on the array, release them,
21 and can then use them in a soluble
22 hybridization assay to enrich target for next-

1 generation sequencing, high-throughput
2 sequencing.
3 In fact, we think that target
4 enrichment and improvement in sample
5 processing are key for the use of these high-
6 throughput sequencing methods in pathogen
7 detection. That was shown already in an
8 earlier work three years ago where we
9 investigated with our colleagues from CDC and
10 South Africa an outbreak of a new rhinovirus,
11 Lujo virus, in South Africa and at the time
12 did next-generation sequencing on the 454
13 platform to identify in 24 hours that agent
14 and characterize it completely genetically.
15 "Completely" means we got a lot of
16 sequence from the next-generation sequencing,
17 but there were certainly gaps that we had to
18 fill with PCR. That is not too surprising if
19 you think about it was serum for liver
20 samples. So, the vast majority of the reads,
21 99.9 percent, are human sequences. Only a
22 very few are actual agents.

1 But, with improved methods, two
2 years later, we then get with the same sample
3 that was used here, the same serum sample, we
4 now get up to 224 coverage throughout the
5 whole genome without any gaps anymore.
6 So, what we are pursuing now are
7 various methods of enrichment that might
8 include physical separation, negative
9 selection against host sequence and positive
10 selection for potential target sequences by
11 various methods.
12 There is also quite a variety of
13 platforms that are used. I think you are
14 aware of that. They have a certain reach of
15 number of sequences that are generated,
16 different timelines that might be relevant in
17 certain scenarios. So, if you think about an
18 outbreak investigation, if you get a result
19 for 14 days after the matter, it might not be
20 that interesting to kill the people. You want
21 something that works quite faster, but you
22 have a tremendous depth of sequencing, a lot

1 of sequences that are generated.
2 Now, for pathogen discovery, we
3 still think that long sequences are very
4 important and very helpful. Because of you
5 have a 300 base-pair read, you can in many
6 cases identify the agent if it is in a
7 phylogenetically-informative species-typic
8 region of the genome.
9 With short sequences, like
10 generated on the Illumina platform of 90 or
11 even shorter base pairs, you might not get an
12 identification. You need overlapping
13 sequences that generate longer context.
14 And again, in the situation of the
15 host background, it is not very likely that we
16 will ever get hundred-fold coverage throughout
17 large genomes of low-titer viruses. So, we
18 might have to live with individual reads that
19 are not necessarily covering the whole genome.
20 And we will come back to that point in a
21 moment.
22 And just to go on with the

1 platforms, they are also different in the
2 processing of the sample, how the sample is
3 amplified and how libraries are built. So, if
4 you want to compare sensitivity and
5 performance, keep in mind that all this
6 depends a little bit on what was the platform
7 and how was the actual material treated.
8 So, that raises a point, what is
9 the overall sensitivity of these systems?
10 That has not been determined really in a
11 thorough way. There is a huge impact of
12 sample quality, sample type. You know, is it
13 urine or spinal fluid versus tissue, very
14 different background scenarios. Also, bigger
15 degree of sequence homology to a known
16 sequence might be very important. So, it is
17 a little difficult to standardize or predict
18 what is the sensitivity for a given agent.
19 Common to all the systems is that
20 they have an extensive sampling handling. So,
21 it is, by far, not a closed system. Although
22 that is now addressed in part by robotic

1 processing and pipetting robots, it still
2 raises the question, what is an appropriate
3 threshold to call a positive? Is a single
4 read really enough? Or do you want larger
5 portions, and what portion of the genome
6 covered? Can it be in spurious sequence an
7 incomplete genome? What do you want as the
8 threshold?
9 To illustrate that small last
10 example that is not a retrovirus that we
11 characterize. Recently, we got a lot of reads
12 that cover well the genome except for these
13 two reading frames. Here there was a gap.
14 Not a single read was generated.
15 Now is it likely that we have such
16 a biased distribution? Not likely. I mean,
17 why would that area be specifically spared?
18 And so, we did PRC across the gap, had the
19 sequence, and then went back into the read
20 data. And sure enough, we found reads all
21 across that region, but those reads were not
22 recognizable by classical glass analysis.

1 Now why is that database there is
2 a certain problem with gene database and other
3 databases? They are not complete and they are
4 not comprehensive.
5 So, if you look in more detail,
6 the gene bank grows exponentially every year,
7 but it only concerns certain families,
8 retrovirus, flaviviruses, picornaviruses, and
9 so on. But the other 59 viral families that
10 are known and characterized, they are utterly
11 underrepresented. And so, that is work that
12 is necessary if next-generation sequencing has
13 to be used on a wide scale, that we get a
14 better distribution and comprehensive
15 representation of sequence.
16 Even worse, if you look at the
17 size distribution, most of the information in
18 the gene bank is present in small amplicon-
19 sized fragments. Whole genomes or larger, R
20 stretches are really the minority. So, that
21 is ongoing work, to beef up some of those
22 databases.

1 And even then, you might run into
2 trouble if you have really distantly-related
3 sequences, like that bunyavirus here that we
4 recently characterized that has 30 percent on
5 the amino acid level to the closest sequence
6 in the gene bank. We can recognize that
7 sequence for one of the segments.
8 Bunyaviruses come to three segments, small,
9 large, and medium. That is the large segment,
10 but we didn't find any read referring to the
11 S or the M, the small or the medium segment.
12 Again, is it likely that there is no read in
13 there? Probably not. We just cannot
14 recognize them with our current tools.
15 And so, that is another arm of the
16 operation at the CII, that we try to develop
17 methods and approaches beyond blast surges and
18 direct sequence comparisons to get clues on
19 what reads that we might find a mean, if they
20 have a long open reading frame and no matching
21 database. What could they possibly mean? Or
22 the other way around, if you have such gaps in

1 transferase, can we identify reads with other
2 methods?
3 This is an example of nucleotide
4 composition analysis. Here again, Amit Kapoor
5 in the lab did an analysis of existing
6 sequences to do nucleotide composition
7 frequency compilation in mammalian viruses,
8 insect viruses, and plant viruses. As you
9 see, they cluster somewhat together. And so,
10 if you have an unknown sequence and ask the
11 sequence composition, you can identify, oh, it
12 is likely an insect virus. And that is
13 particularly important if you think about
14 stool samples or so, where you have a lot of
15 plant material or other food consumption
16 material, in that you can sort out a little
17 bit the relevant agents from just passengers
18 or spurious things.
19 So, in summary, I hope I showed
20 you that next-generation sequencing is a
21 highly-interesting, highly-powerful tool,
22 although currently in pathogen discovery or

1 detection it is mainly a discovery tool, and
2 major obstacles to use it a broader base in
3 diagnostic or screening are the complicated or
4 extensive handling of samples and the
5 complexity of data analysis, which I couldn't
6 allude to, but I think tomorrow there will be
7 some presentations by Matthew Meyerson, and so
8 on, that go into that topic.
9 Then, to improve the overall
10 performance, I think that target enrichment is
11 a major point. You have bias the ratio
12 between actual target and host background
13 better. That would make data analysis and
14 sampling-handling processes also more
15 straightforward and easier and less sensitive
16 to spurious problems.
17 Another topic was multiplex PCR
18 systems. They are on hand. They can be used.
19 They can detect a number of agents
20 simultaneously for low cost with high
21 sensitivity. And so, complementing the
22 discovery arm of the sequencing, once you find

1 something, you can build straightforward
2 assays that cover a large number of agents
3 that might be relevant for your screening
4 purpose.
5 And lastly, microarrays, on the
6 oligonucleotide are based on nucleotide
7 testing. They will have to complete with the
8 down-going costs and the improved performance
9 of next-generation sequencing. So, that might
10 be limited unless you think about reporter-
11 free detection and direct measurement of
12 individual molecules that don't require
13 amplification. So, there might be a niche.
14 The main attraction at the moment
15 I think is in peptide arrays, that you can
16 build currently 3,000, soon 10,000, feature
17 peptide arrays with which one can do serology
18 on a really large multiplex scale. And that
19 will give a lot of important information, I
20 think.
21 And all of that, as you know, is
22 not possible without a lot of contributors in

1 the Center as well as international
2 collaborators that we work with and whom I
3 thank for all their contributions.
4 And thank you for the attention.
5 Thank you.
6 (Applause.)
7 DR. CHIU: Thanks, Tom.
8 I am sure that many of you have
9 questions. We will probably hold that for the
10 panel discussion in the interest of time.
11 Okay. I will be building on
12 talking about some of the topics. I will be
13 kind of going over our experience with the use
14 of microarrays and next-generation sequencing
15 for blood-borne pathogen detection diagnostics
16 and discovery.
17 And really, the motivation for
18 this was the fact that many infectious
19 diseases are due to novel pathogens. In fact,
20 over the past 30 years, arguably, the vast
21 majority of novel agents or unappreciated
22 agents have been novel pathogens, many of them

1 from like animal reservoirs.
2 And so, in 2008, we established a
3 center called the UCSF Abbott Viral
4 Diagnostics and Discovery Center. The goal of
5 this center was really to harness existing
6 technologies, primarily the use of viral or
7 panpathogen microarrays and the use deep-
8 sequencing technologies to be able to identify
9 novel infectious agents in clinical samples.
10 Initially, what we started out
11 with was the use of microarrays. This is the
12 viral chip that was originally developed in
13 the laboratory of Dr. Joseph DeRisi and Don
14 Ganem that analogous to the GreeneChip that
15 Dr. Briese talked to you about.
16 The goal of this, the viral chip,
17 was to basically generate probes that would
18 represent all viral species in GenBank. But,
19 as you know, GenBank roughly doubles every
20 year. So, this has required kind of
21 iterations or different versions of the viral
22 chip. It is currently on viral chip Version

1 5, consisting of 60,000 probes. We have found
2 it to be incredibly useful for the discovery
3 of novel viruses.
4 So, the highlights are that it was
5 used to identify SARS coronavirus, the
6 infamous XMRV, which was detected initially on
7 the viral chip, and a bornavirus in birds
8 called the avian bornavirus, as well as
9 cardioviruses and other viruses.
10 We have also shown that, relative
11 to PCR and NAT testing, that it is also
12 effective as a diagnostic tool. Initially,
13 this was kind of our early experience with the
14 use of microarrays for detection of known
15 agents. So, for diagnosis of respiratory
16 viruses, it had a sensitivity of about 85 to
17 90 percent and a specificity of more than 99
18 percent relative to PCR and NAT testing.
19 In addition, we have designed kind
20 of automated interpretation, because, quickly
21 on, we realized that if we wanted the viral
22 chip to be used as a clinical tool, we really

1 needed it to be interpretable, the data to be
2 interpretable by a laboratorian or a
3 clinician.
4 And subsequently, what we wanted
5 to do is basically design, essentially,
6 automated software that could be run -- and we
7 called it E-Predict -- to interpret and
8 provide a result for the viral chip
9 microarray.
10 The last version is Version 5,
11 which basically incorporates every sequence,
12 it represents every sequence represented in
13 GenBank as of December of 2011.
14 So, this is the general idea
15 behind E-Predict. Imagine if you basically
16 take everything in GenBank and you align those
17 sequences to micro-reprobes on the chip.
18 Well, you can obtain, basically, a theoretical
19 energy profile, shown there. And what that
20 basically means is that, for every single
21 sequence in GenBank, there is a theoretical
22 profile on how it would look on the viral chip

1 microarray.
2 And if you go to the top band, you
3 basically see evidence of kind of a clinical
4 sample. Basically, you can see that, with
5 that clinical sample, that, in turn, will
6 generate by running it on a viral chip a
7 specific microarray signature. And by doing
8 kind of a hybridization pattern profile
9 comparison, you could, then, rank and
10 determine kind of what virus or pathogen is
11 most likely to be present in your sample.
12 So, this is an example of kind of
13 E-Predict for detection of viral pathogens in
14 blood. And you can see here, these are actual
15 clinical samples. Specifically, I chose four
16 blood examples, given kind of the nature of
17 this talk.
18 So, this is basically a blood
19 sample taken from a patient from India with an
20 acute febrile illness. This is also another
21 patient in India that also presented and was
22 admitted to the hospital with acute febrile

1 illness. And these are two cases of acute
2 liver failure in the United States.
3 What we are doing here is we are
4 simply doing a simple extraction followed by
5 a DNase treatment and, then, hybridizing it,
6 and putting the signature on the viral chip.
7 As you can see here by E-Predict, the viral
8 chip is able to detect Chikungunya virus,
9 dengue virus type 1. And if you look here,
10 the significance of the prediction for dengue
11 virus type 1 far outweighs for dengue virus
12 type 2. So, this was an indication that not
13 only could it detect dengue, but it could also
14 subtype it if you went into hepatitis A and
15 hepatitis B.
16 In addition, what is an advantage
17 of a microarray method is, as also Dr. Briese
18 alluded to, the fact that you can add probes
19 on the fly. This is an example where we added
20 2,630 influenza subtyping probes. The goal of
21 this was to enhance the sensitivity of the
22 viral chip to be able to subtype different

1 strains of avian influenza.
2 In short, this is a chart
3 representing essentially all of the
4 characterized strains of influenza that have
5 been previously identified. I actually don't
6 see H7N9, but we actually did test that.
7 And also, we were able to show
8 that, through blinded assignments done using
9 the viral chip, that the chip has 96 percent
10 accuracy in being able to subtype the
11 different strains of influenza. I should also
12 mention that, in addition to that was the
13 capacity to identify bat influenza or other
14 highly-divergent influenza strains.
15 So, with that hand, what we
16 decided to do was basically expand the viral
17 chip and, actually, design what we call the
18 BloodChip, again, kind of analogous to the
19 GreeneChip, where the goal would be not only
20 to detect viruses, but also to detect other
21 non-viral blood-borne pathogens or blood-borne
22 agents.

1 And these targets include kind of
2 the common players, Borrelia burgdorferi,
3 Anaplasma phagocytophilum or Ehrlichia,
4 Rickettsia, Bartonella, Tularemia,
5 Leptospirosis, detection of Babesia, both
6 microti and duncani, toxoplasma, Candida,
7 Cryptococcal species, which are common fungal
8 species that you find in the hospitals, as
9 well as all of the probes on the viral chip
10 that have been used that are associated with
11 blood-borne agents. So, this includes tick-
12 borne encephalitis virus, dengue, and
13 hepatitis A through E.
14 The ones in red, the species, the
15 non-viral species in red are the species that
16 we decided to do validation, given that they
17 were the ones that we considered of greatest
18 concern and for greatest utility for a
19 BloodChip such as this.
20 So, what we did was a series of
21 validation experiments. We took Borrelia
22 burgdorferi, spiked it in serial titers into

1 human blood, and then, made some preliminary
2 estimates of the sensitivity and specificity.
3 And I should mention that this is
4 where the actual procedure is actually very
5 important, the protocols that are used for
6 preparing these samples. These are
7 essentially samples that have simply been
8 spiked into human blood. They have been
9 extracted and, then, randomly amplified. So,
10 we are not using any primers. We are using,
11 actually, strictly speaking, we are actually
12 using a random primer to amplify everything in
13 the sample.
14 And the goal would, then, be to
15 hybridize it and look at the signature on the
16 BloodChip and, then, determine whether or not
17 you can detect and unambigously identify these
18 samples.
19 We were able to show that you can
20 achieve a sensitivity of about 100 copies or
21 10 to the 2 by spiking in serial dilutions of
22 Borrelia burgdorferi, the cause of Lyme

1 disease; Babesia microti, the cause of
2 Babesiosis, and a plasma, as well as looking
3 at human negative controls.
4 But, really, with respect to kind
5 of non-viral pathogens, arguably, the goal
6 would potentially be to improve specificity
7 even beyond 100 copies. So, to do that, what
8 we needed to do was we actually needed to make
9 the assay a little more complicated by
10 coupling this, instead of using random
11 application, to couple it with 16S/18S
12 bacterial ribosomal PCR.
13 So, this gives you an example of
14 how we did it. And we were able to actually
15 get the sensitivity of detection of Babesia
16 down to about 1 genome per mL of whole blood
17 in this case. This is basically using kind of
18 a 16S/18S coupled strategy PCR followed by
19 analysis on the TickChip or BloodChip.
20 And you can see here this is what
21 we call a traditional heat map or cluster map.
22 These are the Babesia probes that are on the

1 microchip. It is only a very small subset of
2 the whole probes on the BloodChip. And these
3 are basically Babesia samples that have been
4 spiked into negative matrix whole blood,
5 negative whole blood, at the concentrations of
6 10 to the 4th down to 1 copy.
7 You can see here that it is
8 obvious, just by looking at it, that is a very
9 strong visual signature. There is no cross-
10 hybridization between these probes with
11 Borrelia, to give you another example. These
12 are spiked Borrelia samples. So, it is very
13 promising, at least early data, to suggest
14 that we achieve sensitive detection using a
15 microarray method.
16 Okay. I am going to move into
17 kind of next-gen sequencing because this is
18 kind of the other arm of my laboratory. I
19 have been very interested in the development
20 of NGS methods, or what we call deep-
21 sequencing or high-throughput sequencing to
22 identify and characterize pathogens.

1 Traditionally, our approach has
2 been the idea of random application. And the
3 reason behind that is we don't want to pre-
4 bias our amplification with kind of known
5 primers to detect known agents. The original
6 goal was actually to detect novel agents or
7 highly-divergent agents.
8 So, as also Dr. Briese mentioned,
9 kind of the capacity is really different. It
10 goes anywhere from about a million reads with
11 454 to now over a billion reads per run on
12 Illumina high seek. There are now protocols
13 for barcoding primers. So, you could actually
14 multiplex hundreds of clinical samples at a
15 time to reduce your cost.
16 But one big issue and one big
17 challenge for this that it really requires the
18 development of both customized protocols for
19 preparing specimens as well as novel
20 informatics pools. And I want to briefly go
21 to bioinformatics. I know that there will be
22 a session tomorrow, but I just want to show

1 you an example of kind how we have dealt with
2 the issue.
3 This is the challenge: the fact
4 is that you have sequenced data being produced
5 at a greater-than-exponential rate. This is
6 actually the rise in the amount of sequenced
7 data, the rate of DNA sequencing, where you
8 can see it is actually an exponential graph on
9 a logarithmic scale.
10 So, it is basically exceeding our
11 ability to actually analyze. The amount of
12 sequenced data being produced is exceeding our
13 ability to analyze the data by Moore's Law.
14 And one of the big issues is,
15 traditionally, what we have done with passage
16 and identification by NGS is we have done what
17 we call computational subtraction. So, what
18 the idea is, you want to sequence everything
19 and, then, eliminate sequences corresponding
20 to the host. Unfortunately, in most cases, if
21 you are studying human samples, it is a human
22 host.

1 The big problem with that, though,
2 is that 99.999 percent of your sequences may
3 be derived from the human host. So, what you
4 need to do is basically a series of steps to
5 kind of get rid of all human host sequences.
6 And, in principle, what it turns out to be is
7 you need to align all your reads against a
8 human genome. But not only do you have to do
9 that, then you actually have to align them
10 against pathogen-specific databases.
11 And in our case, you can choose,
12 say, for instance, a viral database to align
13 against viruses or a bacterial database. But
14 what we wanted to do was we wanted to align to
15 NT, just align it to everything. You don't
16 want to miss potentially any potential agent,
17 eukaryotic pathogen, bacterial pathogen, oral
18 viral pathogen.
19 Unfortunately, this turns out to
20 be a huge computational bottleneck, and
21 traditional algorithms, it takes about days to
22 weeks to do this. And this is something that

1 we have had to deal with.
2 Another important issue to realize
3 is that the magnitude of the bottleneck is
4 highly dependent on the clinical sample type.
5 So, for instance, if you have tissue where the
6 vast majority of your reads are going to come
7 from the human host, you can very quickly get
8 rid of, simply by aligning to the human
9 genome, you can get rid of more than up to 90
10 to 95 percent of your reads right off the bat.
11 And that greatly speeds up the time it
12 actually takes to identify pathogens.
13 Unfortunately, if you are talking
14 about another sample, like stool, for
15 instance, which is essentially an
16 environmental sample, even aligning against
17 human hosts, you only get rid of maybe 20
18 percent of your reads, where 80 percent of
19 your reads remain unidentified.
20 And what even makes the situation
21 even more challenging is the fact that even
22 identifying all known bacterial and viral

1 sequences in the database, you still end up
2 with more than 50 percent of your reads from
3 a typical stool sample that don't align to
4 anything in GenBank. So, how do you handle
5 that? And how do you deal with the fact that
6 this is a huge clinical bottleneck?
7 Well, one way is to design what we
8 call a scalable computational backbone. And
9 our idea has been to design software that
10 would be compatible in any setting -- so, a
11 single research lab, in a large computational
12 cluster, or even if you wanted to put it on a
13 cloud.
14 I have a grant from Amazon EC2 to
15 basically design a cloud computing system to
16 be able to analyze deep-sequencing data. And
17 initially, our idea was to, in effect, do
18 everything in the cloud. The advantage of
19 that is that we are harnessing kind of
20 solutions that have been developed by very
21 well-known tech industries. Instead of
22 reinventing the wheel, why not use computing

1 bioinformatic solutions that have already been
2 developed by them?
3 So, we used a distributed database
4 called Hypertable. I am collaborating with
5 the CEO of Hypertable. Hypertable has an open
6 source implementation of Google's Bigtable.
7 It is essentially the same thing as a
8 distributed database that Google uses.
9 We use a multicore parallel
10 processing algorithm called Hadoop, which is
11 commonly used in the tech industry. Facebook,
12 for instance, uses it. And this is all done
13 under an Amazon.com computing platform.
14 So, how does this actually work in
15 practice? Well, to analyze 100 million
16 sequences, if you take a 16-core server, which
17 is a very powerful computational server, it
18 takes about three months, three months to
19 align everything using traditional BLAST
20 alignments.
21 Now a question you could argue,
22 well, you could go to different algorithms.

1 I will be talking about that in just a second.
2 But if you can actually harness, say, 1,000 to
3 5,000 core, if you can get the time down to
4 six hours, okay, then the problem becomes a
5 matter of cost, though, because the cloud
6 computing cost to do that is about $387 on
7 your run. So, if you want to tack that on top
8 of the cost of the sequencing in a sample
9 prep, well, that becomes kind of cost-
10 prohibitive.
11 So, we also realized that perhaps
12 we needed not only to improve the processing,
13 but we also needed to improve the algorithms
14 themselves. So, this is why we developed
15 basically this algorithm called URPI, which
16 stands for Ultra-Rapid Pathogen
17 Identification. And the goal of this is
18 really to identify pathogens in minutes,
19 characterize genomes of pathogens in minutes.
20 And we use kind of what we call
21 the next generation of alignment, and that
22 being tools -- oops, how do you go back?

1 Sorry about that.
2 Okay. So, it uses, actually, a
3 series of new tools. Some of these tools are
4 published and some of them are not. We use a
5 program called SNAP, which does ultra-rapid
6 nucleotide alignment; a program called
7 Sequedex, which is published, which does kind
8 of protein alignment or identification;
9 another protein called RAPSearch, which does
10 translated nucleotide alignment.
11 And really, you don't have to know
12 the details of this, but what we have been
13 able to do is to take these tools and actually
14 greatly improve the speed at which we can
15 actually analyze the data.
16 So, this gives you an idea of how
17 fast Sequedex, for instance, is against other
18 amino acid aligners. If you take BLASTX, for
19 instance, every algorithm performs well if you
20 are talking about only analyzing a million
21 reads. When you get up to a billion reads,
22 though, BLASTX takes about 28 days. And this

1 is all on the same computational platform.
2 This is a 64-core server in my laboratory.
3 Whereas, Sequedex can still analyze a billion
4 reads in 20 minutes. Okay?
5 We have also implemented kind of
6 another algorithm called SNAP, which is called
7 the Scalable Nucleotide Alignment. The
8 advantage of SNAP is that you could
9 potentially analyze 100 million reads in under
10 five minutes.
11 What we are using SNAP for is to
12 do this rapid computational subtraction of
13 human background reads and, also, for mapping
14 of known viral and bacterial reads. So,
15 basically, SNAP has excellent performance.
16 You can see here that, basically,
17 SNAP also scales very well. If you are
18 talking about, say, a million reads, BLAST,
19 then, takes 17 hours on our server. SNAP can
20 do it in two minutes. This is not even
21 scalable. It is outside the plottable range
22 for BLAST then. But, if you go to a billion

1 reads, SNAP can do a billion reads in about an
2 hour; whereas, these other algorithms that
3 some of you in sequencing may know about, BW
4 and Bowtie, take anywhere from 12 to 38 hours.
5 Okay. So, to give you some
6 applications, this is a diarrheal study that
7 we did in Mexico. This is where we took 16
8 samples. We simply did a simple extraction,
9 put it on the sequencer, generated 100 million
10 reads. And when we ran the assay, we were
11 able to ID basically all of the viruses that
12 were present. They are shown as a series of
13 peaks in Sequedex.
14 We were also able to show that
15 this technique is as sensitive as BLASTX is
16 for detection of novel pathogens. And they
17 are also kind of mechanisms for what we call
18 basically programs that enable automated
19 readout and interpretation.
20 And you can identify, as you can
21 see here, four different viruses, adeno-
22 associated virus, where we can actually mark

1 where it fits on the phylogeny; respiratory
2 syncytial virus, sapovirus, and enterovirus.
3 And now, the computing costs are
4 only $4. And the reason they are only $4 is
5 because the algorithms are so much more
6 efficient, that you don't basically waste time
7 usually cloud computing resources. You can
8 actually get the whole process done in under
9 an hour.
10 In addition, this is another
11 example where I am showing you a phylogenetic
12 map from basically a total of many of our
13 clinical datasets, in total more than a
14 billion reads of data, where you can see you
15 can identify kind of a novel rhabdovirus; titi
16 monkey adenovirus, which is a novel
17 adenovirus; HIV; human parechovirus, shown
18 here; adeno-associated virus, and the subtype.
19 This is human respiratory syncytial virus.
20 And then, finally, Sin Nombre hantavirus,
21 which is actually a sample taken from the
22 recent, a serum sample from an acute patient

1 with hantavirus pulmonary syndrome from the
2 recent Yosemite outbreak.
3 So, what we think we have done is
4 we have solved -- at least we have one
5 solution for this bioinformatics challenge,
6 which is our pipeline which can actually
7 analyze this data, next-generation sequence
8 data, in 10 minutes to one hour.
9 In addition, really, what we
10 wanted to do is also to show that can you
11 apply these techniques for validation. So,
12 this is an example where we are comparing kind
13 of viral titers and sequencing depth.
14 You can see here that, with this
15 type of algorithm, what we can do is we can
16 actually, it seems like, quantify HIV. It
17 turns out to be linearly-dependent to the
18 number of sequencing reads.
19 So, the goal was we were able to
20 show with this particular experiment that you
21 can actually achieve a sensitivity down to
22 about 10 to the 2 copies. This is just

1 randomly amplified serum sample from a patient
2 with HIV infection.
3 Finally, we have also shown that
4 it is applicable kind of in a clinical
5 diagnostic timeframe. These are two examples
6 where we were able to diagnose herpes simplex
7 virus infection, encephalitis, and acute Lyme
8 diseases in patients with basically a
9 turnaround time that is comparable to what we
10 would typically kind of send out tests we
11 would send to Quest Diagnostics and the
12 clinical laboratories. I should also mention
13 that both of these were done in the context of
14 CLIA-certified laboratory.
15 This gives you another application
16 for outbreak investigation. This is the same
17 serum sample from the patient with hantavirus
18 pulmonary syndrome, again, by random
19 application, no primary application. By viral
20 particle purification, you can actually end up
21 with essentially complete coverage or whole
22 genome sequencing directly from a serum

1 sample.
2 I want to give you another
3 example. This is another example. This is a
4 highly-divergent rhabdovirus. So, it was
5 associated with hemorrhagic fever outbreak in
6 central Africa.
7 This is basically, again, a single
8 serum sample from a patient that had acute
9 hemorrhagic fever. You can show here that,
10 with initial detection, we used 454, our
11 collaborator, Dr. O'Dell used 454 sequencing
12 to get one read from this virus, from this
13 patient with acute hemorrhagic fever. And
14 then, by luminous sequencing, from that single
15 read, you can gradually de novo assemble the
16 entire genome of the virus, again, from the
17 serum sample.
18 And this virus is actually so
19 divergent. It only has about 38 percent amino
20 acid identity to any other virus in GenBank at
21 the time. It was so divergent that it would
22 not have been picked up by a PCR assay and may

1 not have been picked up by serological assays,
2 either.
3 So, in summary, I believe that
4 deep sequencing appears to be a promising
5 strategy for blood-borne pathogen surveillance
6 and discovery. The power enables
7 multiplexing, but there is also flexibility
8 for detecting new and emerging targets,
9 especially with NGS, and rapid identification
10 of highly-divergent agents that would elude
11 conventional testing.
12 There are new algorithms that are
13 being developed by my lab and by other labs as
14 well that could greatly improve the
15 computational turnaround time -- we will hear
16 a lot more about that tomorrow -- and, also,
17 make these technologies assessable for
18 clinical laboratories that do not have
19 bioinformatics expertise.
20 Finally, I do think that
21 deployable multiplex platforms based on either
22 microarrays or NGS, or in some cases a

1 combination of both, appear to be feasible
2 options for blood donor screening.
3 Thanks.
4 (Applause.)
5 In the interest of time, I will
6 move on to the next speaker. The next speaker
7 will be Dr. Philip Felgner at the University
8 of California, Irvine.
9 Dr. Felgner is the Director of the
10 Protein Microarray Laboratory at the
11 University of California, Department of
12 Medicine, Division of Infectious Diseases, at
13 UC-Irvine.
14 Recently, his laboratory has been
15 studying the humoral response to infectious
16 disease agents in humans and animals. He has
17 developed approaches to construct and design
18 probes for protein microarrays on a genome-
19 wide scale to characterize antibody responses
20 for medically-relevant infectious
21 microorganisms.
22 Dr. Felgner?

1 DR. FELGNER: Let'S see, can you
2 hear me? Let's see if I can get this to move
3 here.
4 We have been developing and
5 studying these protein microarrays like this
6 shown on this slide here. This is one example
7 of P. falciparum, the array containing 4,500
8 proteins. So, each individual spot on this
9 array is a different protein from the
10 parasite.
11 And you can probe this array with
12 serum from an infected person, and then,
13 detect the antibody that is bound to the
14 different proteins on the array. This is what
15 the array looks like if it is probed with a
16 specimen from a person in the United States
17 who never gets exposed to the malaria
18 parasite, and this is how it looks when a
19 young child from Mali specimen is probed on
20 the array.
21 So, we probe with the serum and,
22 then, detect with the fluorescent secondary

1 antibody, the presence of that antibody on
2 each spot. We have done this type of thing
3 now with all of the different infectious
4 agents that are shown on this summary slide
5 here.
6 There are about 30 different
7 infectious agents, viruses, bacteria,
8 parasites listed here. On the bottom, you may
9 not see this number. Forty-two thousand is
10 what we have as the number of proteins we have
11 made so far and printed out arrays like this.
12 And another point to bring out is
13 that, once we get these arrays, we can, in a
14 very sort of cost-effective way, we can print
15 large numbers of the arrays and, then, probe
16 large numbers of specimens. These numbers are
17 actually outdated now. We have many thousands
18 of serum specimens from all of these different
19 infectious agents that we probed on these
20 different arrays.
21 I am going to show a little bit to
22 give you an idea of who we use them. I will

1 show the example of the Brucella melitensis.
2 And then, I will do some summaries of several
3 other infections that we have studied here.
4 First, to explain a little bit how
5 we go about making these arrays, we start with
6 genomic nucleic acid that has been sequenced.
7 And if it is sequenced, then we can identify
8 PCR primers that would amplify each gene in
9 the genome.
10 We use primers that attach adaptor
11 sequences on each end of the amplicon. The
12 adapters are homologous with the linear vector
13 that we make, which we transform E. coli with
14 this mixture the next day, we get the plasmid
15 in E. coli. We do this at the rate of 384
16 clones a day, and this is an example of the
17 vector that we produce with this approach.
18 So, at this rate, you can see that
19 nominally in a period of a few weeks we can
20 get 4,000 or so clones made that will express
21 all of the proteins from a bacteria like
22 tuberculosis that has that number of genes.

1 Having the plasmid, we add the
2 plasmid to a cell-free in vitro transcription
3 translation reaction, a small, 10-microliter
4 volume. And then, there is a four-hour
5 reaction there. The plate where the reactions
6 are run in 384-Well plates can be put on a
7 microarray printer like this and print the
8 slides. The slides contain a thin layer of
9 nitrocellulose on them. And so, it ends up
10 being like a micro-Western-plot-type
11 arrangement.
12 The arrays, once printed, can be
13 probed. There are chambers that can go over
14 the arrays. This one, we call this a 16-pad
15 array. So, you can do 16 different serum
16 specimens on this type of array, and there are
17 other configurations of the slides, so that
18 you can fit many thousands of spots on one
19 array. And then, after developing the slides,
20 we use a confocal laser scanner like kind of
21 used to be used for DNA microarray analysis.
22 So, this is an example going into

1 the Brucella example. This one has 3,000 or
2 so proteins from the Brucella organism on it.
3 This is the size of the array. This gives you
4 a sense of how efficient the protein
5 expression is that we achieve when we print
6 and probe these slides.
7 And this is zooming down to one of
8 the quadrants. You can see they are arranged
9 in 16 quadrants. So, just looking at one of
10 these quadrants now, you can get an example of
11 what the serology looks like comparing a naive
12 person with a positive person. And while
13 there are some spots that are overlapping
14 between the naive and the positive specimen,
15 there are also reactive antigens that are
16 unique to the cases.
17 And for a group of specimens,
18 then, we can accumulate the data and organize
19 it in this kind of a way, as kind of a heat
20 map. Here is 54 antigens that we identify,
21 those differentially reactive. And in
22 columns, are the individual specimens. So, in

1 this case, there were 42 culture-positive
2 cases and, then, lots of naives from different
3 parts of the world.
4 And you can see that it is clear
5 that there are certain reactive antigens that
6 are more reactive in the cases than the
7 controls. And so, these would be our sera-
8 diagnostic antigens of interest for further
9 study.
10 We plot these in a different way
11 here. Here we are taking the average of all
12 of the cases, all of the specimens, comparing
13 the cases in red against the controls in blue.
14 And it brings out the same point, that there
15 are certain antigens that are far more
16 reactive in cases than in controls.
17 On the secondary axis, we can plot
18 the P-value, so we can see these are very tiny
19 P-values, 10 to the minus 10th and smaller.
20 So, they are very highly differential. They
21 are differentially-reactive sera diagnostic
22 antigens.

1 We can take advantage of other
2 computational approaches to show that we can
3 distinguish the cases from the controls. Here
4 is a PCA analysis that shows that the
5 specimens are distinct. And I think more
6 useful probably is the ROC analysis where we
7 can get sensitivity and specificity.
8 We can look at not just the
9 sensitivity and specificity of single
10 antigens, which we do, but also combinations
11 of antigens and ask how can using several
12 antigens together improve the sensitivity and
13 specificity of the test.
14 And so, having done all of this,
15 then we like to validate the antigens that we
16 have classified this way on a more traditional
17 platform. So, here is an immunostrip-type
18 platform, where we have taken purified
19 antigens that were identified from the
20 microarray and put them on these immunostrips
21 and, then, assay another collection of
22 specimens. And you can see that these are, in

1 fact, good differentially-reactive antigens.
2 We can quantify the bands here and, then,
3 generate ROC curve, and the sensitivity and
4 specificity is 96-97 percent. So, that is
5 really an example of how we have gone through
6 all of the different targets that were on that
7 summary list that I had earlier.
8 One issue, one thing that we can
9 do of sort of scientific and, also, some
10 practical interest is we can try to predict
11 what kinds of antigens would be reactive,
12 based on this kind of analysis. This is
13 showing the entire proteome, entire immuno-
14 proteome from one of our studies. The
15 antigens can be classified into these
16 different functional groups called COGs here.
17 And the red regions on this pie
18 chart show where the reactive antigens tend to
19 be concentrated. Where they are concentrated
20 is in the cell envelopes, so motility and
21 secretion factors, chaperones, heat shock
22 proteins, and other trafficking and secretion

1 categories. Where they are underrepresented
2 is this black one. Transcription factors are
3 not seen by the immune system.
4 There are other computational ways
5 to classify antigens in databases. You can
6 use transmembrane domains, signal peptide,
7 isoelectric point or pSort. And again, you
8 can find antigens that are enriched in some of
9 these classifications.
10 So, overall, can immunoreactivity
11 and sero-diagnostic antigens be predicted from
12 the sequence? Our conclusion is that, yes,
13 they can be. They do share functional and
14 structural features. There are common
15 features now that we have found between many
16 different organisms.
17 The features are listed here, some
18 of them, and other computationally-predicted
19 features here. Kind of the bottom line is
20 that we can predict, out of any infectious
21 agent, we can predict 80 percent of the
22 reactive antigens present usually in about 25

1 percent of the genome. So, right now, that is
2 kind of the level of our prediction accuracy.
3 So, we can make a prediction, but it is not
4 always going to be reactive, and we can
5 exclude a lot of antigens, but some of the
6 ones we exclude will also be reactive. So, we
7 keep working on refining this predictive
8 capability here.
9 So, now in kind of a summary way,
10 I am going to go through more of these. We
11 started to think about the platform we have
12 developed as kind developing what a barcode
13 is. What is a serological barcode of
14 different infections? And so, this could be
15 thought of as if it is a barcode for
16 brucellosis, like I just showed.
17 Here we have another barcode, and
18 this is a malaria barcode. We can even think
19 of these as two-dimensional barcodes. Here is
20 one for Burkholderia, and you can see that we
21 have identified antigens that predict
22 Burkholderia infection. And here is another

1 one for salmonella, distinguishing cases from
2 the controls, like a barcode for the
3 salmonella. And here is one for Lyme disease,
4 either in this type of strip chart or in an
5 array format like that.
6 So, what is the vision here?
7 Well, today we get usually one agent at a
8 time, and the readout is in a visual way. And
9 what we would really like to achieve is to do
10 this same kind of thing in a rapid test, 15
11 minutes or so, but instead of just measuring
12 one signal, measure hundreds of signals and
13 directed against many different infectious
14 agents, still using the same drop of blood
15 that you would use up here.
16 So, we have drawn up a mockup like
17 this. These are actually targets that we
18 would have identified the sero-diagnostic
19 antigens for. And we could imagine putting
20 them on a single array like this and, then,
21 when we get a certain patient specimen, we
22 might see only some of these targets be

1 seroreactive.
2 So, we actually proposed this to
3 our collaborators at the Lawrence Livermore
4 Lab to do a pilot study to demonstrate this.
5 So, I will just show you the results of that
6 here.
7 This is a multiplex sero-
8 diagnostic protein array. It has 144
9 antigens, and we have 18 of the infectious
10 targets, antigens that we discovered by the
11 way that I was explaining. And they are
12 printed on here.
13 These red boxes that you see
14 around, those are negative controls. So, you
15 can get a sense what the negative signal looks
16 like.
17 Here is the list of all of the 18
18 infectious agents that we have on here and a
19 key, so that you can tell which antigens are
20 in which place on the array.
21 So, how did the results of this
22 come out? We had collections of cases and

1 controls. For malaria, for example, in the
2 case of malaria, we had children and adults.
3 So, they were actually all infected. So, you
4 see the blue and the red lines. One is
5 children and one is adults, and they all react
6 to these malaria antigens here. And not too
7 much else that we had on the array.
8 In the next case, salmonella,
9 actually, we had cases and controls. The
10 cases are red and controls are blue. You
11 don't see any blue line here because those
12 were controls, not infected people.
13 So, there are three antigens that
14 were reactive in the salmonella cases and not
15 against any of the other antigens that we had
16 on the array. For Toxoplasma, the cases did
17 react with Toxoplasma. They also had some
18 HSV, and we got some cross-reactivity with a
19 few malaria antigens, which is something that
20 you could weed out in alter iteration of this
21 type of thing.
22 So, here there were, just so that

1 it is clear, you have malaria antigens, HIV,
2 TB, salmonella, Toxoplasma, HSV, dengue. This
3 is CCHF, Crimean Congo Hemorrhagic Fever;
4 Chikungunya, Coccidia, and yellow fever. So,
5 pretty much these antigens that we picked out
6 were specific for the infection.
7 And there is a number of other
8 specimen collections, Bartonella, Brucella,
9 Coxiella, Lepto, and Borrelia. You can come
10 to pretty much the same conclusion about those
11 as well.
12 So, that was a proof-of-concept.
13 It is really the only time we have done this
14 type of multiplex array, but I think it is
15 encouraging and something that we would want
16 to continue to develop.
17 But here I am showing another
18 issue. All of our probing we do with this
19 kind of microarray scanner. It weighs about
20 100 pounds. So, it is not something that
21 would be portable. It would have to be used
22 in some kind of a central laboratory. There

1 would be questions about the throughput, other
2 things.
3 So, here is another possible
4 solution to this. We haven't adapted our
5 technology to this. Actually, this is called
6 Sofia and comes Quidel. We actually are not
7 working with Quidel, but I thought it was a
8 good example to show the types of things that
9 we need and the types of things that we could
10 use.
11 This is small. Obviously, this is
12 a portable device here. It has all of these
13 nice features. Actually, they have an
14 approved influenza product that is used here.
15 This is a device that reads, a
16 lateral flow device that has been developed
17 with a fluorescent secondary antibody. So, it
18 is not something you can use visually. You
19 have to use the scanner in order to read the
20 fluorescent signal, and the slide goes into
21 this little chamber here.
22 And then, it gives you all of this

1 nice quantification. Here is the influenza A
2 and B. It also has these other features that
3 were brought up earlier, that using something
4 electronic like this, we can take advantage of
5 other communication and storage and other
6 issues, that we can use maybe the cloud or
7 some other convenient approach for doing this.
8 This is what the actual slide
9 would look like under a UV light. So, you see
10 this band, and they have scanner in there.
11 So, you can get a graph like this and you can
12 quantify the area under this curve. So, you
13 get quantitative data.
14 This is the type of data we are
15 used to analyzing when we have our microarray.
16 So, basically, this already-deployed platform
17 has many of the features that we would need to
18 extend the type of work we have done from our
19 laboratory environment into something
20 deployable. And really, if you convert these
21 lines into dots, then there would be no limit
22 to the number of antigen signals you could

1 interrogate on a simple platform like this.
2 All of this is much cheaper.
3 Everything about it, it is far more practical
4 to deploy than it would be the large one that
5 we routinely use in the lab.
6 So, the summary is the protein
7 microarrays can detect antibodies against
8 thousands of proteins and serum from infected
9 humans and animals. We can do very large
10 specimen collections. We really do hundreds
11 of specimens at a time to do that. It has a
12 lot of advantages to being able to interrogate
13 and quantify large numbers of specimens
14 because you can achieve statistical
15 conclusions better with a large "N".
16 And we have, like some of the
17 other speakers, talked about we don't collect
18 a single specimen ourselves. All of this
19 specimen collection occurs by other clinicians
20 that are interested in these infections.
21 But if you get very good specimen
22 collections, you can also end up getting very

1 good conclusions from the data. And lots of
2 questions that I think are on people's minds,
3 like can you detect whether you are seeing
4 serology from a person who is acutely infected
5 or is the serology coming from some past
6 infection.
7 And this can all be sorted out if
8 you have very good specimen collections
9 because some antibody responses stick around
10 and are present for decades without any prior
11 or any additional exposure; whereas, others
12 shoot up and come down rapidly. But all of
13 those things can be sorted out if there are
14 good, well-characterized specimen collections.
15 I brought up that sero-diagnostic
16 antigens can be now predicted from the
17 sequence because we have the sequence
18 information and we also have the empirical
19 data that we have generated. I showed that we
20 can make a multiplex array with 18 infectious
21 agents in a successful prototype. And there
22 is a deployable platform that is feasible, and

1 the platform is already in use and has been
2 itself FDA-approved.
3 And so, with that, I will just
4 thank my team at Irvine and thank you very
5 much.
6 (Applause.)
7 DR. CHIU: Our last speaker will
8 be Dr. Indira Hewlett at CBER and the FDA.
9 Dr. Hewlett is the Chief of the Laboratory of
10 Molecular Virology in the Division of Emerging
11 and Transfusion-Transmitted Diseases at the
12 Office of Blood Research and Review at CBER.
13 She will be talking about
14 implementation of nucleic acid tests for
15 infectious diseases testing of donors, CBER's
16 experience.
17 DR. HEWLETT: Thank you, Charles,
18 for the introduction.
19 And good morning, everyone.
20 This is the last talk in this
21 morning's session. So, I will be brief and
22 just get to the message that we are hoping to

1 send from CBER.
2 My talk will take a slightly
3 different focus in that I am not going to be
4 talking about technology so much. You have
5 heard a lot about it from the previous
6 speakers, about the various protein and
7 nucleic acid platforms as well as deep-
8 sequencing technologies that could be used for
9 multiplex for blood-borne pathogens.
10 I am actually going to tell you
11 about CBER's experience in regard to
12 implementation of nucleic acid testing, or
13 NAT, which was put in place to reduce risk
14 from window period donations. And I will be
15 talking about this using HIV as a model. I
16 will also share with you some past and present
17 CBER experiences in regard to research,
18 collaboration, et cetera, that was used to
19 facilitate technology improvements for blood
20 safety and how we can bring some of this to
21 the current discussion in terms of
22 multiplexing.

1 And hopefully, some of the lessons
2 we have learned from implementation of NAT,
3 which was the most recent technology
4 advancement that we put in place almost a
5 decade ago, will actually help us, will inform
6 us about things that would be useful in
7 implementing multiplex NET.
8 You have seen this slide already,
9 but I just want to focus on the technology
10 aspect in regard to HIV. If you recall, in
11 1985, we started out with whole viral lysate
12 ELISAs, followed by Western immunoblots,
13 immunofluorescence assays, and, then, we had
14 recombinant-based anti-HIV-1 and HIV-2 combi
15 assays. We also had p24 antigen capture
16 technology that was put in place for a limited
17 amount of time as an interim measure. And
18 finally, we implemented nucleic acid testing.
19 This is with limited capability for
20 multiplexing.
21 So, what we are talking about
22 today is actually moving from these low-

1 complexity multiplexing to the next level,
2 which is high-complexity multiplexing testing,
3 and how are we going to go about doing that?
4 Obviously, putting all of these NAT tests in
5 place resulted in significant reduction in
6 residual risk and an increase in blood safety.
7 But I would like to take you back
8 a couple of years ago because I am giving you
9 a little bit of historical perspective here in
10 my talk, just to remind you that this is the
11 pre-NAT era. We had actually had data in
12 hand. This is a study that was done by the
13 Red Cross in combination, in collaboration
14 with the CDC.
15 As you can see, for HIV, although
16 the blood supply was pretty safe, since we had
17 introduced very sensitive combi tests, there
18 was still some risk. You can see that the
19 risk is variable, depending on the region
20 where the donors were derived from, going from
21 about 1 in 150,000 in the Southeast to 1 in a
22 million in the central area. And, of course,

1 the overall average was estimated to be about
2 1 in 340,000. So, there was still some fair
3 amount of residual risk remaining at the time.
4 When we looked at where the
5 sources of risk were coming from, it was
6 found, of course, that the window period
7 donations accounted for greater than 90
8 percent of the risk. And so, the focus really
9 was on using technology to try to reduce this
10 window period. Because if we reduced the
11 window period, we could reduce a substantial
12 amount of the risk. So, that is how we went
13 down this road of putting NAT testing in
14 place.
15 And what we found was that there
16 were essentially, for HIV, there were
17 essentially three ways you could do this. It
18 is to culture the virus, to do antigen testing
19 or NAT. Culture, obviously, is impractical in
20 a blood bank setting. Antigen testing was not
21 as sensitive. And so, NAT offered the
22 greatest potential due to high levels of

1 sensitivity that could be achieved down to the
2 1-to-10-copy range.
3 However, there were multiple NAT
4 methods that were essentially research use
5 assays at the time. There is PCR, TMA,
6 Branched DNA, and so on. They were variable
7 in their performance characteristics. They
8 were complex, labor-intensive, lack of
9 automation, and so on. It is sort of the
10 kinds of issues we are talking about today in
11 terms of multiplex platforms.
12 So, in 1994, the FDA proactively
13 organized a workshop very similar to what we
14 are doing today. The focus there was to try
15 to explore the feasibility of genetic
16 technology to close the HIV window period in
17 blood screening. So, we will be using, again,
18 HIV as a model to bring in new technology.
19 And at this conference, like we
20 are doing today, we had a lot of data
21 presentations -- and this is just one of them
22 -- in regard to reduction in the window

1 period. This is data from Dr. Busch's group.
2 Actually, what they showed was that, by using
3 RNA PCR, one could reduce the window period by
4 an additional 11 days relative to the
5 sensitive combi tests that were in use at the
6 time.
7 So, as an outcome of this
8 workshop, NAT was identified as the most
9 appropriate strategy to reduce risk from
10 window period transmissions. The NAT formats
11 existing at the time were found to be not
12 suitable for mass screening of individual
13 donations, what we call ID-NAT.
14 So, the concept of pooling small
15 numbers of specimens, or mini-pool NAT, as we
16 call it today, was considered more feasible
17 for large-scale implementation. So, some new
18 ideas came out of these discussions.
19 And a key development actually was
20 the funding that was put forward by the
21 National Heart, Lung, and Blood Institute of
22 the NIH to expedite development of NAT for

1 blood donor testing. Without this, obviously,
2 we would not have the platforms we have today.
3 Now, while all of this was going
4 on in regard to the platforms and NAT, we at
5 CBER were also engaged from a laboratory point
6 of view. You heard that we do research to
7 prepare for, to transition to these
8 technologies. In our labs, we were looking at
9 the need for standardization because, as I
10 said earlier, there are multiple different NAT
11 technologies. They are all variable. And we
12 needed to be able to compare results from one
13 platform to another. So, this, of course,
14 called for standardization.
15 And CBER collaborated with WHO to
16 develop NAT standards for HIV, HCV, and so on;
17 to facilitate technologies standardization.
18 We also developed lot release and reference
19 panels and defined things to these standards
20 for NAT. And nationwide screening was
21 initiated. Of course, the platforms were
22 licensed eventually in 2001-2002. That is

1 almost a decade ago.
2 A key point that should be made is
3 the availability of these NAT platforms or the
4 preexistence of them in routine use in blood
5 banking, of course, expedited West Nile virus
6 NAT implementation in 2003, and its eventual
7 licensure in 2005.
8 Of course, since then, there have
9 been several multiplex NAT platforms that have
10 been licensed with limited capability for
11 multiplexing. So, what we are focusing on in
12 today's discussion is to expand that to be
13 able to address emerging challenges.
14 And you have seen this data
15 already as well, but I just want to show that
16 we went from the 1 in 340,000 risks to 1 in 2
17 million donations for HIV. So, this is a vast
18 improvement in blood safety.
19 In addition to the research or the
20 laboratory work to do with standardization, I
21 just wanted to point out that we actually were
22 involved in proof-of-concept work, which is

1 shown on this slide. These are just four
2 papers from several years ago where we showed
3 proof-of-concept of multiplexing here in CBER.
4 And, actually, the work with the HCV and HIV
5 was in some ways a model for some of the
6 platforms or the assays that came forward
7 eventually for blood donor testing.
8 So, obviously, we have achieved a
9 fair amount of improvement in safety in regard
10 to certain agents, such as HIV, HCV, and HBV,
11 and so on, and West Nile as well. But we
12 continue to face challenges. Of course, these
13 challenges offer future opportunities in blood
14 donation testing.
15 So, I have listed off some of the
16 challenges here. You have heard a lot about
17 them already, but I will just quickly run
18 through them.
19 There is globalization, leading to
20 emerging/re-emerging pathogens such as dengue
21 and Babesia. There is a limited ability for
22 the current platforms for multiplexing and for

1 rapid adaptability to detection of multiple
2 emerging agents.
3 We have also heard about need for
4 improvements in NAT sensitivity to detect low
5 levels of viremia, such as with West Nile
6 virus, as well as parasitemia. Also, there is
7 a need for whole blood and cell-based sample
8 preparation methods to detect cell-associated
9 pathogens, example parasites, and cell-
10 associated viruses.
11 So, the opportunity lies in
12 designing new platforms that can be easily
13 modified to detect these new pathogens, new
14 variants of a pathogen using different sample
15 types and, ideally, with a rapid turnaround
16 time.
17 So, here we are in today's
18 workshop, and we are talking about new,
19 emerging detection technologies. We have
20 already heard about protein and nucleic acid
21 microarrays earlier in this session. There is
22 nanotechnology and fusion of micro- and

1 nanotechnologies. And I will present some
2 work that we are doing in our own lab at CBER.
3 And then, there is next-gen sequencing that
4 has been talked about by Dr. Chiu and others.
5 Of course, there is a need for
6 miniaturization. We will probably hear more
7 about this as we go along in test development.
8 And this is, of course, to reduce the volume
9 of sample that is used for testing. And
10 finally, of course, there is a need to focus
11 on bioinformatics because these algorithms
12 will be needed for data analysis, in
13 particular, with highly-multiplex platforms.
14 So, we in our Division, the DETTD
15 that Dr. Nakhasi heads in the CBER, we have,
16 as our research priority, evaluation and
17 adaptation of emerging technologies, just
18 nanotechnology, deep sequencing, et cetera,
19 for high-sensitivity detection.
20 And our Division is actually
21 conducting research on multiple technologies.
22 Microarray is a favorite. Many of us are

1 working on microarray platforms. There is the
2 open array for NAT which you already heard
3 about from Dr. Nakhasi's presentation. I will
4 tell you a little bit about nanoparticle-based
5 microarrays that we are using in our lab.
6 Resequencing arrays, you will hear about that
7 later in this workshop, ultra deep-sequencing
8 techniques.
9 And, of course, we want all these
10 techniques to be adaptable to both central
11 laboratory -- so, there is a central
12 laboratory setting -- and, ideally, for point-
13 of-care use as well, if possible.
14 In the next couple of slides, I am
15 just going to show you some research that we
16 are doing in the area of new technologies. I
17 have selected one, which is nano-diagnostics
18 using influenza as a model.
19 The identification of highly-
20 pathogen flu caused concern for the blood
21 supply due to potential viremia. Some of
22 these viruses have been detected both in the

1 respiratory tract as well as in blood. And
2 there is the challenge of ongoing reassortment
3 and mutation which generates new, emerging
4 strains. So, there is a need for platforms
5 that can be easily adapted to detection of new
6 strains as part of pandemic preparedness in
7 the context of the blood supply.
8 Nanotechnology has the potential
9 has the potential to provide sensitivity
10 enhancements and facilitate multiplexing, and
11 nano-materials combined with conventional
12 detection methods could provide new platforms
13 that are sensitive as well as capable of
14 multiplexing.
15 So, this slide shows a gold
16 nanoparticle and silver-enhanced whole genomic
17 microarray to detect and discriminate some of
18 the major influenza strains. We have
19 attempted to also modify this array to detect
20 the swine flu agent when it was discovered in
21 2009. And this array could be adapted and
22 modified very quickly. We further went on to

1 adapt this to detection of various influenza
2 A strains and B strain. And we have adapted
3 it, also, to detection of a blood-borne
4 pathogen, HIV, and multiple biodefense
5 pathogens such as Ebola, Lassa, and Marburg
6 viruses.
7 So, we have, of course, an
8 interest in screening technologies, such as
9 the microarrays, but we thought we should also
10 look at next-gen sequencing, both as a
11 potential tool for multiplex diagnostics,
12 which I am not going to talk about today
13 because Dr. Chiu has shown you a lot of data
14 on that, and we have only just started going
15 down that road, but I will present some data
16 on how we use it to characterize and confirm
17 a pathogen that has been identified on, for
18 instance, a microarray platform as being
19 reactive or potentially, say, H3N2 virus.
20 We want to confirm the identity of
21 this. So, we do deep sequencing, and this
22 slide shows the identity of two different flu

1 strains, H5N1 and H3N2. This is the Luminisic
2 platform. The folks in my lab have actually
3 done mixing experiments to show how well they
4 can resolve these at very low detection
5 limits.
6 So, that is nucleic acid
7 detection, and in the next couple of slides I
8 will show you some data showing fluorescent
9 Europium nanoparticles. These are not metal,
10 but they are fluorescent particles that
11 enhance the sensitivity of the immunoassays.
12 This is detection of antigens.
13 So, you can see there is a antibody that coats
14 the well, and you capture the target. The
15 second antibody, the biotin labels anti-second
16 antibody. And really, a sensitivity comes
17 from the signal amplification achieved by the
18 Europium stripped out of the nanoparticle
19 complexes.
20 This is just a proof-of-concept to
21 show that we could improve the sensitivity of
22 a protein such as p24. This is actually a

1 limit of detection that is about 50 times
2 better than the current, 50 to 100 times
3 better than the current ELISA techniques.
4 When this assay was used to test
5 samples that were p24-positive -- actually,
6 these are all NAT-positive samples on which we
7 ran p24 -- you can see that the ENIA, which is
8 the Europium nanoparticle immunoassay,
9 actually has the highest detection rate. So,
10 this basically shows that nanoparticle
11 technology actually can be very useful in
12 trying to enhance the sensitivity of these
13 current platforms and should be explored as
14 part of these platforms.
15 So, to summarize this part of the
16 work, new, emerging technologies facilitate
17 multiplex pathogen detection. Nanoparticle-
18 based whole genomic arrays could potentially
19 offer a new platform or provide a new platform
20 for sensitive multiplex detection of blood-
21 borne and biodefense pathogens.
22 Incorporation of nanoparticles

1 significantly enhance the sensitivity of
2 conventional ELISA-based techniques. We feel
3 that next-gen sequencing obviously is going to
4 be useful for screening, but it would also be
5 extremely useful to both screen and identify
6 the pathogen at the same time.
7 I would like to conclude by saying
8 that CBER has in the past actively engaged and
9 is now currently actively engaging, through
10 this workshop, in defining strategies to
11 implement new technology to advance public
12 health and blood safety. Implementation of
13 new platforms adaptable to higher levels of
14 multiplex testing and the current platforms
15 are needed to address blood safety concerns
16 regarding emerging infectious agents.
17 Multiplex detection should include
18 maintaining high levels of sensitivity and
19 specificity that is needed for blood safety.
20 And finally, I will close by
21 saying that our Division in CBER is conducting
22 research to inform about the technology as

1 well as to help facilitate implementation of
2 these technologies. These are people that are
3 the people in the lab that are involved with
4 this work. It is a multi-office and multi-
5 laboratory collaboration.
6 I would also like to acknowledge
7 the funding organizations without whom we
8 would not have been able to perform this
9 research.
10 Thank you.
11 (Applause.)
12 DR. CHIU: Thanks, Indira.
13 So, at this point, I would like to
14 invite the speakers both from Sessions I and
15 II to come up to the stage. We will begin the
16 panel discussion.
17 We do have some set questions, and
18 I think that we will start with the set
19 questions and, then, open it up to questions
20 from the audience.
21 Okay. So, we have a little more
22 than an hour to actually go through the

1 session. What I would like is for the session
2 to be interactive and also involve significant
3 audience input.
4 We will quickly go through the set
5 questions. I think it would be good to get
6 kind of different perspectives. So, in other
7 words, to get various members of the panel to
8 basically answer the same questions.
9 So, we will start with the first,
10 which the first question is: "How can the
11 current epidemiological surveillance methods
12 and pathogen discovery technologies help to
13 predict the risk to blood safety from new and
14 emerging pathogens or variant forms of
15 existing pathogens?"
16 So, why don't we start by having
17 Dr. Nathan Wolfe? Nathan, do you want to
18 tackle that?
19 DR. WOLFE: Sure. I am happy to.
20 And I guess this is a followup
21 from the talk earlier. My take is that,
22 really, we are talking about global risks. I

1 think there is a certain way in which, by
2 waiting for things to hit the U.S., we are
3 waiting too long. And so, my take is that we
4 should be working with blood supplies around
5 the world.
6 Personally, I would push us
7 towards trying to intensify our activities on
8 blood supplies and blood banks in sort of
9 hotspot regions around the world. I think
10 something that has happened over the last
11 really two to three years -- and this is
12 partially with the USAID sort of PREDICT
13 program that Thomas talked about, but with
14 other programs as well -- is we are starting
15 to really get the human/animal interface. But
16 now we have to get sort of a little bit
17 downstream from that entry point into the
18 human population. And I think blood banks
19 would be sort of an obvious way to do that.
20 I mean, certainly from our
21 perspective, we have started to sample from
22 heavily-transfused individuals. And again,

1 this just sort of increases the capacity as a
2 sentinel population, certainly, if we are
3 talking about chronic agents.
4 Now acute agents, obviously,
5 present different sorts of challenges. But I
6 think if you want to do chronic agents, then
7 there certainly can be some creative ways to
8 approach sentinel surveillance to really focus
9 on individuals that are likely to have been
10 exposed to many, many units of blood during
11 the course of their lifespans.
12 I think that, at least from our
13 perspective, we have kind of voted with our
14 feet and started doing that some years ago.
15 But, again, it is a very sort of deployable
16 strategy, that we could certainly pick that up
17 and move it to a number of different
18 locations.
19 But I think my take is global. I
20 do think that we need to have some sentinel
21 surveillance and really focus on the right
22 populations. And then, we should probably be

1 coordinated with regards to how we approach
2 the testing of these kinds of specimens that
3 we are pulling in.
4 DR. KATZ: Well, that question
5 came from me, by the way.
6 (Laughter.)
7 One of the things, Sue referred to
8 our transfusion supplement, where we kind of
9 went like this and picked 68 bugs and said we
10 think these are potential threats.
11 And one of the striking things in
12 that effort, which took quite a long time to
13 pull together was the paucity of evidence with
14 regard to the presence of absence of viremia
15 from multiple, multiple, multiple pathogens.
16 And so, I would ask the people
17 that are engaged in these surveillance
18 projects, how do we move that forward, both
19 from the standpoint of a yes/no answer, but
20 also the kinetics of "bugemia," which it is a
21 virus or a parasite or a bacterium. What are
22 the barriers that you guys see to answering

1 the question about the presence of the
2 pathogen in blood?
3 DR. NAKHASI: So, I think I see it
4 two ways. One is the surveillance part with
5 what is coming into the country. I think Dr.
6 Wolfe really elegantly pointed out that we
7 need to be looking out there. But the
8 question is how practical that is from the
9 perspective of the blood banks. You cannot be
10 doing all sorts of surveillance data, but
11 there are CDC type of labs which can be
12 focused onto the surveillance part of it.
13 Should that be intertwined, the surveillance,
14 with organizations like Dr. Wolfe's, so that
15 we could be constantly looking at those
16 pathogens coming into the system and at the
17 same time have the capacity, and the
18 manufacturers' developments looking at these
19 things, and then, practicality in the blood
20 establishment.
21 So, I would like to get the
22 perspective from both the blood establishment

1 as well as probably the surveillance group,
2 and maybe I think CDC, I guess Matt is here.
3 I would like to hear Matt's input onto this
4 information.
5 DR. STRAMER: If I may start,
6 obviously, surveillance, global horizon
7 scanning is a key to understanding what is
8 facing us in the future.
9 In blood donor screening, we are a
10 niche or we are a subset of the bigger
11 picture. And for us, I mean, obviously, there
12 are hundreds or thousands or tens of thousands
13 of agents or variants which exist and
14 potentially could be human pathogens. But,
15 again, "potentially" could be human pathogens.
16 So, the issue for us really is I
17 don't think we can jump through the same type
18 of hoops that we went through for XMRV with
19 every single agent that is potentially a human
20 pathogen. We need to answer and go through
21 some critical questions, which we are trying
22 to develop and add to our toolkit once these

1 horizon-scanning efforts do reveal potential
2 pathogens.
3 I mean, Lou already brought up the
4 concept, are these agents present in blood?
5 Do they survive blood storage conditions or
6 processing conditions? Are they transfusion-
7 transmitted? Do they cause clinical disease?
8 So, I think we have to, again, not
9 to discourage global surveillance because it
10 is certainly something we need to do, is a
11 step that we need to be able to distill down
12 what is relevant to blood centers. We need to
13 be very careful in the processes that we use,
14 but, overall, I don't think we are going to be
15 implementing interventions for tens of
16 thousands of agents because there is no public
17 health reason or there is no value to
18 recipients in potentially doing such.
19 DR. WOLFE: I mean, one question I
20 might like to pose to the folks on the panel
21 that have been thinking a lot about
22 biodiversity is at this moment, right, if you

1 looked at -- Charles, you showed the different
2 graphs comparing sort of gut specimens with
3 blood specimens. Obviously, the diversity of
4 agents present in the blood is a lot fewer
5 than other sorts of tissues we could look at.
6 Like what is the total number of
7 viruses in human blood globally at this
8 moment? While I am letting you think about
9 the answer to that question, you know, another
10 interesting thing that might be applied to
11 this is there has been some very interesting
12 studies with influenza, sort of
13 phylogeographic methods where, basically, if
14 you get good sort of temporal and geolocation
15 data on enough different specimens, and you do
16 full-length sequencing, at least the sort of
17 phylogenetic community is starting to claim
18 that they can get a sense of transmissibility
19 and origin. I know those would have to be
20 adapted to the blood community.
21 But it would be very interesting
22 if we could try to use some molecular data to

1 answer questions about transmissibility of
2 things that you would find in multiple
3 individuals that you would be sampling through
4 this kind of surveillance.
5 DR. STRAMER: I think molecular
6 methods are one such method we use. But we
7 have to, again, be very careful about the
8 findings of extraneous RNA or DNA. Do they
9 relate to an organism or an agent? And then,
10 do they translate to transmissibility or
11 infectivity? So, certainly, a part of the
12 puzzle.
13 DR. KATZ: And our niche, it is
14 fascinating. In 2009, H5N1, as Indira has
15 referred to already, as we were doing pandemic
16 planning for the blood community in the United
17 States, we kept asking the question, what is
18 the magnitude of viremia risk with each agent?
19 And we actually knew a little bit about H5N1
20 from literally the case report level and old
21 studies from the sixties. The half-life of
22 truth being several years, we don't know

1 whether to believe them anymore or not, but
2 that is another story.
3 But we struggle with the fact that
4 the samples collected from many of the things
5 that we become concerned were respiratory
6 specimens, or this or that. And I am kind of
7 interested in how we get you the resources you
8 need to have blood samples over a wide variety
9 as well, which is a much more difficult
10 undertaking in many settings. I supposed
11 blood spots and whatnot. But we are really
12 interested in you telling us from Southeast
13 Asia whether that bug is in the blood or not.
14 DR. BUSCH: Yes, I am going to
15 jump in. It is kind of interesting, two
16 blood-bankers on one side and the discovery
17 folks on the other.
18 (Laughter.)
19 But, you know, in terms of this
20 specific discussion of blood versus tissue
21 and, also, humans versus animals, one is I
22 think we all have to be attending to all of

1 it. We have to be aware of the discoveries
2 going on in animals.
3 And the issue of blood, you know,
4 I think that healthy donors are very unusually
5 chronically or acutely viremic. And a number
6 of the organisms that we worry about, if you
7 were to strictly look at symptomatic cases of
8 West Nile disease, there would be very little
9 virus in the blood because, by the time they
10 are symptomatic, the viremia has cleared.
11 So, I think in terms of whether
12 you are focused on agents that are exclusively
13 known to be in blood, I think we have to be
14 very aware that many of these tissue disease
15 samples that are the basis for the discovery
16 and characterization, that many of these
17 agents go through at least a transient blood
18 phase. So, I think we have to tend to the
19 blood. And getting the samples from the
20 donors may not be the place to find the agents
21 and prove that, in fact, there was a viremic
22 phase.

1 So, I like what Nathan said about
2 focusing on sort of sentinel recipient
3 populations because I tend to think that the
4 donor pool, you know, the blood itself is
5 essentially sterile, at least for pathogens,
6 by virtue of the immune response and the
7 capacity to control recipients who are
8 extensively exposed. So, I would like to
9 really explore building in regions of the
10 world highly-exposed recipient cohorts and
11 capturing samples of blood and potentially
12 even tissues from those groups to try to sort
13 out the perennial risk in those groups.
14 DR. CHIU: Yes, I would like to
15 chime-in a little bit. I completely agree
16 with what Dr. Busch basically described, in
17 the sense that, I mean, I guess the question
18 is that we have limited resources; everyone
19 does. And I don't think it is really the
20 purpose of blood banks to do pathogen
21 discovery.
22 I can tell you that really the

1 issue with pathogen discovery, having worked
2 in the field for many years, is that very
3 often it can take months to years to actually
4 link a novel pathogen that you discover, just
5 say a novel sequence or a novel virus that you
6 discover, to disease.
7 Very often, it is only years later
8 where you finally realize what is the extent
9 of disease, how prevalent is it. That really
10 involves, as Dr. Briese mentioned earlier,
11 that involves just traditional molecular
12 biology, traditional virology. And really, it
13 is very difficult. That involves animal
14 models. It involves culturing, if you can.
15 Many viruses and other agents aren't
16 culturable. And so, this is something that is
17 not really practical, let's say, in kind of a
18 days-to-weeks kind of timeframe.
19 So, I guess my question to bring
20 back to the FDA is, should the focus be really
21 on surveillance of known agents that we
22 already know are in the blood supply in

1 transfusion-transmitted cases, should the
2 focus really be on surveillance, multiplex
3 surveillance of known agents, say Babesia,
4 dengue, et cetera, that we do know are already
5 being transmitted in this fashion, already
6 found in blood? And should discovery be
7 restricted, say, to sentinel populations,
8 populations with disease, outbreak regions,
9 you know, regions where there is a high
10 probability?
11 Because I have a feeling that it
12 would be a waste of resources for us to do,
13 say, next-gen sequencing or proteomics, as Dr.
14 Felgner mentioned, on everything. It may be
15 eventually possible to do that, but I think
16 currently we really don't have the resources
17 to be able to screen routine blood donations
18 using these technologies.
19 But I would be interested in
20 getting other thoughts on this.
21 DR. NAKHASI: So, I see the two
22 parts. One is that, as again said, the

1 horizon scanning and what is also applicable
2 in the blood establishment.
3 We already know there have been
4 several agents which I mentioned in my talk
5 that we would like to have donor screening
6 because we feel -- not that we feel -- there
7 is significant donor loss. They are
8 transmitted.
9 But we do not know what is the
10 next one coming around the corner which will
11 be transmitted or not. As I said, the West
12 Nile, you know, nobody thought about it until,
13 all of a sudden, it became from 1999 to 2002
14 when there was this activity going on. Nobody
15 knew that it was transmitted because there
16 were no tools to detect that, and there was no
17 scientific basis and data to do that.
18 And so, therefore, we have all
19 become very sensitive to that issue, that when
20 there is a no agent lurking somewhere, we need
21 to get ready. And how do we become ready is
22 basically if there was some kind of an

1 activity going on, surveillance activity,
2 which establishes those. And therefore, we
3 have, then, on the platforms, multiplex
4 platforms, such agents. And if tomorrow that
5 agent becomes a blood safety issue, then we
6 can implement that testing quickly.
7 So, I think that is where we need
8 to be. You know, it is not, okay, we need to
9 put the resources here and not there, but I
10 think it has to be that we need to look at
11 both issues. We need to be continuing to do
12 some kind of surveillance which tells if that
13 particular may or may not be coming down the
14 road and an issue in plasma safety.
15 DR. STRAMER: You have to go
16 through a process. So, the process starts
17 with the unknown, I mean as we have talked
18 about.
19 So, we do horizon scanning and we
20 pick the correct populations for which to do
21 that, whether it is multiply-transfused
22 patients, transplant recipients, ID user.

1 There are a lot of sentinels that we can use
2 and have been used for viral discovery, and
3 that is a good thing.
4 But when it comes to known agents,
5 then really the challenge is what
6 technologies. I mean, we all need horizon
7 scanning anymore. It just goes through the
8 questions that I kind of posed, as we will for
9 the EID toolkit. You know, is an intervention
10 needed, once we answer a certain number of
11 questions that we say we need to trigger an
12 intervention?
13 And then, we need to work on the
14 availability of those interventions. Some may
15 not be needed, but that is the part of the
16 research that the blood centers will
17 participate in, answering those questions:
18 are the interventions needed? What is the
19 appropriate intervention and how is that
20 implemented?
21 I think one thing -- and this is
22 really off-topic, and it may be part of

1 another question -- I just feel this urge to
2 talk about it. When I was listening to Dr.
3 Felgner's talk, it just struck me, you know,
4 we have blood donor screening. And then, we
5 have the rest of what we do.
6 What do we do with reactive
7 samples prior to donor notification, prior to
8 consignee notification? We have a bunch of
9 disparate tasks, and losing players that are
10 interested in it, it would be a cool idea, at
11 least in my mind -- we put all of our reactive
12 samples together. Once a week we put it on a
13 protein array. Donors have already consented.
14 We already know they are potentially infected.
15 We have all known agents on an array.
16 And rather than doing, God help
17 us, an HIV Western blot or reba, which is no
18 longer there, or a test for HTLV, which we are
19 struggling with, we have a nice little chip or
20 microarray. Once a week all the reactive
21 samples on the United States get blasted
22 through on a couple of these, and we have all

1 the answers.
2 So, that may be a method of
3 introducing technology because right now we
4 are just grappling at the back-end of the
5 feeding chain, so to speak, with what do we do
6 with these reactive donors. I really don't
7 see this technology today for thousands of
8 pathogens, whether they are known or unknown,
9 being introduced in the blood centers. I will
10 talk about this tomorrow, but who is going to
11 pay for it?
12 DR. CHIU: Phil, do you want to
13 comment?
14 DR. FELGNER: Well, yes. I think
15 one of the things that I think about is that,
16 when we have one organism that we study,
17 pretty much we are studying that in the
18 laboratory. So, we are not deploying this
19 into any test that would become FDA approved.
20 But when I think about doing that,
21 I think that, you know, it is feasible because
22 we do this one a time. We can imagine how

1 people in your group have been doing that.
2 And so, any of the technical issues about
3 getting one test to be validated that way
4 would work.
5 But now you are talking about
6 making a multiplex test where each agent on
7 there needs to go through that kind of
8 vigorous validation. And then, you do that
9 for each individual test. And now, you have
10 to put it on a multiplex platform, and,
11 basically, you have to go through the whole
12 thing again to get it all integrated. So, I
13 think I see it as a feasible thing to do, but
14 it is a serious commitment to do that.
15 Your suggestion to use it as more
16 of an exploratory thing where you may not be
17 asking for FDA approval for it -- I think that
18 is what you are saying, right?
19 DR. STRAMER: Well, I think the
20 mechanisms are important, but let's not get
21 lost in the details. And I don't mean to say
22 the FDA is a detail.

1 (Laughter.)
2 But, you know, first talk about
3 the feasibility. It is an aspect of the
4 industry that we are struggling with right
5 now, and talking about cost-effectiveness, I
6 don't think any of these technologies would
7 see the light of day for blood donation
8 screening.
9 So, I thought about the
10 feasibility, whether first in research mode,
11 which would prove feasibility, you know, as a
12 way to really introduce the next technology,
13 have the technology available, familiarity
14 with the platform. You know, that is the way
15 a lot of these things get started in the
16 diagnostics arena or in smaller niche areas
17 before we could adapt them, then, to blood
18 donor screening.
19 DR. FELGNER: Yes. So, I think
20 that that sounds feasible to me, and it also
21 gets around the technical and logistical
22 problems connected with actually making a test

1 like that applicable to blood donor screening.
2 DR. CHIU: Okay. We have some
3 comments from the audience.
4 DR. ALTER: Harvey Alter from NIH.
5 Currently, there are probably 20
6 pathogens that had been identified that could
7 be re-identified on a multiplex assay. The
8 technology, as we have heard, is very close to
9 being able to do that, if it isn't already
10 here. A lot of regulatory things and stuff to
11 go through.
12 So, as these 20 agents, maybe 30
13 agents, face us -- these are agents of known
14 pathogenicity -- as they face us, we have two
15 approaches at a blood bank. One is to get a
16 robust pathogen activation that would be
17 applied to every unit, and the other is to do
18 this multiplex assay. But we can't keep
19 adding tests for every one of these agents.
20 So, these are our two routes forward.
21 It seems to me that multiplexing
22 is right now the one that is most conceivable

1 in the short-term. So, I would encourage that
2 we push in that way.
3 But the other comment I want to
4 make is that, if you are talking about the
5 detection of novel sequences, clearly, one,
6 that is not the blood bank's job. But
7 detection of novel sequences in a vacuum, in
8 the absence of a disease that they are
9 associated with, is fraught with a lot of
10 problems.
11 And we have been through in the
12 blood bank world, I have been through,
13 regrettably, HGV and SEN virus and TTV virus,
14 none of which proved to have pathogenic
15 potential, but they may be part of the normal
16 viral flora, and they may be very prevalent,
17 like TTV virus. So that you are most likely
18 to pick up the agents that are most prevalent,
19 but maybe least pathogenic, these normal four
20 agents.
21 So, I am all in favor of horizon
22 screening and picking up things ahead of time,

1 and then, finding out if they have relevance.
2 But I think working from the nucleic acid
3 sequence outward is a tough road compared to
4 working from a disease backwards to find out
5 what is causing that disease. So, that is
6 just my experience.
7 Thank you.
8 DR. KATZ: I might say, "Amen,
9 Brother Alter" to that. Or I might not.
10 (Laughter.)
11 I want to bring something up
12 because it has to come up at least once during
13 the conference. I was actually kind of
14 surprised when Jay showed his slide deck that
15 there was cost consideration on one of the
16 slides, which we think is entirely
17 appropriate. But we also know that the FDA
18 has a very difficult statutory hurdle to get
19 over to, in fact, address cost or cost/benefit
20 or any of that sort of thing.
21 And it was striking during the
22 coffee break that the people from the

1 companies that we rely on to build these
2 devices came to me and said, "Yes, but what
3 about Chagas disease?"
4 And for the people that don't
5 understand that experience, basically, we
6 said, if you build it, we will use it, and we
7 started screening all the donors in 2007 for
8 Chagas disease with an assay that was built at
9 considerable expense by a company. A couple
10 of years later, we pulled the plug out,
11 reduced their volume by 20 percent, and they
12 feel we did a bit of bait and switch. Eighty
13 percent of their volume that they did their
14 projections on went away.
15 And so, one of the things that has
16 to happen -- I am from Iowa; we can do PCR in
17 Iowa. If you can do PCR in Iowa, you can do
18 PCR anywhere.
19 (Laughter.)
20 We will do it in blood safety is
21 probably a truism.
22 But the connection between our

1 risk assessments and FDA's analysis of the
2 platforms to the reimbursement mechanisms in
3 U.S. healthcare and other systems as well in
4 the world, but especially here, doesn't exist.
5 And that is kind of the elephant in the room
6 that the people who build these things for us
7 are asking about.
8 DR. BRIESE: So, I think the
9 screening of existing positive blood donors,
10 and so on, is certainly a way to go into the
11 surveillance and horizon-scanning procedure,
12 which is one arm of the whole operation, to
13 find out what might be new agents that play a
14 role.
15 But in your routine testing of all
16 samples, that is not the way you want to go
17 there. You want to select really important
18 agents. As Harvey Alter said, it might also
19 be a problem in the end, how much do you want
20 to know. You don't want to know maybe things
21 that are not relevant.
22 But, to come back to Charles'

1 question with sequencing, if one would
2 sequence, let's say, every sample and could
3 get all the information, what are all the
4 pathogens in there? What are all the blood
5 markers, HLA types, and whatever is in the
6 sample?
7 So, if you could replace all the
8 different testing that is currently done with
9 one universal test, what then? Could that be
10 cost-effective? Could that be doable? Would
11 that be desirable? That, I think, is also a
12 question to look into.
13 DR. STRAMER: I don't think we
14 have -- I mean, we can take all of the
15 pathogens that we screen for today and add the
16 20 or so that we have concerns about,
17 relevance concerns. But I think what we are
18 struggling with is who is going to do it. Who
19 is going to put it on a platform, so we have
20 that platform that we can do serology on, we
21 can do nucleic acid on? So, where is that?
22 I don't think we are opposed to evaluating or

1 implementing it. It just isn't there.
2 You know, it is one thing to have
3 research assays be able to do next-generation
4 sequencing, but that is not what we do today.
5 It is going to take a long time to translate
6 that into what we do today.
7 So, if we take all the serologic
8 assays, we use nucleic acid, supplemental
9 tests, and put it together all in one nice
10 platform, that is great; bring it on, you
11 know. More power to you. Bring on pathogen
12 reduction. Bring them all on.
13 (Laughter.)
14 But someone has to pay for it.
15 You know, it is just we are all struggling
16 with that.
17 DR. WOLFE: I think that is
18 exactly right, right? We are having two
19 different discussions here. One is what
20 should be implemented on a regular basis for
21 screening within facilities. And the other is
22 what kind of research should be done globally,

1 right?
2 Because the reality is -- and I am
3 quite open for some debate because, right,
4 these things are fun if you get a little bit
5 of debate -- but I think, look, XMRV, a very
6 interesting situation, right? Disease is very
7 difficult to determine. Okay? Let's say that
8 we agree with that.
9 Having said that, we are seeing
10 some of these really interesting results
11 coming out of this EPT PREDICT program where
12 you are actually starting to create viral
13 discovery curves -- you guys are doing some of
14 this -- where you can start for a species to
15 get to the point where you have some sense
16 that you have discovered all the viruses that
17 are present in a particular tax within a
18 particular species.
19 Now we may not be there yet with
20 regards to the technology for doing, say,
21 blood in humans. But, even if we are
22 screening through and we are determining, you

1 know, here are all the agents that are
2 present, and we don't know for sure to what
3 extent they are deadly -- we assume many of
4 them are not -- we get a sense of that normal
5 flora.
6 And what that means is, when there
7 is perturbation, when things change in the
8 future, we have a baseline. I don't know if
9 we are ready yet to start achieving that
10 baseline, but I think, from a research
11 perspective, there is a lot of utility to
12 thinking about it and moving in that
13 direction.
14 DR. BRIESE: I think, yes, that is
15 precisely the point. We want to have a
16 baseline and know eventually what is out there
17 and what you have to deal with. And then, you
18 can take the next step and evaluate how
19 dangerous or not is each of those. And then,
20 you get to a more comprehensive picture.
21 But the question, then, still is
22 that is the one part to really know everything

1 you might have to deal with. But what do you
2 put on the routine assays? Do you want to
3 select just the ones you think are important,
4 and maybe miss some unknowns, or do you want
5 to put on, more or less, everything, all
6 global, with the option that you might not be
7 able to miss anything because everything is on
8 the test?
9 And what is the sweet spot of
10 cost-efficiency and productiveness of the
11 system? That is the question.
12 DR. WOLFE: Yes, and I am not
13 going to propose an answer to that question.
14 But I will say, like between disease and
15 transmissibility, right, disease is
16 notoriously very difficult to catch. But,
17 having said that, whether it is 1,000 or
18 10,000 or 100,000 viruses that are regularly
19 circulating in human blood, we can start to
20 get a sense of what they are.
21 And certainly, transmissibility
22 should be an easier assay to determine, right?

1 And I am not saying -- maybe that there is a
2 dynamic that is going back and forth with
3 regards to things that are entering and,
4 sadly, perhaps with Chagas, things that are
5 leaving. But to have that, you have to have
6 at least some research surveillance going on
7 where you are monitoring, and you are
8 monitoring a larger set of things in your
9 research surveillance network. And you have
10 to be very strategic and sentinel about that.
11 And then, ideally, it is this sort of back-
12 and-forth dialog.
13 DR. CHIU: I am watching and
14 wondering if it is possible to kill two birds
15 with one stone, where clearly both kind of the
16 protein assays that Dr. Felgner introduced and
17 kind of the deep-sequencing assays that we
18 have been discussing here, I mean, they
19 clearly have the potential to identify any
20 agent.
21 Well, in a more restricted sense,
22 I can tell you that, for instance, unbiased,

1 next-generation sequencing, assuming with a
2 standardized protocol that we have in my
3 laboratory we have been able to show that we
4 can detect 100 varians, viral capsids from any
5 virus.
6 And we have validated this for 15
7 agents now that are found in blood. The
8 sensitivity is about the same. It is at least
9 100 copies. In some cases, it is better than
10 100 copies per mL.
11 So, I guess my question would be,
12 could the FDA envision perhaps validating or
13 approving an assay like this where part of it
14 would be validated, but the interpretation
15 would be, say, for the 20 agents. And that
16 would be the validated aspect of it, but it
17 would also give you more information, both for
18 surveillance, for the purposes of identifying
19 new agents with the exact same assay.
20 In other words, we are not
21 repeating like one assay to do surveillance
22 and another assay to kind of deal the

1 regulatory issues of standardized multiplex
2 testing. But can we potentially kill two
3 birds with one stone? I don't know if that is
4 something that would be feasible.
5 DR. NAKHASI: So, I think I just
6 want to put another point in perspective here.
7 Diagnostic is a part of this whole equation.
8 As many of you know, there is multiplexing of
9 the diagnostic assays. You will hear tomorrow
10 some of the discussion from our sister Center,
11 CDRH, and what needs to be part of validation
12 and things like that.
13 So, obviously, if we have
14 platforms like that where we have all of the
15 opportunity for the diagnostic, then the
16 question is, how we, then, go from there to
17 blood donor screening? I think that will be
18 an important movement from one step to another
19 step. And it answers your question. We will
20 ask -- many of these questions will be
21 answered -- if we have validated for 20, if
22 you see something else, what do we do for

1 that? We have some of the answers, probable
2 answers, for that, and you will hear more from
3 CDRH about that.
4 So, I think the question is we
5 need to be prepared. We are at a stage now,
6 I think, basically, a point was made by Lou
7 that cost is an important issue. Obviously,
8 I put in my slides and Jay put in his slides
9 also the cost-effectiveness because we cannot
10 be going and adding more and more in.
11 But we are at a cusp of
12 development where we have to come with some
13 solutions, that is the multiplexing a way to
14 put many agents together, including knowns
15 where we need tests or some which we want in
16 the future, to, then, at the same time have
17 the validation for certain and, then, wait for
18 the validations for others when we need those
19 samples.
20 And the important thing is not
21 only just the validation, but I think what I
22 see is having those samples. Because I think

1 the samples which Nathan's group is
2 collecting, and Tom's group is collecting,
3 that is very valuable to me, at least for your
4 consideration when we do the validation of
5 these assays at least. And then, obviously,
6 have donor screening validation. But at least
7 validation from those samples.
8 So, the sample collection is a
9 very, very important aspect. We shouldn't
10 forget that. And that comes only from
11 surveillance. I think that is important.
12 DR. BUSCH: I am going to jump in
13 because I think a great discussion, and to
14 link this back to what Sue said earlier about
15 the positive donors that we pick up, and the
16 challenge we are facing on confirming and
17 being accurately able to notify those donors,
18 but, actually, those infected donors, and
19 particularly those who are acutely infected,
20 be it from sexually-transmitted or IDU or a
21 mosquito, those serve as an incredible
22 surveillance funnel. Those are people who

1 just got exposed to one agent that we picked
2 up, but who knows what other agents they may
3 have?
4 So, they actually, unlike what I
5 was saying earlier, focus on the recipients.
6 The infected donors are an incredible
7 resource, not only in the U.S., but globally.
8 If we could capture those -- but the dilemma
9 is some of the consent issues that we are
10 faced with, both Red Cross and FDA. Everybody
11 has got language that says you can test for
12 these things, but you can't do genetic
13 testing. I think that is a big mistake that
14 we have to get past, that we have to consent
15 all of our donors to enable us to do the kind
16 of work that we need to do to maintain safety
17 and surveillance.
18 And then, we can just dovetail off
19 those positives. And you can quickly, as you
20 get a reactive, you can not only get the
21 plasma, but you can get the cells. Those
22 tubes are sitting there.

1 So, if we put into place a system,
2 we could actually use our routine screening to
3 identify a very small proportion of donors who
4 they are potentially very informative to
5 discovery and surveillance.
6 DR. STRAMER: That was the point I
7 was trying to make, not only confirmatory,
8 but, I mean, we would test them for all
9 agents, I mean at the same time. And not only
10 would it be efficient, it would be
11 standardized. I mean, we are on a downhill
12 spiral with confirmatory testing right now.
13 DR. NEDJAR: Yes, Charles, may I
14 ask a question?
15 Dr. Felgner, I was noticing in
16 your table you showed that, when you had one
17 antigen, sensitivity was close to 98 percent.
18 Then, you came up to 35 antigens; it came down
19 to 93 percent, and the specificity dropped as
20 well. So, more antigens may not be always
21 better.
22 So, I will just make the comment

1 here that the tools he will use for pathogen
2 discovery or antigen discovery, but when you
3 bring it down to your sero barcode or even DNA
4 barcode, the known antigens may be, actually,
5 very established antigens which are being
6 currently used with known characteristics. It
7 may not be too bad actually. That may give
8 you more with respect to the picture,
9 actually.
10 DR. FELGNER: Well, it depends on
11 the particular infection. Yes, what happens
12 is some antigens, some individual antigens
13 give rise to a better sensitivity and
14 specificity than others do.
15 So, what happens is, when you
16 combine in the bad ones with the good ones,
17 you reduce the sensitivity and specificity of
18 the tests. But if you have a collection of
19 equally-good antigens, usually putting them
20 together would improve the total outcome.
21 So, I think the thing to come away
22 with is that this gives you an empirical way

1 of getting to the point where you want to get
2 to, the most accurate test, yes.
3 AUDIENCE MEMBER: If I could get
4 back to the question of testing multiple
5 targets, with the focus on multiplex assays,
6 what are your thoughts from a regulatory
7 perspective in regard to assays that are
8 directed both to targets that are considered
9 donor screening, such as HBV, CV, and IV, and
10 to targets that are currently treated as in-
11 process controls, such as HAV and parvo?
12 I guess my question is, for a
13 company developing such a test, what would be
14 the regulatory process? Because those are two
15 different things right now.
16 DR. NAKHASI: Well, stay tuned.
17 We will hear tomorrow about that discussion.
18 (Laughter.)
19 DR. KUEHNERT: Matt Kuehnert from
20 CDC.
21 I totally agree with the
22 sentiments of many people who have commented

1 already on just the idea of trying to test for
2 everything. I mean, to me, that is comparable
3 to an outbreak investigation where you go in
4 and you do environmental sampling, except you
5 sample, you swab everything, and you try to
6 test for everything.
7 So, you are going to get answers,
8 and you are going to get a lot of answers you
9 really don't want to know. And so, what we
10 try to do is target testing to the
11 epidemiology. So, I think that is what
12 everyone is sort of coming to. Well, you
13 know, you have to horizon scan. You have to
14 come up with a hypothesis.
15 But you don't want to wait until
16 there is a cluster of a whole bunch of peoples
17 who have died. You want to do something
18 before that.
19 So, this gets to the point I
20 thought was very good about a baseline and
21 having baseline data for what you would
22 expect, and then see if things change.

1 But my question to the panel is,
2 well, is that baseline different depending on
3 the population? We have talked about people
4 in developing countries. We have talked about
5 frequently-transfused individuals. We have
6 talked about transplant patients, which I
7 think are very important because those are
8 often our so-called canaries in terms of being
9 particularly susceptible to disease before
10 other people may be.
11 So, how do you look at what that
12 spectrum is in terms of the baseline? And
13 when you say, well, now it has changed in the
14 population we are looking at, and then, you
15 have a hypothesis as to that it has changed,
16 and whether it has changed in either donors or
17 in recipients.
18 DR. WOLFE: I love the question.
19 My short answer is let's have a look.
20 But, look, the first time I heard
21 the word "virome" must have been 12 years ago
22 or something, right? Maybe it goes back

1 further than that. Within the last 12 years
2 -- you know, when you heard it originally, it
3 was such an intuitive idea, right? There is
4 at any one moment a global human blood virome,
5 a global human skin -- et cetera, et cetera.
6 What is it? Well, 12 years ago,
7 it was totally pie-in-the-sky to even imagine
8 that you could capture what it was in an
9 individual, let alone in a population, let
10 alone globally.
11 We are a lot closer, but we still
12 may have that feeling of like maybe we can do
13 it, but we are perhaps not quite that close.
14 But the point is we can start doing this.
15 But, to begin to answer that question, we are
16 going to have to go out and figure out some
17 way of cutting up the world into different
18 pockets, whether it is subpopulations or
19 geographic locations or risk populations. Go
20 out and do some surveys.
21 Start getting the information in a
22 small scale, not like having to burden blood

1 banks that are doing really important work
2 with necessarily things that are going to
3 complicate their lives. But, once you start
4 figuring that out, I think it is an iterative
5 process to answer the question, but you have
6 to start somewhere, start just measuring it.
7 And a lot of stuff will be sort of
8 natural. Many of it will be transmissible.
9 I think like the TTVs, the GBVs, those will be
10 very interesting agents, and it becomes just
11 part of this baseline that you can detect
12 signal on top of.
13 DR. KUEHNERT: Yes, I think it
14 will be great tomorrow to talk about what
15 those categories should be because there is so
16 much; there are so many directions to go. I
17 think you're right, we have to start somewhere
18 and try to figure out, okay, what are the
19 different spheres that we want to look at
20 where we would expect populations to be
21 different and then see how different they are.
22 DR. McLOUGHLIN: Kevin McLoughlin

1 from Lawrence Livermore.
2 I was interested in Indira's talk
3 where she was talking about doing mini-pools
4 or pooled samples from multiple blood donors,
5 and the idea of using that for a surveillance
6 strategy. And so, what I am wondering is --
7 and this is partly addressing Sue's issue
8 about the cost of implementing this large-
9 scale, multiplex screening -- so, what I am
10 wondering about is, what are the requirements,
11 both in terms of performance and regulatory
12 issues, for a multiplex assay that would be
13 used in this surveillance capacity on pooled
14 samples with, presumably, reach-back, so that
15 you could do confirmatory assays on individual
16 blood donations?
17 DR. HEWLETT: Actually,
18 surveillance assays are not regulated by us.
19 So, you are essentially free to do the
20 research. In fact, you know, some comments I
21 was hoping to make was to somehow bridge all
22 of these different repositories. You have got

1 donor repositories. You have got all of these
2 global epidemiologic collections of samples.
3 And you have got discovery assays and
4 detection assays, right, with known pathogens,
5 and, of course, all of these degenerate,
6 consensus, and random primer, those types of
7 approaches that are used to fish out pathogens
8 from high-risk populations.
9 So, for those types of uses, you
10 don't need to worry about coming to us. In
11 fact, those are the studies that are going to
12 inform us about the need to do blood donation
13 testing or for diagnostics or disease
14 association, and so on and so forth.
15 So, I hope that answers your
16 question. It is a good one.
17 DR. McLOUGHLIN: But I think what
18 I am asking is, could you foresee a strategy
19 where this became part of the regular blood-
20 screening process, to do pooled screening of
21 samples on a regular basis, rather than just
22 as part of a research study?

1 DR. HEWLETT: Well, it is already
2 in place in blood banks. You know, we are
3 already screening for pathogens, a limited
4 number of pathogens in pool sizes that are
5 variable, anywhere from pools of 6 to 24, and
6 in the source plasma they are much higher in
7 number.
8 So, that practice is already going
9 on, and it is just a question of doing the
10 sort of pre-clinical-level validation of your
11 systems, which you obviously would be doing,
12 you know, in order to make sense of the
13 results you get when you do your discovery.
14 Some type of laboratory-level of
15 validation would be helpful, so that data
16 actually means something when you come to us
17 for blood safety or blood-donation testing.
18 Is that helpful?
19 DR. McLOUGHLIN: Uh-hum.
20 DR. STRAMER: Yes, I was just
21 going to say maybe the link wasn't clear, but
22 after David Kessler gave a talk at the 1994

1 FDA workshop that we have to do more, I mean,
2 for those of us who were there it is very
3 memorable and really started us down the
4 pathway of doing mini-pool testing where we do
5 pool samples. And that was the only
6 reasonable way that we could implement nucleic
7 acid testing for HIV, HCV, and HBV, because
8 the technology wasn't out there to do single-
9 unit testing.
10 And now that we have implemented
11 mini-pool NAT, it seems to be very effective.
12 The only agent for which it is really not
13 highly effective is West Nile. So, when we
14 see West Nile outbreaks, we convert to ID-NAT,
15 to single-unit testing, and then, go back to
16 mini-pool NAT, as I call it, as a surveillance
17 tool. And that is really how we use mini-pool
18 NAT for West Nile. But the other ones are
19 remarkably effective.
20 DR. HEWLETT: Yes, and that is
21 actually a very good point, and depending on
22 the pathogen. You know, let's say it is a

1 parasite and you can't find it. Obviously,
2 you may want to do it on your entire sample.
3 So, it depends on the level of pathogen found
4 in the particular sample you are testing.
5 Obviously, in blood donation we test plasma
6 and serum.
7 DR. CHIU: In the interest of
8 actually getting through the questions,
9 because I think a lot of these points will be
10 covered in other sessions as well, I
11 definitely want to actually ask the people on
12 the panel, and the audience as well, about
13 Question No. 2.
14 So, Question No. 2 is, "What are
15 the pros and cons of nucleic-acid-based versus
16 protein-based testing?" And then, also, would
17 it be desirable and feasible to try to combine
18 both approaches? And what do you see are the
19 challenges that are involved? And how easy or
20 difficult would it be to actually try to
21 combine both of these approaches?
22 So, why don't we start out with

1 perhaps Dr. Briese to comment on this?
2 DR. BRIESE: Well, I think it was
3 already mentioned that there are some
4 differences in timing of detection between
5 nucleic acid detection tests, which go after
6 the nucleic acid of the agent, and maybe more
7 indirect either antibody or protein, which has
8 sensitivity or delay issues.
9 So, I think, as also pointed out
10 in one of the examples, in West Nile, the
11 assays have different strengths and
12 weaknesses. So, you might not want to rely on
13 one platform necessarily, but use the
14 complementarity of the detection. Whether you
15 can do that with time, effort, and cost is a
16 different question. But, whenever you can, I
17 think it is a good idea to do so.
18 Now, combining detection into one
19 assay platform, I think there are certain
20 problems in there because the preparation is
21 quite different for the different targets.
22 So, with nucleic acid, one of the advantages

1 why we also like to use it in many of the
2 merging disease studies is you can work with
3 non-infectious material. It can be, you know,
4 extracted nucleic acid, which is essentially
5 a chemical. It doesn't pose any risk.
6 But, if you complete in natural
7 proteins, then you can in many cases not
8 detect much with them. So, there you usually
9 have to work with more native preparations or
10 more complex mixtures.
11 So, to combine that in a single
12 platform might be possible. There are also
13 platforms that are already looking into that,
14 but I think they are a little further out
15 timewise and not really in a stage where we
16 can think about application because we also
17 don't know much about their performance and
18 how is the interaction or crosstalk or
19 influence on either site, protein or nucleic
20 acid detection, in those systems. So that it
21 will probably be another five or so years
22 until we get more information on that topic.

1 DR. CHIU: Okay. And, Dr.
2 Felgner, can you comment as well on this
3 question?
4 DR. FELGNER: I think it is pretty
5 much the same as what Tom said. I mean, that
6 platform that I did show, the Sofia Quidel
7 platform, for example, they do nucleic acid
8 detection on the same platform. They will do
9 antibody detection. They will do antigen
10 detection. And they have also demonstrated
11 that they can do a multiplex test, a fourplex
12 antigen test.
13 But I think some of the things we
14 are talking about are a little bit more
15 complicated engineering-wise and the fluidics
16 and all of the other issues. So, there are
17 technical issues, but I guess the fact that
18 they are able to use the one platform to do
19 several different types of tests is
20 encouraging.
21 DR. BRIESE: Yes, but, I mean, you
22 can view the topic in two ways. I mean, can

1 you use the same platform in different setups
2 or batches to do different things or do you
3 have one platform which does simultaneously at
4 the same time both detections? So, that is
5 different.
6 DR. KATZ: I have an operational
7 view of this that comes from having run a
8 blood center laboratory for many years. And
9 that is: show me the data.
10 And we saw some very interesting
11 data. I am not sure that the non-blood-
12 bankers in the room understand how the FDA and
13 industry have spoiled blood banks, however.
14 And we saw a very nice, almost
15 square ROC curve with a 96-percent
16 specificity. And I wouldn't let that in my
17 State, much less in my laboratory.
18 We need assays with 99.94 percent
19 specificity because we screen 1,000 donors a
20 day, and I can't be telling four donors a day
21 in my donor base, "Well, you had this signal.
22 We think it is non-specific. You will be

1 fine. Go away and don't ever come back,"
2 because that is the message that the donors
3 get.
4 And so, we need performance
5 characteristics that are incredible. And I
6 don't care whether it is a protein-based assay
7 or a NAT assay, but we need that level of
8 performance in order to screen thousands and
9 thousands of donors every day in populations
10 with very low risk of the things we are
11 looking for. It is Bayesian.
12 DR. STRAMER: Or an algorithm that
13 gets us to that level of specificity. But
14 anyone who is reactive, we are required to
15 give them an explanation. And you may have
16 1,000, but we have 22,000 a day.
17 So, there are a lot of donors, a
18 lot of donor physicians, a lot of donor
19 attorneys who don't understand what any of
20 this means.
21 DR. NAKHASI: So, the next
22 question to go to in the loop: what if there

1 is a platform like the one which we envisage
2 here as a nucleic acid and a protein, not just
3 mixed together, but at least have a platform
4 where you have a chance to do the nucleic acid
5 testing? And then, you have a confirmatory
6 assay built onto that, so that you can, one,
7 it will reduce the cost, obviously, because
8 you don't have to have two separate platforms.
9 I don't know operationally how much it will --
10 you know, obviously, you have to do twice the
11 testing. It is not just one-time testing.
12 But, instead of having it
13 separately, can that be a feasible issue in
14 the situation where you can have one screening
15 assay and, then, a confirmatory which could be
16 much more highly-specific? And that way, it
17 can reduce the cost as well as the donor --
18 DR. STRAMER: I mean, all of that
19 would be great if, in fact, the performance
20 was as we talk about and costs were
21 manageable.
22 DR. NAKHASI: Uh-hum.

1 DR. STRAMER: I think, though, the
2 issue is, even with NATs now, we do the
3 multiplex assay, and you may either through
4 multi-dye have a result or through just
5 separate discriminatory assay have a result.
6 We still have this urge to do more, in that
7 when we notify a donor, we want to make sure.
8 So, we still will take an
9 independent sample and we will do confirmation
10 of that. We will enroll a donor in followup,
11 and we will make sure of the specificity or
12 the positive predictive value of the result.
13 So, it is a great start to have
14 that, but it doesn't mean that we will
15 eliminate doing other things to be able to
16 understand, maybe from a research perspective
17 or from a next-generation perspective, the
18 quality of the results that we are generating.
19 But it would be great. Why wouldn't we want
20 one system that does everything? A black box,
21 you put a sample in; you get all the results
22 out. Who cares if it is protein or nucleic

1 acid?

2 AUDIENCE MEMBER: Sue, I would

3 argue pretty strongly that you do not want a

4 single platform to do everything. And the

5 reason for that here is that, for a naive

6 party interested in bringing a serology system

7 or a protein system to the market, they have

8 very significant barriers right now because,

9 in order for them to get any business with

10 either of the two large lab systems in the

11 United States, you have to develop a system

12 and get six assays through clinical trials

13 before you can get a return of even $1.

14 If you add to that a NAT system,

15 you are adding another four assays that go

16 through clinical trial. And you are narrowing

17 the window of opportunities for other

18 companies to bring some technology to market.

19 So, I think your savings would be

20 superficial. In the long-run, you would pay

21 a whole lot more by relying on fewer and fewer

22 suppliers.

1 DR. STRAMER: But, Brian, if you
2 took cost -- I mean, that is not reality --
3 but if you took cost out of the issue, what
4 would be the ideal world? The ideal world is,
5 it is just like we walk into a laboratory, but
6 we don't need a laboratory; we have a black
7 box. You put the sample in and you get the
8 result out.
9 I mean, that may not be
10 reasonable. It may not be reasonable to have
11 next-generation serology instruments because
12 return on investment will be so low. But I
13 was just speaking to an idealized world where
14 cost doesn't count.
15 AUDIENCE MEMBER: I understand.
16 And if you are talking to someone who is
17 running a lab, it would be ideal. But I think
18 it is not in the best interest of the blood-
19 banking industry to choke-down what few
20 opportunities there exist for companies to
21 bring technology to the table today.
22 DR. STRAMER: Agree.

1 DR. CHIU: Okay. That, actually,
2 is a very nice segue into Question No. 3,
3 because Question No. 3 basically states that:
4 "Are the potential performance
5 characteristics, sensitivity and specificity,
6 of the available nucleic-acid-based new tests,
7 next-generation sequencing arrays, and
8 protein-based tests, such as next-generation
9 tests, emerging tests such as proteomics and
10 arrays, are they comparable to the performance
11 characteristics of some currently-licensed
12 donor-screening tests?"
13 So, why don't we start out by,
14 again, sort of asking, what are the minimal
15 performance characteristics that you would
16 expect for any new test to be licensable?
17 DR. STRAMER: That is easy. You
18 could look at FDA guidance. Sorry.
19 (Laughter.)
20 Whenever companies ask me this
21 question, I say, "Pick up a package insert.
22 Look at a currently-licensed test. Look at

1 the sensitivity characteristics. Look at the
2 specificity. Look at reproducibility. Look
3 at the types of populations." We talked about
4 drug-use population or sentinels. All of
5 these tests have to go through high-risk
6 populations as well as low-risk populations to
7 define their performance characteristics.
8 So, since for the last two decades
9 we have had -- you know, pick any manufacturer
10 you want and pull out a package insert, and
11 all of the information is there.
12 I mean, but the short answer is,
13 as Lou said before, we are used to performance
14 characteristics, be them sensitivity or
15 specificity, in excess of 99 percent. I don't
16 think anything could be licensed below that.
17 I mean, an HIV assay, if you went to eight
18 patients and you didn't pick up 100 percent of
19 them, I think you would be hard-pressed to get
20 a license.
21 DR. KATZ: There might be a little
22 wiggle room for an emerging pathogen that does

1 something really rotten. We lived with the
2 original HIV assays for a while. We lived
3 with first-generation HCV for a while.
4 But, certainly, for the things
5 that we are analyzing now, we need 99.9-
6 something for all of them because of the
7 number of extraordinarily low-risk donors that
8 we test every day.
9 DR. STRAMER: We need to say there
10 is one exception, and that is bacterial
11 contamination of platelets. We don't have
12 anywhere close to that performance, and that
13 is maybe a little bit of a different paradigm.
14 We are not doing donation screening. We are
15 doing product screening, growth of organisms
16 in platelets.
17 But we have tolerated, as an
18 industry, 50 to 75 percent sensitivity for
19 bacteria in platelets. So, for something like
20 pathogen reduction, that is, you know, the
21 obvious target.
22 DR. CHIU: But that brings up the

1 question as to, well, what exactly are you
2 using as your gold standard for sensitivity
3 and specificity? So, what are you using as
4 your gold standard? When you say 99 percent
5 sensitive, versus what?
6 DR. STRAMER: Or higher. Well,
7 for sensitivity, it has to be disease states.
8 I mean, how else would you define sensitivity?
9 Either prospectively in high-risk populations
10 or known characterized samples. And for
11 specificity, it has to be in low-risk
12 populations like blood donors. I mean, that
13 is who the tests will be applied to. So, that
14 is where you get the efficacy data. At least
15 that is our view on the world. There may, of
16 course, be others.
17 DR. KATZ: And I look at it a
18 little bit as, how good can you do at the
19 time? I come from a clinical background. So,
20 I am the greatest good for the greatest number
21 sort of person.
22 And as I said, we lived with

1 terrible assays by today's standards for HIV.
2 She is showed you a CIE.
3 DR. STRAMER: Watch it. I need
4 those assays.
5 (Laughter.)
6 DR. KATZ: She showed you
7 counterimmunoelectroporesis for B surface
8 antigen. It was the best we could do at the
9 time.
10 DR. STRAMER: Right.
11 DR. KATZ: And it got a lot better
12 over time.
13 So, really, I don't have a number
14 in my brain. For specificity, I do. I can't
15 have false-positives because I have to talk to
16 those donors. In my former life, I had to
17 talk to those donors.
18 But it is a moving target,
19 depending on what you can do with the
20 technology that is available.
21 DR. STRAMER: And to add to that,
22 again, it depends on the agent. If there is

1 a dire agent and we have no method of
2 screening, and we only can get 90 or 95
3 percent, certainly that is 90 or 95 percent
4 better than we had without the screening test.
5 DR. NAKHASI: I think that is
6 exactly what our understanding at this point
7 is. But I think the important thing is that,
8 with the new technologies which we have now,
9 we should be, as you pointed out, Tom, in
10 yours and other presentations, we should be
11 able to get very highly-sensitive and meet
12 those requirements. You know, Ouchterlony
13 plates, you couldn't do that. Antigens, you
14 can only choose it. But now, that is the
15 purpose of this workshop, that we live in 21st
16 century technology, and we should be achieving
17 that kind of highly, highly-sensitive specific
18 assays, right?
19 DR. CHIU: Yes, because, I mean, a
20 lot of the validation hasn't been done, but I
21 can already tell you I am very confident that
22 next-gen sequencing, just even unbiased,

1 primary, dependent, next-gen sequencing is as
2 good as NAT assays that have been FDA approved
3 and validated and tested.
4 All of the data that we have
5 generated so far has suggested that it has
6 equivalent sensitivity/specificity. In some
7 cases, you might argue that it has better
8 sensitivity. And we have actually shown that,
9 you know, that we could actually get better
10 sensitivity because we are probing for all
11 parts of the genome as opposed to just the
12 small, little segment of the genome that you
13 typically do with PCR.
14 DR. NAKHASI: Well, I think it is
15 important not to forget the specificity. I
16 think it is not only sensitivity was fine, and
17 then, when you start screening people, you
18 need to make sure of the specificity. And
19 then, on top of that, if we are doing
20 multiplexing -- and I think, as was pointed
21 out, that there may be a good pathogen which
22 is a bad pathogen, as we know from our other

1 experiences. As you keep on multiplexing,
2 there is some drop in the sensitivity and
3 specificity.
4 So, I think we need to be
5 cognizant of that, too. But, obviously, we
6 would like to achieve, what we would want to
7 achieve is the highly, highly-sensitive and
8 specific tests, so that we do not -- you know,
9 the risk versus benefit of testing is
10 achieved.
11 DR. HEWLETT: Yes, I just wanted
12 to make a comment about your question of how
13 do we know or how do we compare. So, this is
14 where we should probably be thinking about
15 standardization of these platforms.
16 This is coming up in a number of
17 other discussions to do with next-gen
18 sequencing in the context of diagnostics or
19 genotyping, and so on. So, if there is a way
20 that there could be some studies done with
21 some of these platforms using reference
22 materials or standards, we get a better sense,

1 well, how exactly they compare.
2 Because we have been down this
3 road 20 years ago, you know, looking at
4 standards and international units and copies,
5 and all of these things, for NAT testing. So,
6 this is a good time, actually, for those with
7 platforms to sort of have a collaborative
8 group that can think about these issues, to
9 see that you can actually compare these
10 platforms amongst themselves and see how they
11 compare with the NAT tests that Sue and Lou
12 Katz seem to like very much in terms of
13 sensitivity and specificity.
14 DR. KASARSKIS: So, one question
15 that sort of comes to me, and it is very
16 interesting -- my name is Andrew Kasarskis.
17 I am from the Mount Sinai Medical Center in
18 New York.
19 So, Dr. Felgner's talk was
20 interesting to me in many ways, but
21 particularly because he was able to predict
22 some degree of immunogenicity from sequence.

1 And he was able to do that because he actually
2 had a good, rich set of data on a set of
3 samples that span both nucleic acid and
4 protein.
5 What I think would be interesting
6 relative to this question about sensitivity
7 and specificity, as well as the general
8 question of how it is that we get from
9 sequence, which is relatively easy to detect
10 for novel pathogens, to an estimate of
11 pathogenicity, which we seem to agree is very
12 difficult, and the question would be, in
13 addition to standards, what sort of data, what
14 types of data, what types of modalities are
15 needed to be able to make those predictions
16 and make them better? And how do we go about
17 getting those data?
18 So, it is taking sort of what has
19 come to be known in the internet industry as
20 a big-data approach to understanding the
21 environment in which the test is done.
22 DR. FELGNER: I guess one part of

1 the question is how would we be able to better
2 predict sero-diagnostic antigens and doing it
3 all bioinformatically rather than doing it
4 empirically. Is that pretty much --
5 DR. KASARSKIS: Well, basically,
6 what additional data would allow you to make
7 better predictions? And then, how could you
8 extend that not just to predicting
9 immunogenicity, but actually going down toward
10 pathogenicity? How would we get data that
11 would actually allow us to make predictions
12 that you could actually go more quickly from
13 a next-generation, phased detection of a
14 nucleic acid to thinking about a test or an
15 association at least to how it actually might
16 function physiologically?
17 DR. FELGNER: Yes, those are kind
18 of separate questions. So, one is, how can we
19 do a better prediction? What that allows you
20 as a practical thing, it allows you to, in
21 some cases, you can decide not to clone an
22 entire proteome, clone and express an entire

1 proteome to start doing protein microarray
2 experiments. We do that sometimes.
3 But I think for this meeting it
4 would be more, how can this information be
5 useful for this application that we are
6 talking about here, blood banking and the
7 blood-banking community and leading to more
8 accurate tests?
9 I guess what I think is that it is
10 getting more accurate, well-characterized
11 specimens. You know, the thing that you were
12 saying about how our test was almost like a
13 square, but it was actually only 96 percent,
14 but the specimens we had, they did the job for
15 what we needed to do. We needed to identify
16 the sensitive antigens that would be good
17 predictors.
18 But, as far as deciding whether
19 any of those things are going to be useful for
20 blood banking, that is a whole other kind of
21 activity that would need to be invested in.
22 That is why I was interested in your sort of

1 bridging idea, to use it as a surveillance
2 tool to get something that you said didn't
3 require all of the same approvals as an FDA-
4 approved product would require.
5 DR. HEWLETT: Yes, I think the two
6 samples that we have been saying, the donors
7 and the surveillance samples, these studies
8 are really going to inform us about the need
9 for testing.
10 DR. KASARSKIS: And just to
11 clarify a bit for myself, I mean, it sounds to
12 me like we are quite comfortable with the very
13 sensitive next-generation sequencing-based
14 tests, other technologies that are good at
15 detecting things, probably better than
16 existing technologies.
17 And I guess the question that I
18 still don't know the answer to is, to what
19 extent do we feel like we currently today get
20 access to the right specimen collections to be
21 able to develop better tests? Or to what
22 extent do we need to be actually prospectively

1 identifying different collections of
2 specimens?
3 DR. NAKHASI: I think that is a
4 very important point. I think that is where
5 you need to sample 18 panels, which is very
6 important, to validate not only because those
7 panels have to be predictive of the disease
8 and those panels, there are certain
9 seroconversion panels. You know, in the
10 validation establishment, they will tell you
11 we have in guidances also what is needed to
12 really validate a particular assay.
13 So, obviously, for certain
14 infections, which is why I think the sample
15 collection will come from the surveillance
16 data, that those are valuable sample sets, to
17 then go where we know that the disease is
18 there and we have the samples. Now what is
19 the sensitivity and the specificity of these
20 assays or the platforms with those samples to
21 begin with? And then, you go into the donor
22 population.

1 DR. KASARSKIS: Thank you.
2 DR. DUNCAN: I would like to make
3 a comment. This is Robert Duncan from CBER.
4 On the question of performance
5 characteristics, the information that has been
6 presented so far, and some that is yet to
7 come, suggests that these new devices have a
8 sensitivity level that is comparable to
9 current nucleic acid testing. But all the
10 data that I have seen so far is individual-
11 unit testing, individual-sample testing.
12 And as Indira pointed out, the
13 real breakthrough in nucleic acid testing for
14 blood screening was pooled testing. So, I
15 think the frontier for multiplex testing is to
16 show sensitivity in pooled sample testing.
17 Having made that comment, I would
18 like to ask a completely different question.
19 It was my hope that out of this early session
20 we would establish the need for multiplex
21 testing as a way forward in blood donor
22 screening. And a lot of good points have been

1 made, especially in the world of emerging
2 agents.
3 But whenever a preamble to a
4 discussion about multiplex testing is made,
5 there are usually other things that are
6 mentioned: streamlining, ease of testing,
7 using less volume from the sample, and a few
8 other items. I would like to hear some
9 comments from the blood-bankers, in
10 particular. Are there other aspects like
11 those that you see as a need that could be
12 addressed by multiplex testing platforms?
13 DR. STRAMER: Maybe I am senile,
14 but I forgot the first part of the question.
15 Regarding advantages like smaller
16 sample volume, I think one of the real
17 challenges, maybe something we will need to
18 focus on, is the sensitivity, sensitivity if
19 you say comparable to individual donation NAT
20 now. You know, if we take these microarrays
21 with a gazillion different areas for different
22 pathogens -- so, we are going to say now they

1 have about 5- or 10-copy-per-mL level
2 sensitivity. And if we are adding less volume
3 to those, and the anolyte isn't present in the
4 sample, I mean, it doesn't matter what the
5 analytic sensitivity is. I mean, a lot of
6 what we do now, what we are limited by, is
7 that the anolyte may not be present in high
8 enough concentrations in the sample, and our
9 sample is just devoid of anolytes, so, you
10 know, boson distribution.
11 So, I think that is one of the
12 real issues. Will we have to ultra-centrifuge
13 down 5 mLs of sample to ensure that we get a
14 valid sample to test on some of these systems?
15 So, I think, for me, that is where some of the
16 challenge still resides.
17 DR. KATZ: And the corollary to
18 that is, if you have a concentration
19 technique, how much does that tax my
20 laboratory staff to do? Or is it completely
21 automated? And if I put it in at this end of
22 the instrument, and then down at that end of

1 the instrument it all comes out, the
2 advantages of multiplexing are quite obvious.
3 The devil is in the details. What
4 I am hearing here is I think that is technical
5 issues that I won't be -- I mean, it sounds a
6 little cavalier, but we are used to seeing the
7 technical issues overcome. And then, you get
8 down to the stochastic issue that Sue raised.
9 DR. STRAMER: I think if we figure
10 out a model to bring some of these
11 technologies into blood centers, we will work
12 through the logistics. And that is why,
13 again, I go back to supplemental testing. I
14 mean, we may not have consent to do human
15 genetic studies, but we have consent to do
16 blood safety studies. So, I mean, there is a
17 lot we could do and a lot we can do to work
18 with companies who are interested in bringing
19 these technologies forward.
20 DR. ALTER: Harvey Alter, NIH.
21 I consider myself a sensitive old
22 man and want to give you my perspective on

1 sensitivity.
2 (Laughter.)
3 Sensitivity has to be judged in
4 timing, timing in relationship to the
5 prevalence of that agent that exists at that
6 time.
7 And I bring it up because I would
8 argue that Ouchterlony testing had more impact
9 on blood-transmitted diseases than any test
10 that has come since that time. First-
11 generation anti-ACV testing had much more
12 impact than NAT testing on HCV transmission.
13 The more sensitive tests are just
14 mopping up the small minority of cases that
15 weren't picked up by the very insensitive
16 tests. So, while ultimate sensitivity is what
17 we want to get to, but in terms of impact,
18 even a very poor test can have an enormous
19 impact. And that has been our history.
20 So, were a new agent to come along
21 that was bad, I wouldn't wait until you had
22 the best test available, but I do whatever we

1 had at that time.
2 DR. CHIU: So, it is time for
3 lunch. We will go ahead and conclude this
4 session.
5 I would just like to thank all of
6 the panel members for their insights.
7 Oh, actually, go ahead.
8 DR. EPSTEIN: Thank you.
9 DR. CHIU: Go ahead, Jay.
10 DR. EPSTEIN: I am often accustomed
11 to the last word. So, I appreciate it.
12 I just wanted to separate the
13 issue of the here and now from down the road.
14 You know, the here and now is that we have
15 unmet challenges for agents of known concern.
16 You know, it has been mentioned that we don't
17 have a test for malaria; we don't have a test
18 for Babesia; we are worried about virus
19 variants, especially for HIV.
20 And the prospect is that we don't
21 want to keep introducing individual tests.
22 So, the goal is to try to encompass the here-

1 and-now concerns into multiplex tests. So, I
2 would start with the question of, how can
3 these new technologies and these new platforms
4 get us to where we need to be with the current
5 agents of concern?
6 The second issue, then, is
7 flexibility, because we know that over time
8 the anolytes may improve. We may discover a
9 better protein target for detecting an
10 antibody. We may come up with a better
11 nucleic acid target.
12 But we don't want to have to start
13 all over again revalidating from square one.
14 So, the second issue is, how do we leverage
15 the technologies that we put in place for
16 multiplexing, so that they are also flexible
17 toward the future?
18 And then, I think that the longer-
19 range question is the most difficult one,
20 which is bridging discoveries coming out of
21 epidemiological surveillance with decisions on
22 safety strategies and, then, readily-

1 implemented platforms?
2 So, I just think if we stage this
3 on a timeline, you know, what is it we are
4 trying to do in the next one to two years?
5 What do we need to fix looking forward to the
6 next five to ten years? And I think we have
7 a here-and-now challenge to use the 21st
8 century technologies to solve the unsolved
9 problems of today, which are already well-
10 defined. It is the adaptation, you know, the
11 translational question of the technologies
12 that needs to be grappled with, not to forget
13 the down-the-road issues, but we have here-
14 and-now problems.
15 DR. CHIU: Okay. That sounds great.
16 So, I would like to thank all of
17 the panel members and the audience for their
18 feedback and their insights.
19 (Applause.)
20 (Whereupon, the foregoing matter
21 went off the record for lunch at 1:19 p.m. and
22 went back on the record at 2:33 p.m.)

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

2 2:33 p.m.

3 DR. KUMAR: I hope you guys had a

4 nice lunch, so we can get started now.

5 So, this brings us to our Session

6 III, and Dr. Connie Westhoff from the New York

7 Blood Center is going to chair this session

8 and give the first talk, also, right?

9 So, Dr. Westhoff, she is Director

10 of Immunohematology and Genomics at the New

11 York Blood Center. She is also Adjunct

12 Assistant Professor of the Division of

13 Transfusion Medicine, the University of

14 Pennsylvania.

15 Dr. Westhoff received her Ph.D. in

16 molecular genetics studying the evolution of

17 Rh genes and did postdoctoral work in

18 immunology, studying autoimmunity in mouse

19 models.

20 I guess I am not going to read all

21 of this, not take the time of it.

22 So, I will let Dr. Westhoff begin.

1 DR. WESTHOFF: Thank you, Sanjai.
2 And thanks to the organizers for
3 including the red cell and HLA folks in this
4 conference.
5 I am really pleased to be here,
6 and I hope you all agree that we belong here,
7 talking about multiplex testing of donors. We
8 hope to convince you this afternoon that we do
9 belong here and that we have some of the same
10 issues and some of the same concerns and some
11 of the same technology that can be applied to
12 both areas.
13 So, this is a brief outline of
14 what we hope to today. I promised Sanjai that
15 I will keep us on time.
16 I have been asked to just give a
17 general overview of the field, the current
18 state of the art, so to speak. So, after all
19 those wonderful presentations this morning
20 about where technology is going, I am going
21 back to basics and what we are doing now for
22 blood cell typing.

1 And I am also going to talk a
2 little bit about a platform I am very familiar
3 with, the one for genotyping for minor red
4 cell antigens that is a product of BioArray
5 and Immucore.
6 Then, we will go to Mary Sue
7 Leffell from Hopkins on current approaches for
8 HLA.
9 We will move, then, to Christian
10 Gabriel, who will also give a 30-minute talk,
11 talking about genomics-based approaches for
12 HLA and red cell typing.
13 And then, Gorka Ochoa will talk
14 about molecular assays developed by Progenika.
15 And then, we will finish with a
16 15-minute with Greg Denomme from the Blood
17 Center of Wisconsin, who will talk about
18 technology they are using, the open array.
19 We will have a short break, and we
20 hope you will come back after the break to
21 join the panel discussion. It looks pretty
22 tempting to me there, but maybe traffic is too

1 bad.
2 And Bill Flegel from NIH will join
3 us on the panel.
4 So, with that introduction, my
5 first goal is just to review current
6 approaches for minor antigen typing and to
7 define, actually, when we talk about minor
8 antigens, we are talking about other than ABO
9 and Rh. And we will talk about those later.
10 But minor antigens in this area are other than
11 ABO and Rh.
12 Talk a little bit about how we are
13 doing them now serologically. We have been
14 using also DNA approach for over about 10
15 years now as laboratory-developed tests.
16 Review the experience with
17 BioArray, which has been available since 2006,
18 a platform that is research use only.
19 Talk a little bit about the
20 benefits and why we are excited to move this
21 technology forward and to use it more
22 routinely. Because, first of all, it allows

1 us for the first time to type in an automated
2 kind of platform with somewhat high
3 throughput, and the fact that we are really
4 plagued by this problem of limited antibody
5 reagents for the typing we want to do.
6 There are some challenges
7 remaining I want to touch on briefly and,
8 then, talk a little bit about the synergy and
9 contrast with infectious disease testing.
10 So, actually, this all started in
11 the 1990s, when the genes that carry the blood
12 group antigens were cloned. And lucky for us,
13 most of them are simple SNPs. So, I will be
14 talking about SNPs today, single nucleotide
15 polymorphisms. And in the lab we talk about
16 these as testing or SNPing for single
17 nucleotide polymorphisms.
18 I often get this question: is
19 this genetic testing, because you are using
20 DNA to do something on a patient? New York
21 State has determined that it is blood group
22 typing by a different method, and I think most

1 regulatory bodies agree now that it is not
2 genetic testing for a disease. We are not
3 looking for disease here. We are looking for
4 expression of blood group antigens. So, I am
5 not aware of anyone that is getting a special
6 content for this typing.
7 There are great advantages here.
8 We are able to type now from any cellular
9 source. We are type a fetus without trying to
10 get a blood sample. These assays are designed
11 so blood transfusion doesn't interfere. And
12 the reason it doesn't interfere is because all
13 these assays are designed with the primers to
14 amplify a region of the gene that is common to
15 all alleles and, then, do the downstream
16 analysis. So, any contaminating donor DNA,
17 then, gets swamped out in the PCR and becomes
18 insignificant.
19 As Jay has mentioned there is more
20 than 300 red cell antigens. Are we interested
21 in all of them? Maybe about 30. I was happy
22 to see Harvey mention 20 targets maybe for

1 infectious disease testing that you are
2 interested in. We are interested in about 20
3 to 30. So, I think we might be compatible
4 there on a similar platform as far as space
5 goes.
6 And really, the problem is we have
7 commercial antibodies only for about 18 of
8 these. These are the 18. There may be a 19th
9 out there. CW may be out there. But these
10 are the ones we have antibodies for.
11 Currently, most serologic testing
12 is done by a manual tube technique yet. Kind
13 of like Susan's mention of the Ouchterlony, it
14 is a method that works. We haven't changed
15 technologies because we haven't wanted to. We
16 haven't changed technologies because it worked
17 and was relatively cheap and reliable.
18 But manual assays have some
19 drawbacks. But the majority of minor antigen
20 typing in the U.S. is still done by very
21 manual methods.
22 In the hospital, we do have some

1 automation now coming online with the ProVue,
2 the Tango, and Immucore's products. But the
3 only antigens, minor antigens, that are
4 licensed, reagents that are licensed for
5 typing for minor antigens are the CcEe's and
6 the Kells. So, the others aren't licensed for
7 typing other than by manual methods. So, that
8 is our challenge.
9 In our donor centers, a lot of
10 typing is done on the PK7300. It is used for
11 over 90 percent of the North American blood
12 supply. And again, this does ABO and Rh, as
13 well as CMV and the syphilis test. And it,
14 too, is only licensed for CcEe and Kell, not
15 any of the other typing serum.
16 So, again, even in the donor
17 center, many reference labs are still doing a
18 lot of minor drop a reagent in a tube, adding
19 a drop of cells, reading it manually.
20 So, why do we care about changing
21 this? There is a lot of movement in the area
22 to prevent alloimmunizations. So, when a

1 patient becomes alloimmunized, it increases
2 the cost of future transfusions, both the
3 patient workup and the product. There are the
4 negative outcomes with transfusion reactions,
5 and it is a significant risk, then, for that
6 patient. They are not able to have an
7 emergency transfusion.
8 So, we are looking to improve
9 transfusion therapy means to prevent
10 alloimmunization rather than just living with
11 the effects of alloimmunization, especially
12 for those that are chronically-transfused.
13 And much of this is going on in
14 the sickle cell and thalassemic world where
15 patients, if they are matched for CcEe and
16 Kell have a much lower alloimmunization rate.
17 But that means we need donor
18 centers to have more information on all of
19 their donors, not just a few units that they
20 sell as CcEc and Kell-negative. So, we need
21 to provide this minor antigen typing, I
22 strongly feel, on the donor unit labeling and

1 avoid costly repeat testing by the hospital.
2 So, if it is left to the hospital
3 to do CcEe and Kell typing, that never gets
4 associated with the donor record, and that
5 testing has to be repeated over and over and
6 over again. That is not a very productive,
7 cost-effective way to do things, in my
8 opinion.
9 The problem is donor centers often
10 charge an additional $50 to $100 per antigen
11 per unit because they are often doing this
12 manually. It is not high throughput and it is
13 labor-intensive.
14 So, hospitals, then, don't buy
15 these. They try to type them themselves. To
16 me, we are caught in this vicious circle. My
17 hospitals can't afford my antigen-negative
18 blood, and I can't afford to continue to do
19 all of the workups for them on the patients
20 who become alloimmunized because that is not
21 a revenue-generating stream, either. So, this
22 is economic, in my mind, economic as well as

1 improved patient care. And I think we will
2 all be winners.
3 So, we have to find a way to do
4 this, get it on the unit label in a cost-
5 effective manner. What is it costing now to
6 do this, if you tried to type out a donor?
7 These 18 reagents vary in cost, depending upon
8 who you buy it from and what your contract
9 involves, but you will notice some on here are
10 as high as $7 a single drop of antisera,
11 pretty expensive testing.
12 And if it is all done by manual,
13 we are talking about $100 per reagents per
14 sample to do, and that is without the labor or
15 without the controls and without all the
16 supplies. If you are in the Midwest, the
17 labor probably adds a little bit. If you are
18 in my neck of the woods, it doubles the cost
19 certainly in New York.
20 So, that is kind of our comparison
21 state of the art, so to speak. We have now
22 over 10 years' experience using DNA in the

1 lab. Most of early testing was done by manual
2 methods, lab-developed tests.
3 We have, then, evolved to real-
4 time PCR. And now, we will be talking today
5 about some of these automated platforms that
6 are now available to do this in a little bit
7 more high-throughput method. And I will be
8 talking today about the BioArray product, and
9 you will hear, also, from the HLA world that
10 are using Luminex and, also, for blood group
11 typing from Progenika, and, then, the Blood
12 Center Wisconsin platform.
13 So, this automated potential,
14 then, and interpretation has now made this
15 possible throughout the community because it
16 takes away the high-complexity designing of
17 assays and interpretation that is needed with
18 laboratory-developed tests. And so, now it
19 moves it to the mainstream by giving an
20 automated determination of assay results.
21 So, this path to automation hasn't
22 been an easy one. Dr. Reid and I for many

1 years were trying to get the NAT and
2 infectious disease manufacturers to talk about
3 is there a way that we can put blood group
4 typing on this. And that is why I am
5 particularly pleased to be here, because now
6 we are talking.
7 At that time, there wasn't much
8 interest. There was a lot going on in the NAT
9 world. But there was a New York Blood Center
10 researcher whose wife was working for a small
11 company called BioArray, and they had a
12 technology that came out of Bell Labs that was
13 to miniaturize the colored bead assay
14 technology, akin to the Luminex assay, only
15 miniaturize it.
16 They were doing this by putting
17 these miniaturized beads on a silicone wafer
18 chip on a glass slide. And they had set it up
19 for HLA typing and they were looking for other
20 applications. So, this was a great boon to us
21 to be able to have a company that was
22 interested in looking forward to this

1 technology because everyone we had talked to
2 outside the industry says the same thing: if
3 you are not testing every donor every time and
4 it is not a high-volume area of testing, then
5 there is not a lot of dollars available for
6 companies to put into new technology in this
7 area.
8 So, this uses, then, these colored
9 beads, these small colored beads coated with
10 the DNA oligos on this wafer chip mounted on
11 a slide. And this can run eight samples.
12 These are very -- the resolution
13 is pretty poor up there (referring to
14 PowerPoint) -- these still are somewhat manual
15 methods, in that all the pipe heading is high-
16 precision manual pipe heading. This isn't
17 done by machine, but it is able to do higher
18 throughput.
19 So, these beads now are on this
20 silicone chip. They are not addressed. In
21 other words, each silicone chip is different.
22 So, the analysis and reading the signal with

1 the original template has to be done to
2 associate because these aren't put on here in
3 an addressed manner. But up to 123 different
4 aligoes probes, then, can be on this platform,
5 20 to 30 probes for each antigen, each allele.
6 So, the steps involved: isolating
7 the DNA from the sample, multiplexing the PCR,
8 all of the targets, in one tube. So, they
9 tell me they are at almost the max of their
10 single tube PCR and having 38 different primer
11 pairs in this single reaction.
12 The product, then, needs to be
13 cleaned up and hybridized to the bead chip,
14 which is about a 40-minute cleanup and 40-
15 minute hybridization. And then, there is an
16 on-chip extension reaction that takes about 30
17 minutes. And then, you wash and read.
18 Now this is what we have kind of
19 been talking about today. So, this has been
20 validated for these 38 primer pairs. And so,
21 I want Yta in here. I want the new Vel in
22 here. Of course, it would have to go through

1 validation and all of these things to throw
2 another primer pair in here. So, that is our
3 challenge. It is not expandable. It is what
4 it is.
5 So, here is how it works. Here is
6 a bead with the oligos with the Duffy A and
7 the Duffy B sequence on it. And the end-
8 nucleotide is a single SNP that determines
9 whether you are Duffy A or Duffy B.
10 Here I come with my PCR products
11 that hybridize to the bead chips. And here I
12 come with my enzyme. So, if the patient's
13 product is an AT match, it will extend. But
14 if there is not a match here, they don't have
15 the Duffy A allele, it won't extend.
16 So, here now I have a signal
17 attached to the Duffy B bead, but there was no
18 product from the Duffy A locus. So, I have no
19 signal attached to the Duffy A locus, and that
20 is the basis of the signal to bead read.
21 Now the assay image actually needs
22 to associate the signal with where the beads

1 are located, where Duffy A and Duffy B are
2 located. This is done over the internet. So,
3 this is new for us in this field, in that the
4 software is not in-house and the allele
5 intensity calls are not in-house. This is
6 done over the internet and sent right back.
7 So, it is a separate internet line.
8 The computing power, for two
9 reasons, isn't necessarily available in a lot
10 of hospitals or even in a donor center where
11 we have a regulated environment. Putting the
12 software on to make all of these calls not
13 only is a space issue, but a regulatory issue.
14 So, this is done over the internet and the
15 results come back.
16 So, there is also a 96 well plate.
17 This is our high throughput available. So,
18 this matches with your 96 well DNA extractor,
19 again, in a templated kind of run-through.
20 This automatically reads. So, the
21 operator loads the sample template, and they
22 scan the barcode on the slide that belongs

1 with the sample template. This is automated
2 signal acquisition. And then, like I said,
3 the signal is downloaded via the internet for
4 decoding and analysis and comes back in a
5 matter of minutes, because you have to be able
6 to associate the chip number with the sample
7 template and, also, with the signals.
8 Okay. So, like I said, partnered
9 with the 96 well plate extractor, this is
10 somewhat high throughput. But you will notice
11 here this is not a black box. We are sample
12 in, results out. You have got to extract your
13 DNA. This is manual pipe heading for the PCR
14 and then precision pipe heading onto the
15 plate. This is a glass plate and, of course,
16 it is thousands of dollars.
17 So, precision handling, but we
18 still call it high throughput because we can
19 do a lot of assays, 3,072 antigens in six to
20 eight hours with our extraction and running
21 the plate.
22 Hemoglobin S is also on here. We

1 did that because at the New York Blood Center
2 hemoglobin S is treated like an antigen, even
3 though it is not an antigen. So many people
4 order hemoglobin S negative blood, that we
5 have a large call for hemoglobin S negative
6 units. And we also don't label our units as
7 leuko-reduced if they have a hemoglobin S
8 marker on them, because so many of them fail
9 leuko-reduction. So, that is the reason we
10 put it on here.
11 We are not using it right now
12 because we haven't completely validated it.
13 And we are trying to decide if that means it
14 is a genetic test. Because it tests for
15 hemoglobin S, is that a genetic test and we
16 have to get consent?
17 But, then, we are doing a lot of
18 hemoglobin S screening in the blood centers
19 now by solubility testing. And I am not sure
20 anybody is getting consent to test a donor for
21 hemoglobin S. I am not sure; maybe you are,
22 but that is just one of our issues.

1 Okay. So, this is what the
2 printout looks like. You can't see it very
3 well. We have got 35 antigens here, and here
4 they are, but you will notice we have no ABO
5 and Rh on here. And I am going to talk a
6 little bit about that. Thirty-five different
7 antigens.
8 So, just to review a little bit
9 without a lot of detail our experience with
10 this platform, like I said, it was available
11 in 2006. It was first published in 2007 by
12 Hashmi and Reid where they determined 25 minor
13 antigens in 10 systems for 2,000 donors. They
14 found confirmation of 4,510. They found eight
15 discordant due to silencing of hemoglobin S.
16 So, they typed as S pos, but they are really
17 S neg. That was, then, changed on the
18 platform from this initial study.
19 Sixteen errors were in recording
20 results and data entry. And so, this is a
21 common theme when we are starting to compare
22 DNA testing with manual serologic testing.

1 Okay? So, the majority of errors are manual
2 serologic posting results, just what you would
3 expect from manual testing. You are going to
4 have some data entry errors.
5 They were able to identify 25 rare
6 donors. So, this has been a real boon. In an
7 eight-hour shift, you can process two plates.
8 So, the new version, then, to correct the
9 glyco for an S typing was 1.2.
10 Now, in the U.S., BioArray tells
11 me, as of March of 2013, almost 700,000 donors
12 and patients have been tested on this
13 platform. So, because the analysis goes back
14 to the mainframe at BioArray, they know how
15 many tests are being performed.
16 Twenty-five donor centers are
17 using this, 14 hospitals, 3 reference labs,
18 and 10 countries outside the U.S. And this is
19 a platform that is CcEe marked in Europe.
20 So, I often say that this is
21 probably the more mature platform and the most
22 experienced platform for testing for minor red

1 cell antigens.
2 And they also have data on what
3 has been coming through. You can tell from
4 the high prevalence of these markers that are
5 in minority donors that a lot of people are
6 using this to test their minority donors or to
7 screen their minority donors. This is not a
8 licensed test. It is research-use-only. So,
9 people will use this to screen, and, then,
10 they will confirm anything that is screened
11 negative; they will confirm it with a licensed
12 manual usually serologic test.
13 One thing else I wanted to mention
14 is what has come to light since using this
15 throughout the U.S. is there is a lot of Duffy
16 weak antigens out there. We estimate about 2
17 percent of Caucasians have a weak Duffy B that
18 is not detected by the current monoclonal
19 anti-Duffy B's that are on the market. And it
20 has an additional mutation which is called
21 Duffy X. But we are able to detect these on
22 the platform.

1 So, my experience personally,
2 though, is in testing about 18,000 samples now
3 at New York Blood Center and testing 8,000
4 samples when I was with the American Red Cross
5 from 2008 to 2010.
6 And I have just summarized here
7 what we found in both of those centers is the
8 discrepancies between when we compare the
9 serology to the DNA are, first and foremost,
10 due to manual serologic testing or recording
11 errors. And that is not because folks are
12 doing a bad job. I think a CAP survey a
13 number of years ago showed that any manual
14 test has about a 1 percent to as high as 2
15 percent error rate somewhere in the process,
16 just because of human error, et cetera. These
17 kinds of things show get it computerized, get
18 it automated, and it improves significantly.
19 The rest are due to these genetic
20 variants. The fact is, and we all know,
21 serology and antibodies don't detect some weak
22 antigens. We have known this for a long time.

1 The Duffy B I talked about, which has a
2 prevalence of 2 percent in Caucasians, is
3 right up there. We have some weak E's, big
4 E's, that aren't detected with our serologic
5 reagents and our detected, then, in a
6 genotype.
7 There is also cases of false-
8 positive C typing. We have now learned that
9 samples with this partial D4A are typing as C-
10 positive with one manufacturer's serologic
11 reagent.
12 But I am not here to go into these
13 in any detail. There is DNA on the other
14 side, doesn't detect some rare or new silenced
15 alleles that aren't on the platform. If it is
16 a silenced allele and it is not on the
17 platform, it is not going to be detected.
18 We are finding that in the kids'
19 system, especially in Blacks, there are some
20 things we go serologically-silenced Duffy A
21 and Duffy B genes. They type negative, but
22 they have, again, low levels of protein. Like

1 I said, I am not here to go into them in any
2 detail.
3 Actual users' experience, the
4 Children's Hospital in Philadelphia has been
5 typing their children, 340 children. They do
6 a pre-transfusion test for 13 antigens,
7 compared it to the platform. Thirty-eight
8 discordant results have a result of 0.86. I
9 think that is pretty low. In repeat testing,
10 they were all concordant with the DNA
11 genotype, again, just showing the manual error
12 rate in their testing. And the CHOP folks say
13 the results so far are batting a thousand for
14 the HEA typing.
15 But they get additional
16 information. They get to know what kids are
17 lacking high-prevalence antigens and at risk
18 to produce these. This is where we improve
19 patient care.
20 They identify patients on this
21 platform with a partial-C antigen. So, they
22 know they are at risk for big C, and they

1 transfuse these kids with C-negative units.
2 So, they are actually changing practice based
3 on the results.
4 And the other nice thing that
5 comes up on this platform is we know which
6 kids aren't at risk for Duffy B because they
7 have the GATA mutation. So, with this
8 additional information, it is very helpful.
9 Things we can't do with serology that we can
10 do with DNA.
11 So, this just summarizes the
12 majority of discrepancies. We are in a good
13 position with the serology and the DNA, in
14 that we have something to compare and to
15 validate. And so, that is the beauty of
16 moving to DNA. We can validate it with the
17 serology, and all the same potential reasons
18 for discrepancies, these due to the reagents
19 and the antibodies, and these due to
20 additional changes in the alleles that aren't
21 targeted on the platform.
22 But here is our dilemma:

1 population differences, we are always going to
2 run into population differences. How do we
3 design these platforms to pick up all of the
4 population variations we are interested in?
5 In my neck of the woods in New York, all of
6 these diverse kids are present in copious
7 amounts in Queens, et cetera. So, I want all
8 of the ethnic SNPs on a platform. Whereas, in
9 many areas of Europe, et cetera, they are
10 quite able to not go that in-depth in the
11 number of SNPs they are analyzing.
12 So, there are limitations. If we
13 are only doing a SNP typing and we are only
14 detecting changes for a Duffy A or a Duffy B,
15 what about the silenced? What risk does this
16 make if I miss a silenced allele? You will
17 get a false-positive then. Is that a problem
18 for donor typing? No, because I am always
19 looking for an antigen-negative unit. If it
20 comes up as positive, I am going to keep
21 looking for an antigen-negative unit.
22 In patient typing, no, it is not

1 problematic, in my mind, because if I don't
2 use their antigen-positive status to say they
3 don't have the antibody, then I am not in
4 trouble. So, I need to continue to perform
5 the antibody screen to avoid any risk.
6 The same with false-negatives. I
7 am running out of time. But, again, a false-
8 negative is problematic on a donor, but,
9 hopefully, it would be detected in an
10 incompatible cross-match. So, I want to avoid
11 false-negatives, but for the patient there is
12 not a significant risk.
13 So, beneficial for really
14 increasing the number of rare units and for
15 patients, giving us a comprehensive antigen
16 profile.
17 Just two words about ABO. I
18 didn't mention any ABO and Rh typing on these
19 platforms. There are many different ways to
20 be a Group O. So, a single SNP or a number of
21 SNPs are not going to suffice here. We don't
22 know all the ways that mutations cause a Group

1 O.

2 The same with Rh negative. Most

3 Rh negative lack the D gene, but you can't use

4 the presence of absence of the D gene to

5 determine the D type because there is a number

6 of silenced. So, the gene is there, but it is

7 not active and expressing. So, the gene is

8 there; you can't call it positive in case it

9 is silenced because there are some serious

10 consequences to typing Rh negative as Rh

11 positive.

12 But we can use it for confirmatory

13 testing. In this Red Cross pilot study, when

14 I was with the Red Cross, we had 150 donors

15 that fell into this category of NTDs. I am

16 sure you all face the same kinds of issues.

17 They are usually donors that are, then,

18 deferred because their type can't be

19 determined because what happens is their front

20 types and their back types are discordant.

21 So, they often get deferred.

22 We showed that 30 percent of these

1 were actually Group O. By using a single PCR
2 in an RFLP analysis, we could show which ones
3 were homozygous for O alleles, and we would
4 like to consider this as a confirmatory
5 testing because the red cells were front-typed
6 as Group O. So, there won't be any retype
7 problem at the hospital center. And these
8 low-titer anti-A and anti-B products are
9 worthwhile for plasma products.
10 The same with RHD. We typed a
11 number of samples with NTDs due to D. People
12 I think shy away from confirming that their Rh
13 negative donors are truly negative because we
14 don't want to lose any more negative donors in
15 the donor pool. But we actually were able to
16 show, yes, many of them are a weak D, but a
17 lot of them don't even have a D gene or any
18 reason to have been false-positive on a Pk.
19 So, we were able to actually retain 20 percent
20 of those donors.
21 But the potential for ABO and Rh
22 typing, as you will hear later on, probably

1 lies in next-gen sequencing, especially for
2 ABO as a confirmatory test.
3 So, in summary, this is our first
4 new technology since monoclonal antibodies in
5 the eighties. The equipment and testing
6 environment is very different than blood banks
7 are used to. So, this certainly does affect
8 adoption.
9 But we are able to also
10 understanding that a new technology used at
11 the donor center has to be somewhat concordant
12 with what testing is going on at the hospital.
13 So, that is a little different than infectious
14 disease testing, where someone is not
15 rechecking your results or reconfirming.
16 Test turnaround time here is very
17 different than our ABO and Rh typing, which
18 takes minutes in a tube, six to eight hours.
19 So, one of the issues, then, is, can this be
20 one-time testing for patients and part of
21 their electronic medical health record?
22 So, lastly, we will talk a little

1 bit more about this. The testing, though, I
2 think is going to improve patient care. SNP
3 typing is really highly correlated, and I feel
4 it falls to donor centers to provide this
5 information to our hospital customers because
6 we can do it cost-effectively. We can do it
7 high throughput, and we can tie it back to the
8 donor, so that information is not lost every
9 time a segment is typed at the hospital.
10 And also, our leuko-reduction
11 methods are very effective. So, it is not
12 like the hospital could do the DNA testing on
13 the donor units as they come in the door
14 because there is just not enough DNA in most
15 of those segments.
16 So, with that, I will close, and
17 we will introduce the next speaker.
18 And I apologize, I am five minutes
19 off, Sanjai. I promised you I would be on
20 time.
21 Okay. So, Mary Sue Leffell. Mary
22 Sue is Professor of Medicine and Director of

1 Immunogenetics at Johns Hopkins University.
2 She has been very active in ASHI and a Past
3 President of ASHI. In 2011, she was honored
4 with the Distinguished Scientist Award from
5 ASHI.
6 Her active research has been in
7 the clinical histocompatibility and
8 transplantation for over 30 years. So, she is
9 certainly an expert. And she works on the
10 incidence and impact of HLA-specifics
11 alloimmunization among patients also requiring
12 platelet support.
13 Mary Sue?
14 DR. LEFFELL: Thank you, Connie,
15 and I would like to thank the organizers for
16 inviting me to participate in this exciting
17 workshop.
18 As Connie said, I am going to be
19 talking to you about the current techniques
20 and approaches we use for HLA testing or,
21 probably more correctly termed,
22 histocompatibility testing in HLA.

1 I think it is important to focus
2 on the clinical needs for HLA testing. The
3 major needs are, obviously, for patients who
4 are sensitized and need transfusion support
5 and, also, for the detection of HLA antibodies
6 in prevention of TRALI.
7 There are, of course, other
8 indications in patients, fortunately somewhat
9 rare, potential transfusion-related graft
10 versus host disease, but these are the major
11 indications.
12 To do this, we need to do two
13 things. We need to, first, type our donors
14 and recipients. And secondly, we need to have
15 sensitive and accurate methods to identify and
16 detect HLA-specific antibodies.
17 I will get this right in a moment.
18 To do this, our current testing
19 platforms are molecular-based typing, PCR-
20 based typing for HLA antigens, alleles, and
21 allele groups. These are the major methods
22 that are currently in use: sequence-specific

1 primary implication, reverse sequence-specific
2 aligonucleotide probe hybridization, and, of
3 course, sequencing-based typing, which by many
4 people is considered sort of the gold standard
5 currently. That is probably going to be soon
6 replaced by next-generation sequencing.
7 And then, for solid-phase
8 immunoassays, we have a number of different
9 platforms. I will tell you a little bit more
10 about those later on in the talk. But these
11 have revolutionalized our detection of HLA-
12 specific antibodies.
13 Now the major confounding factor,
14 whether we are trying to type or whether we
15 are trying to define HLA-specific antibodies,
16 is simply the extreme polymorphism of the HLA
17 system. As of January this year, the IMGT
18 January report, there were close to 9,000
19 reported HLA alleles, 7,000 Class 1, and
20 around close to 2,000 in Class 2, Class 1 loci
21 being what we refer to HLA A, B, and C; Class
22 2, including the DR, DQ, and DP antigens and

1 alleles.
2 I am sorry, this seems to be slow
3 in responding (referring to PowerPoint).
4 Fortunately, of these close to
5 9,000 alleles, the ones that are probably
6 clinically-relevant really only make up about
7 13 to 14 percent. These fall into the
8 category of what we call, refer to as common
9 and well-documented. And there is now
10 actually an international committee that
11 considers and updates this listing.
12 And the latest update just came
13 out last month. We now include around 1100
14 alleles in this category. If they are common,
15 they have a definable frequency, usually
16 determined by a large population-based study
17 of generally around 1500 individuals. If they
18 are considered well-documented, if they have
19 been reported by a technique such as
20 sequencing-based typing and confirmed by SBT
21 in at least five individuals, and preferably
22 in multiple populations, this establishes that

1 it is not just a single occurrence, because
2 the HLA system is still evolving. We detect
3 new alleles on the average -- one of the last
4 reports I heard from the WHO Nomenclature
5 Committee was an average of three to four new
6 alleles being reported weekly.
7 To type, it is important to
8 remember for transfusion support that we may
9 need to type at a different level than we do
10 for other clinical indications. For
11 hematopoietic stem cell transplantation, bone
12 marrow transplantation, we try to type an
13 allele level or an allele group level. We do
14 the same level of typing if we are typing for
15 adverse drug reactions with specific allele
16 associations.
17 Solid organ transplantation, we do
18 very little matching. And so, we really only
19 need to type on an antigen level, and we have
20 to type on an antigen level to be able to find
21 relevant HLA-specific antibodies.
22 And this is really the level that

1 is needed for transfusion support. But the
2 problem is very complex because translating
3 9,000 alleles into about 100 relevant antigens
4 is a very complex process because many alleles
5 will encode the same serologically-defined or
6 immunogenic antigen.
7 Am I doing something wrong that
8 this is not advancing (referring to
9 PowerPoint)? I think I will switch to the
10 arrows. Okay. We will just stick with the
11 arrows.
12 This slide illustrates the targets
13 for HLA typing that we use for our molecular
14 typing. Most of the variation that occurs in
15 the HLA alleles occurs in those domains that
16 encode the antigen recognition site. Now the
17 antigen recognition site are the membrane
18 distal domains that encode the peptide-binding
19 region and the region that interacts with the
20 T cell receptor. And for Class 1 antigens,
21 these are exons 2 and 3, and for Class 2
22 antigens, we have to consider both the primary

1 polymorphism is located in exon 2, but we have
2 to consider both the alpha and beta chains,
3 with the exception of DR antigens, where only
4 the beta chain is considered to be very
5 polymorphic.
6 However, there are a growing
7 number of alleles that differ outside exons 2
8 and 3 for Class 1 and exon 2 for the Class 2.
9 And these can be variants that occur in the
10 transmembrane domains or the site of plasmic
11 tail or in the case of Class 1 in exon 4,
12 which encodes the alpha 3 domain.
13 These are often clinically-
14 relevant. In many cases, we are required by
15 accreditation standards to define these
16 alleles.
17 So, while there are many
18 advantages to molecular typing -- it is
19 accurate; it is reproducible; it is far better
20 than the old serologic typing we used to do --
21 there are some specific limitations. And some
22 of these limitations would have to be taken

1 into consideration if HLA were going to be
2 incorporated into a multiplex platform for
3 blood donation.
4 And one of the major factors is
5 the fact that we are dealing with a moving
6 target. There are an increasing number of
7 recognized alleles. This means that we have
8 to update our sequence libraries periodically.
9 We have to update our analysis software, and,
10 of course, it requires definition and
11 validation of new reagents. Currently, we are
12 updating our sequence library at least every
13 six months.
14 We also have to deal with a major
15 problem that is brought about by HLA
16 polymorphism, and that is this issue of typing
17 ambiguities. And this can occur in one of two
18 ways. You can simply have alleles that differ
19 outside the exons that are the primary target
20 for our molecular assays, that is, exons 2 or
21 3. And I have given you three examples here
22 of null alleles that differ in exon 4 and the

1 C locus allele, which is actually fairly
2 common. To identify this allele, we have to
3 probe or sequence all the way through exon 7.
4 And then, there is the
5 particularly thorny problem that we refer to
6 as ambiguous heterozygotes. Let me try to
7 explain briefly what this term refers to.
8 The HLA system, the genes evolved
9 by a process of gene duplication, but the
10 polymorphism, the diversity was and is still
11 being generated by either intragenic
12 recombination, some gene conversion, or in
13 some cases simple point mutations.
14 But this diversity has resulted in
15 polymorphic motifs or limited sequences that
16 have been literally shuffled around among the
17 HLA genes. And so, the system is referred to
18 having this patchwork motif that identifies
19 certain allele groups and in some cases
20 crosses allele groups with these different
21 patterns of these motifs that I have tried to
22 illustrate here just by the different colored

1 bars within these gray sequences that would
2 represent sequence homology.
3 So, when we type, if we type by a
4 probe hybridization or even an SSP, and
5 certainly by sequencing-based typing, we are
6 generally typing heterozygous. We don't
7 separate the strands.
8 And so, if we have two individuals
9 who have sets of alleles that share these
10 sequence motifs, but differ in the phase,
11 differ in the strand that has a particular
12 sequence motif. When we type an heterozygous
13 state, both of these individuals are going to
14 be positive for these four probes for these
15 different sequence motifs.
16 And the same thing would occur if
17 we do sequencing-based typing, which is
18 generally done in the heterozygous state, and
19 sequencing in both the forward and reverse
20 directions, we would get this result where we
21 have at these particular polymorphic sites
22 these heterozygous results.

1 And the only way, if these
2 particular sequences are shared in allele
3 combinations, the only way to decipher that is
4 to do a hemizygous typing. The way that this
5 is most often done in HLA labs today is to do
6 what is called group-specific amplification,
7 which simply uses either group-specific
8 primers prior to sequencing that would be
9 specific for a particular motif -- in this
10 case, the primer would be set to recognize the
11 GTG sequence. And so, you would amplify only
12 one strand and set the phase of that
13 particular motif and could, therefore, then,
14 determine which of the allele combinations was
15 correct.
16 This is an extreme problem. It
17 occurs roughly anywhere in 50 to 60 percent of
18 the cases we are doing where we have to do
19 some sort of subtyping or group-specific
20 amplification.
21 This is an actual example. I know
22 you can see the sequence. But this shows

1 sequences for two allelic combinations, a B7
2 allele with a B48 allele versus a much-less-
3 common, but still possible combination of B48
4 with a B81 allele.
5 The sequence for the more common
6 pair, the B7/48, is shown up here at the top,
7 and the boxes indicate the corresponding
8 sequence that would be for this pair of
9 potential alleles.
10 What you really need to appreciate
11 is these shaded areas, which are the areas
12 where we see the polymorphic substitutions.
13 This part of the sequence, positions 80
14 through 340, comprise exon 2. This bottom
15 half refers to the sequence that we would see
16 in exon 3.
17 And what I would like to point out
18 to you is the read length that it would take
19 to resolve this even with a group-specific
20 amplification or with next-gen sequencing
21 where you could do linear sequencing of a
22 single strand. At a minimum, you would have

1 to sequence from 280 of exon 2 through 420 of
2 exon 3 plus the intervening intronic sequence.
3 This would be a minimum read length of 365
4 base pairs. And we are not quite there yet
5 with next-gen.
6 So, the major typing methods
7 currently in use are, I have mentioned SSP and
8 sequencing-based typing. Probably the most
9 common technique in routine use in clinical
10 HLA laboratories is a reverse SSOP. I am
11 going to tell you a little bit more about this
12 assay because, looking at some of the
13 limitations and advantages of this technique
14 I think will introduce some principles that
15 would be applicable to trying to move to a
16 multiplex platform with other systems.
17 As I mentioned, Dr. Gabriel, who
18 is going to be following me, will tell you
19 more about next-generation sequencing for HLA.
20 It is not quite there yet for routine use in
21 clinical labs, but I think it is getting
22 close. I think Dr. Gabriel will be,

1 hopefully, giving us some insights about where
2 we are with this technology.
3 The most common amplification of
4 the reverse SSOP is on a Luminex platform.
5 So, it is a micro-suspension bead array. I am
6 not going to go into any details of the
7 technique. The primers include a reporter
8 molecule that is incorporated into your
9 amplified test DNA. And then, this is
10 hybridized with multiplex probes on the
11 microarray bead platform.
12 It has many advantages. You can
13 rapidly achieve what we call an intermediate
14 resolution typing at an allele grouper antigen
15 level. It can be semi-automated. It has a
16 relatively-fast turnaround, and it is
17 relatively inexpensive.
18 It does have some limitations. As
19 you can imagine with the complexity of the HLA
20 system, to put everything on a single
21 molecular platform, you have to go with one
22 set of hybridization and wash conditions. And

1 for many of the probes, these are going to be
2 less than optimal for both hybridization and
3 the stringency of the wash steps. So, we are
4 going to have to live with some false-positive
5 and negative reactions.
6 And it requires multiple probe
7 sets for resolution of typing ambiguities.
8 The Luminex platform is powerful, but in many
9 cases you have to go to a subtyping routine to
10 resolve some of the ambiguities.
11 And a very real problem, and I
12 think one that may well be a stumbling block,
13 is the lack of reference reagents,
14 particularly for rare alleles.
15 This just gives you an example of,
16 again, the complexity. This simply shows from
17 one commercial system a probe set, a probe
18 known as 072. And these are the alleles that
19 will react with that particular probe. The
20 sequence would bind to that particular probe
21 sequence. So, to accurately type, you have to
22 use multiplex probes to get a single typing.

1 This shows the readout from one of
2 the commercial systems by One Lambda,
3 Incorporated. And you can see in this lower
4 lefthand quadrant the signal strength of the
5 different beads and corresponding probes that
6 react with this with this particular test DNA,
7 the signal strength being measured as either,
8 depending on your system, a median or mean
9 fluorescence intensity.
10 You will see that this green bar
11 is flagged as a potential false-positive
12 reaction. And the quality control data for
13 this particular bead, Bead 99, is shown in
14 this quadrant. And it shows positive
15 reactions with certain reference samples that
16 allows the manufacturer to set a cutoff for
17 what they would consider as positive.
18 And these are the reactive scores
19 on the clinical samples on this run. The red
20 bar shows this particular test sample, and it
21 is flagging this B99 as a possible false-
22 positive reaction. And the reason for this is

1 that, when the software analyzes this, it
2 gives a combination of a relatively-common B7
3 allele with a relatively-rare B40 allele.
4 Now the manufacturers allows some
5 subjective intervention in interpreting the
6 software, and our technologists can actually
7 go in and they can look at this QC data. They
8 can say, "Well, that bead was just barely over
9 the cutoff." And you can raise the cutoff.
10 If you do this, now the software will give you
11 a more common typing.
12 This is the more likely result
13 because the B40:03, I think it was, is
14 relatively rare compared to this allele
15 referred to as 40:01.
16 This is software from the other
17 major manufacturer of reverse SSOP,
18 LIFECODES/Immucore, formerly known as
19 Gen-Probe. And this is just an example of
20 their software. Remember, I referred to
21 common, well-documented alleles. Their
22 software will flag common, well-documented

1 alleles, and they are highlighted in yellow.
2 In the typing results for this
3 particular typing, there were five possible
4 combinations of alleles. All of them were
5 exact matches with the probes.
6 So, you can make an educated guess
7 that this first pair, 39 and this 40:01, is
8 the more likely assignment, but you can't be
9 absolutely sure because these others were all
10 exact matches. This software also gives you
11 the serological equivalent for the 40, which
12 is known antigenically as B60.
13 There are cases where, if we are
14 trying to type at an antigen level, that our
15 reverse SSOP gives us serologic ambiguities.
16 And what I mean by this is it comes up with
17 allele combinations that cross antigen groups.
18 This is just one example. It shows typing
19 results for some C locus alleles. In this
20 case, there are 24 possible combinations.
21 Again, the common, well-documented are
22 highlighted in yellow. This would most likely

1 be the most probable assignment.
2 But you will notice down here,
3 also paired with this common Cw12 allele is a
4 C5 allele. And it is rare, but it is still a
5 real possibility. So, this is a case where we
6 can't differentiate the C8 alleles from the C5
7 alleles. And this occurs with all the current
8 commercially-available methods. This is
9 another example from One Lambda showing
10 exactly the same kind of ambiguity, not being
11 able to differentiate the C5 from the C8
12 allele.
13 And this diagram simply points out
14 the lack of reference reagents. Remember, I
15 showed you that you could look at the QC data
16 for a given bead. In this particular example,
17 bead 56, the manufacturer had no reference
18 reagents. So, there were no positive samples
19 to set a cutoff for this particular bead.
20 So, these are just some of the
21 problems we have run into with our molecular
22 typing. Don't get me wrong, it is wonderful.

1 It really has revolutionized the field of
2 typing, particularly for bone marrow and
3 hematopoietic stem cell transplantation. But
4 it does require some different typing
5 strategies.
6 In our lab, we currently use
7 basically anything we can to resolve some of
8 the ambiguities. We make a lot of our own
9 sequencing primers and do subtyping. For the
10 reverse SSOP, there are extended probe sets.
11 I have already mentioned the fact that we have
12 to do group-specific amplification. And
13 again, we also combine information from two
14 systems to rule out possible combinations.
15 I would like to turn now for the
16 time I have left and very briefly give you
17 some insight into the world of detection and
18 identification of HLA-specific antibodies.
19 And I wanted to start by pointing out that
20 this is still a real problem when it comes to
21 transfusion medicine, even in the age of
22 leuko-reduced transfusion products.

1 These are some studies. This was
2 a study that came out of the National Marrow
3 Donor Program, a retrospective study looking
4 at the incidence of HLA-specific antibody in
5 bone marrow transplant candidates.
6 This next series of slides are all
7 from our institution up the road in Baltimore
8 done with colleagues in both bone marrow
9 transplantation and our colleagues in the
10 hemapheresis transfusion service. And this is
11 data from the National Organ Procurement
12 Transplant Network. All together, these
13 figures point out anywhere from a 20 to 30
14 percent incidence of HLA-specific antibodies
15 in candidates who may need transfusion when we
16 assay with the sensitive solid-phase
17 immunoassays we have available today. So, it
18 is still very much a real problem.
19 Now the antibody assays, also --
20 and I am going to tell you about some of their
21 limitations -- but I want to set the scene in
22 pointing out that they are lightyears better

1 than what we were using up until about 15
2 years ago, which were cell-based serologic
3 assays. And these required viable cells.
4 They required separation of T and B
5 lymphocytes to be able to differentiate HLA
6 Class-2 from Class-1-specific antibodies. And
7 they were relatively non-specific.
8 And so, the solid-phase platforms
9 we have available today have really
10 revolutionized antibody testing. They are
11 very sensitive, and they are very specific.
12 The most widely used, again, is on the Luminex
13 platform. They are relatively-high
14 throughput. They can be semi- to fully
15 automated.
16 And they have allowed us in the
17 field of transplantation to do something
18 called virtual cross-matching. This has been
19 particularly useful in heart and lung and
20 liver transplantation where time is of the
21 essence, and the transplant surgeons very
22 often would like to know if they are likely to

1 have a negative cross-match, so that they can
2 go ahead with procurement and actual
3 transplant without having to wait for an
4 actual cross-match.
5 The formats that are available,
6 there are three general types. There are
7 pooled HLA antigens. These are usually from
8 multiple donors, 1,000 or more donors. It is
9 an inexpensive platform. It has the weakness
10 of really not providing any great specificity,
11 but it is great just for screening for the
12 presence of antibody. And it can be done high
13 throughput and very cheaply.
14 The phenotype assays are a single
15 Class 1 or Class 2 antigen display from a
16 given individual. So, it would be a Class 1
17 phenotype, including HLA A, B, and C antigens,
18 and for Class 2, DR, DQ, and DP.
19 This is the approach that really
20 comes close to giving us what we see in real
21 life. We don't see a single antigen. We see
22 an array of antigens.

1 In our experience -- and we have
2 published extensively on this from our lab in
3 Hopkins -- it gives us the best gauge of the
4 actual relative strength of antibodies, and it
5 correlates beautifully with our cross-match
6 test.
7 Single antigens are a platform
8 that is widely, widely used. These are
9 single, purified, in many cases recombinant
10 HLA antigens. They are great for confirming
11 the presence or absence of a particular
12 antibody. But these are very sensitive tests,
13 and they are highly susceptible to
14 interference and false reactions.
15 Some of the limitations: there is
16 a great variability in the amount of antigen
17 actually on each bead. Some of these vary or
18 are distorted to so degree. So, we can get
19 false-positive or negative reactions.
20 Because these tests are so
21 sensitive, there is an inherent amount of
22 variability. We see tech-to-tech variability

1 between assays and extensive variation among
2 laboratories. This is improving as people are
3 making -- we are getting help from our
4 commercial colleagues in reducing this
5 variability.
6 And I have already mentioned that
7 they are subject to interference. This can
8 include autoantibodies, high-GM antibodies,
9 and in some cases even therapeutic antibodies.
10 Rituximab, thymoglobulin are known to
11 interfere with these assays.
12 They are semi-quantitative. The
13 readout is in a decreasing scale of reaction
14 strength, again, recorded as either median or
15 mean fluorescence intensity. And you can
16 relate to ranges of reactivity.
17 And I would like to stress,
18 however, they are semi-quantitative, again,
19 because of the variation between assay-to-
20 assay. And there are currently no reference
21 reagents. So, they cannot be used on an
22 absolute quantitative scale.

1 But you can establish relative
2 ranges of reactivity. And we have done this
3 and published this. This is data from a
4 number of years ago.
5 The top panel shows correlations
6 of a single-antigen panel reactivity with a
7 flow cytometric cross-match. The lower panel
8 shows the correlation with a phenotype panel,
9 again, with the actual results from flow
10 cytometric cross-matches.
11 We got a relatively-good
12 correlation with the phenotype panels, and I
13 think you can appreciate there is more
14 variability with the single-antigen assay.
15 The limitations of these, just to
16 summarize now:
17 The test validation and the
18 interpretation for these antibody assays needs
19 to be both assay- and antigen-specific. Just
20 two quick examples. We have established that
21 values for antibodies to DQ antigens, the
22 values will be twice that what we see for

1 corresponding levels of reactivity with
2 antibodies to DR antigens. We have also
3 observed similar correlations with antibodies
4 to C locus antigens and to DP.
5 There is also no currently-
6 established level of antibody that is
7 clinically-relevant. These assays are
8 extremely expensive and they can detect very
9 low levels, and there are many complications
10 out there that suggest that, at least in the
11 short-term, these are not clinically-relevant.
12 I have already mentioned that there are no
13 reference standards.
14 And I think you should point out
15 that there was a recent international
16 consensus conference. And despite the power
17 of these assays, one of the important
18 recommendations from this conference was that,
19 at least in transplantation for the time-
20 being, these assays should be used in
21 conjunction with a cell-based cross-match
22 test.

1 The final point I would like to
2 make is that it takes a fair amount of
3 expertise to interpret these assays because of
4 just the alphanumeric soup that goes into HLA
5 nomenclature. These are just some examples of
6 allele groups that have corresponding alleles
7 that differ in their antigenicity.
8 For those of you who are familiar
9 with HLA nomenclature, the first two digits
10 generally refer to a corresponding antigen
11 group. A02 refers to a very common antigen
12 known as A2. This would be a prototype allele
13 in that group. This particular allele, 02:03,
14 has a different antigenicity. It reacts
15 totally differently serologically. And these
16 are just some other common examples. The B15
17 group varies extensively in their different
18 antigens. So, this is something that has to
19 be taken into account in terms of interpreting
20 and correlating molecular typing and
21 correlating it with very sensitive antibody
22 assays.

1 So, in closing, these are the
2 considerations that I think would be relevant
3 for future multiplexing of HLA testing. I
4 think all of us believe and are looking
5 forward to next-generation sequencing because
6 it will address the problem of these ambiguous
7 heterozygotes. However, it still will have to
8 deal with an ever-growing number of HLA
9 alleles and the fact that we don't have
10 adequate reference reagents.
11 With regard to HLA-specific
12 antibodies, currently, the microarray
13 technique is, without a doubt, the best assay
14 available. But, again, I would point out that
15 it is a complex system. And so, it requires
16 a certain amount of expertise and validation
17 to be able to interpret these results.
18 And I thank you for your
19 attention.
20 (Applause.)
21 DR. WESTHOFF: Thank you, Sue.
22 So, before the day is over, I am

1 going to put you and Christian on the spot to
2 say, how many spots on a multiplex do you
3 really need for transfusion care?
4 Our next speaker is Christian
5 Gabriel, who is with us from Innsbruck,
6 Austria. He is currently Medical Director of
7 the Red Cross Transfusion Service in Upper
8 Austria.
9 His research interest is focused
10 on various applications of next-gen sequencing
11 for blood and tissue products as well as
12 bacterial contamination and viral infection.
13 And welcome.
14 DR. GABRIEL: So, can I just get
15 the slides on? Oh, yes. Thank you very much.
16 So, I want to thank the FDA for
17 inviting me to come over here, over the great
18 pond, and to tell you a little bit about what
19 we have done so far in next-generation
20 sequencing.
21 I have, also, the intention to
22 tell about validation because we have this

1 method since June last year, accredited by the
2 European Federation of Immunogenetics.
3 So, coming back, what is the
4 purpose of next-gen sequencing? Well, it is
5 primarily these techniques have been designed
6 for biology and metagenomics and plant
7 genomics. So, these instruments have been
8 developed for high genomic complexity, long
9 ranges of repeated sequences, and multiple
10 overlaying genomes.
11 Next-gen is primarily designed for
12 total genomes, for high-throughput massive
13 parallel sequencing. And I think from the
14 manufacturer's side there was no primary
15 intention to produce these devices for medical
16 applications.
17 Now, looking into the literature
18 now today, you see that next-gen sequencing is
19 used in immunogenetics, in virology, for ultra
20 deep sequencing, as well as susceptibility
21 testing. Immunology, for instance, we are
22 tapping for IgH and TCRs; oncology, where

1 there are a lot of groups now looking for
2 multi-polymorphisms and areas which are
3 predictive for therapy or for regions of
4 resistance polymorphisms.
5 Now it is somehow in the medical
6 field using next-gen sequencing is like
7 plucking a daisy with a chainsaw. So, what we
8 are looking for is finding populations with
9 low numbers, but high relevance for the
10 patient's fate. For instance, in deep
11 sequencing for HIV resistance, it is quite
12 important. Or we are looking, also, for
13 higher sensitivity in comparison to Sanger
14 sequencing, detecting multiple polymorphisms
15 as in immunogenetics or compound mutations.
16 And we would like to find haplotypes.
17 So, we do not use these
18 technologies in the design they were built
19 for, but we still have an urgent need to
20 pursue this technology, especially in virology
21 and oncology. So, what is better, use a
22 system which is usually designed for plant

1 genomics or to use it also for medical
2 applications?
3 So, just to describe shortly how
4 this system works, it is somehow bringing
5 order to the alphabet soup. Usually, you have
6 an emulsion PCR and very many different DNAs
7 amplified, and then you bring the order by the
8 different systems can select out what patient
9 had what sequence.
10 However, what is very important,
11 if you are considering our next-gen sequencing
12 and validating these applications, is usually
13 the fact that you are combining a primer
14 together with an identifier which is
15 identifying the patient as well as the
16 adapter. So, this is a very long
17 oligonucleotide, and it usually doesn't work
18 all the times on every sequence. So, it is
19 not very easy to design these complex
20 oligonucleotides and it requires, also, an
21 extensive validation.
22 As well, you have to do a forward

1 and reverse sequencing, and there is also some
2 consideration ongoing from different systems.
3 If you look on this system, for instance,
4 which has a picotiter plate, and you have
5 different beads in this picotiter plate where
6 the sequences are read out, it can happen that
7 the density of the beads may vary from time to
8 time. It can happen that stretches of the
9 sequence which are homopolymers may not be
10 detected in a way which is required.
11 However, we get a different
12 readout, a so-called flowgram, in the four
13 system, for instance. And you can get a huge
14 amount of sequences which are read together in
15 one stack. And then, you can align them to
16 context.
17 And what we are using in
18 immunogenetics is a software which is now
19 already available for Sanger sequencing and
20 has been adapted for next-gen sequencing. So,
21 you get all the sequences which have already
22 fulfilled the quality reads and the quality

1 filters, and then, you can get the sequences
2 stacked together and find out in which amount
3 these sequences are coming together.
4 So, approximately here, you see a
5 DRB1070101 combined with a DRB11105. This is
6 the most common sequence read which has been
7 brought up, but sometimes it may happen that
8 you get also other sequences showing up in
9 this list. And that may happen because the
10 Taq polymerase has sometimes misreadings, and
11 these misreading may cause, also, different
12 sequences coming up, and they are also shown
13 here. So, it is also important to distinguish
14 between wrong and right sequences or common
15 and uncommon ones.
16 So, what can we accomplish from
17 next-gen sequencing in comparison to Sanger
18 sequences? Well, all sequences are shown.
19 So, you can see them. You can quantify.
20 So, you can get a percentage, how
21 many sequences are belonging to what type.
22 You have a higher sensitivity to find diverse

1 populations in low numbers. There has been a
2 recent publication on the transplantation
3 side. As well, you have the possibility to
4 make a high-throughput sequencing, but you
5 have, also, the possibility to use a
6 meticulous quality control on the sequences
7 you read.
8 So, what we have developed so far
9 is two types of next-gen sequencing in
10 immunogenetics. One is for registry and blood
11 donor typing, so-called low resolution for
12 digit typing, as we do for A-B-C-DRB1, DQB1.
13 It includes 9 to 10 amplicons for 100 to 200
14 patients on one plate.
15 The other one is, as we call it, a
16 very high-resolution typing for hematopoietic
17 stem cell transplantation. Ambiguity-free
18 includes also A-B-C-DRB1, DQB1, with 16 to 18
19 amplicons with a maximum of 50 patients in one
20 plate. Usually, we are typing about 10
21 patients in a smaller format.
22 So, the questions are arising:

1 well, is next-gen sequencing ready for
2 transfusion medicine? Is it reliable? Are
3 there any data? Is there usability in
4 different labs? And is there a good
5 scalability?
6 So, I just want to show you some
7 data of a so-called alpha site study which has
8 been done from various labs between Europe and
9 the U.S. What has been done there is that the
10 amplicon generation, as well as the chemistry
11 and the sequencing, was performed on the same
12 protocol in different labs.
13 What was really astonishing in the
14 first moment, because some labs had not that
15 year-long experience on next-gen sequencing,
16 was the fact that the agreement between the
17 labs on the sequences on the first run was
18 very high. So, you see from HLA, A was an 89
19 percent agreement between different sequences,
20 but also in DQA 100 percent.
21 And it is very interesting that
22 manual editing was required for the A and B's,

1 but for the other ones, it was very low. So,
2 this is a big difference to Sanger sequencing
3 where manual editing is quite common and very
4 often required.
5 So, I just want to show you, also,
6 data on our validation study we did in Europe
7 for the European Federation of Immunogenetics.
8 This is the setup of our amplicons we use.
9 So, for Class 1, we have on HLA, A exons 2, 3,
10 and 4; for B, 1, 2, 3, and 4; for C, 1, 2, 3,
11 4, 6, and 7; the exons 2 and 2 and 3 for DQ as
12 well for DR for exons 2 and 3.
13 If you look on the raw wells how
14 much data is extracted out of next-gen
15 sequencing, you can see that it is quite
16 varying. It might happen that you are using
17 usually research-use-only reagents. However,
18 it is a quite complex process which you are
19 using. It depends highly, also, on skilled
20 staff. And you see, also, that there are some
21 days where you don't get any readout because
22 something has happened in the manual process.

1 However, what is really counting
2 and what is really very important is to have
3 these so-called high-quality reads, so reads
4 you can really use for bioinformatics. And
5 this is quite drastically reduced if you have
6 not done a quite good normalization or
7 something has gone wrong in the lab.
8 And so, it needs some experience
9 to get next-gen sequencing running and to have
10 a robustness on an everyday process, to have
11 the same amount of sequences which can be read
12 out.
13 This is now the depiction of how
14 it is relating to the different alleles. You
15 see that is quite varying, that the raw wells
16 in comparison to the high-quality reads may
17 vary very much. This has to do with the
18 amplification efficiency.
19 So, it comes back to, let's say,
20 boots on the ground, that it is very important
21 in next-gen sequencing to find out the right
22 spot where you place your amplicon. It is

1 also very important to have a good combination
2 of these MIDs with the amplicons to retest it
3 all the time. So, for instance, you see here
4 the exon 7 in C has a very low amplification
5 efficiency, which is shown here.
6 The variation is quite high. So,
7 the CV rates for raw wells, high-quality
8 reads, are quite high. And so, it is
9 important to have a very high level of next-
10 gen sequencing reads, so you are on the safe
11 side. So, you can also compute all the reads
12 which are coming up after they have gone
13 through the quality filters.
14 So, we tested 173 samples in
15 Austria. All these samples have been
16 qualified as ambiguous. So, we took the hard
17 tour and we just have been looking on all
18 those samples which have required family
19 testing or which have been sent out by other
20 labs where ambiguities were not resolvable.
21 And so, what came up is that, from
22 these 173 samples, we have some insufficient

1 read counts, but it is interesting that there
2 are approximately 97 to 100 percent of these
3 samples tested successfully, and we had a
4 concordance rate with the combination of
5 Sanger sequencing and family typing of 100
6 percent.
7 So, this shows the power of next-
8 gen sequencing in very high resolution. This
9 graph also depicts, the dark-level graphs show
10 the ambiguity rates on these samples, and all
11 that is less colored is the remaining
12 ambiguities. So, it comes down that the
13 remaining ambiguity is about 0.6 percent down
14 to 0.9 percent in HLA Class C.
15 So, next-gen sequencing is
16 important. You need long reads, we heard this
17 morning already. So, this ensures higher
18 yield of usable reads. You have a better
19 readout quality. It gives more safety for
20 bioinformatics. So, you have a longer
21 overlapping read. And you also need high
22 coverage and a good design of primers.

1 This is, I think, also very valid
2 for virology. So, it is important to know
3 that next-gen sequencing cannot be transferred
4 just from Sanger sequencing.
5 This is just a short description,
6 how long it takes to get this running in a
7 lab. So, it takes about five days, including
8 with all this automation that you come up with
9 your results.
10 So, automation is very important.
11 So, we installed it and we sold it, also, with
12 a very impressive software tool in the
13 background.
14 So, I am coming now to RHD
15 sequencing and genotyping. What we are doing
16 in Austria is that we are doing donor
17 screening which is quite usual and
18 conventional, but we are doing, also, a full
19 PCR of Rh negatives and the big C's. After
20 that, if something is showing up that the Rh
21 is not negative, we do molecular typing by
22 sequencing or exon screening by a PCR which is

1 running over the 10 exons of the RHD.
2 So, it is a very interesting fact
3 that we have a lot of weak D's in Austria.
4 And so, we decided, also, to put this onto the
5 next-gen sequencing level.
6 Here again, you see depicted the
7 combination of the adapters, the MIDs, and the
8 target-specific primers, but in total we made
9 a short validation on 26 patients. So, there
10 are four MIDs required with 20 primers. So,
11 you have to design about 80 primers in total,
12 and that is a very long workload or a very big
13 workload.
14 So, this is how the amplicon
15 strategy was chosen. I just want to tell you
16 that was not very successful for specific
17 exons.
18 So, we selected 26 samples, 25
19 blood donors, one proficiency testing sample.
20 All patients were routinely tested on Sanger
21 sequencing and, then, subsequent RC genotyping
22 by NGS.

1 And it is also possible to use
2 scaffolds on these plates and to divide them,
3 so you don't have to design so many MIDs. You
4 can split them up.
5 Yes, here I show you the selection
6 of the patients we used. So, there were 14
7 different weak D, two D categories, two
8 partial D's, and a DEL, as well as RHD3. And
9 we chose, also, homo- or hemizygous or
10 heterozygous mutations.
11 So, 33 of 35 SNPs were correctly
12 identified. What is very important to know,
13 in next-gen sequencing, you can do clonal
14 sequencing. And there was heter- and
15 homozygocity in concordance with next-gen and
16 Sanger sequencing found out.
17 But we had one problem, that exon
18 2, the primer was not designed very specific
19 for RHD. So, heterozygocity with RC was also
20 caught here.
21 And this shows that it is very
22 important to design very high-quality primers

1 and to revalidate these primers in next-gen
2 sequencing.
3 But there were, also, two
4 polymorphisms missed in exon 3. We had a
5 failure in the forward-sequence in 18 of 26
6 patients. So, this is also important to
7 reiterate to see how the primers are working
8 and redesign where it is also important to be
9 done.
10 But, again, coming back to the
11 power of this method, we were able to
12 distinguish in one sequencing run a combined
13 weak D Type 4 and a D Category 3 Type 6, as
14 well, a Weak D Type 4 combined with RHD3. And
15 this is, I think, a very nice tool to make
16 reference sequencing for very problematic RHD
17 types.
18 Yes, another example was the
19 IVS3-19. It includes, also, 37 base pair
20 duplication. You can read it out quite
21 easily, and you can select it by next-gen
22 sequencing because you can split out two

1 different populations you see in your
2 sequences runs, and then, you know this is
3 also included in the sequence.
4 So, for blood grouping, you cannot
5 exploit the possibilities or the capacity of
6 the next-gen sequencing. So, it is like it is
7 a very nice tool to clone sequencing. You can
8 possibly put up to 500 patients if you are
9 going for rapid sequencing to a plate.
10 HLA typing, I think we came to the
11 conclusion that the possible maximum is 48
12 patients with 14 amplicons, so to be in the
13 safe range for coverage. And there is some
14 room open now for ABO genotyping and to amend,
15 also, the amplicons for blood group
16 genotyping.
17 So, coming to the conclusion that
18 primers from the Sanger sequencing are not
19 easily transferrable. All these primers needs
20 new designs. Clone sequencing by next-gen
21 sequencing enables the resolution of
22 cis/trans-linkage of SNPs as well as clear

1 identification of duplications.
2 You can also clearly identify de
3 novo alterations. That is quite clear. And
4 next-gen sequencing is suitable for high-
5 throughput genotyping. It is providing us
6 also results with higher quality.
7 And with this, I want to conclude.
8 This is my team which has been working now for
9 five years on this project, and I want to
10 thank you for the attendance.
11 (Applause.)
12 DR. WESTHOFF: Good timing.
13 Our next speaker is Gorka Ochoa.
14 He is Research Manager at Progenika in
15 Medford, Mass. He studied medicine and
16 surgery at the University in the Basque
17 country in Spain, and he received a Ph.D. in
18 microbiology and immunology from the
19 University of Southern California, and did
20 postdoc work at the NIAID in Rockville.
21 And like I said, he is the
22 Research Manager with the Progenika.

1 Gorka?
2 DR. OCHOA: The presentation is
3 going to start with a brief introduction to
4 the strengths and limitations of molecular
5 genotyping. It is going to be followed by a
6 description of two Progenika products that are
7 going to be released shortly for genotyping
8 red blood cell and platelet antigens.
9 Then, I will go into the bulk of
10 the presentation, which is about the procedure
11 to be followed for genotyping with these two
12 products. Then, I will mention the content,
13 that is to say, the antigens interrogated by
14 both tests. And I will conclude with some
15 performance characteristics of the products
16 and their throughput.
17 Much of this has already been
18 said. I will go quickly through it.
19 Key strengths of molecular typing
20 at the clinical level include:
21 It is not limited by reagent
22 availability, and this applies not only to

1 FDA-approved reagents, but commercial reagents
2 in general. It is not affected by interfering
3 antibodies or by recent and multiple
4 transfusions. And it is not challenged in the
5 way serology is by difficult antigens, such as
6 weak or partial forms.
7 At the operational level, it is
8 multiplexing nature in a single PCR. You can
9 amplify everything that needs to be amplified
10 to determine numerous antigens. And the
11 coverage in the tests that I am going to
12 present is about 40 minor and rare antigens.
13 Another advantage that has been
14 briefly mentioned this morning is the
15 possibility to standardize this technology as
16 opposed to the agglutination assays that do
17 not lend themselves to so much of a
18 standardization procedure.
19 And finally, molecular typing is
20 more cost-effective as you increase the
21 antigens that you interrogate.
22 Among the limitations of the

1 molecular assays, these are common to most, if
2 not all, platforms, including sequencing, and
3 they refer to polymorphisms at primer or probe
4 binding sites; post-transcriptional or post-
5 translational modifications that affect the
6 phenotype -- these are very rare -- and
7 finally, unreported variants.
8 These are the two products I am
9 going to be referring to during the
10 presentation. The first one, ID-CORE XT,
11 interrogates 37 red blood cell antigens in 10
12 blood groups, and ID-HPA XT interrogates 18
13 encoded by 12 antigens. Both tests for
14 research use only.
15 Now I start the bulk of the
16 presentation. This is the workflow of the
17 assay, and I have represented it as a tree
18 with two branches that converge into late
19 steps and move from hybridization at that
20 point to data analysis.
21 I am going to start with the top
22 branch of the tree, which are two steps

1 devoted to preparing the sample DNA. And
2 these two steps are performed by the platform
3 user.
4 The first step is DNA extraction
5 from whole blood, which, in turn, involves
6 three steps. The first one is cell lysis, and
7 this is usually applied to blood collected in
8 EDTA, although other sources of DNA can also
9 be used.
10 The second step is the critical
11 step in the purification of DNA and involves
12 binding of the lysed material to a matrix that
13 is specific for DNA. The key element in that
14 specificity is the spin column.
15 And finally, the DNA is eluded
16 after removal of all unbound biomolecules by
17 passing an aqueous solution through the
18 column. This process can be done manually or
19 for higher throughput it can also be
20 automated.
21 The second test to be performed to
22 prepare the sample DNA is amplification. And

1 this is done in a multiplex PCR that consists
2 of the following elements: obviously, the
3 genomic DNA that has been purified from blood;
4 DNA polymerase; a primer mix, and that mix
5 contains multiple pairs, each pair specific
6 for one segment of one of the many blood
7 groups interrogated by the test.
8 In addition, the PCR amplification
9 step is used to introduce into the DNA a
10 biotin molecule. And the function of that
11 biotin will be evident in a few slides.
12 The bottom part of the tree deals
13 with two other elements of the assay, the
14 beads and the probes. These are prepared by
15 Progenika and provided as part of the product.
16 The probes are short
17 oligonucleotide sequences, 17 to 27
18 nucleotides in length, and they are designed
19 to bind to either the common allele or the
20 rare allele at each one of the polymorphic
21 positions. Since there are in the red blood
22 cell test 29 polymorphic positions, there are

1 29 primer co-pairs as well.
2 The second element is the
3 fluorescent beads which are manufactured by
4 Luminex. These are polystyrene micron-sized
5 beads that are filled with a combination of
6 two fluorescent dyes, and those two dyes are
7 mixed inside the beads in different
8 proportions, such that up to 100 different
9 bead species or bead types can be generated.
10 Each one of those bead species or
11 bead types is conjugated to one type of probe.
12 So there is a one-one correlation between the
13 two.
14 The binding of the probe to the
15 bead is performed by a simple EDC chemistry,
16 and the product of it is a stable bond.
17 Finally, the two elements, the
18 prepared DNA and the provided bead
19 proconjugate, are put together at the
20 hybridization step. The hybridization step is
21 a very simple incubation at 52 degrees for 30
22 minutes in a heat block or in the thermal

1 cycler. And what comes out of it is a
2 fluorescent bead conjugated to a nucleotide
3 probe, bound to a purified DNA that has a
4 biotin label on it.
5 This complex is incubated with a
6 second fluorophore, phycoerythrin, which binds
7 to biotin through streptavidin moiety. The
8 two fluorophore complex is, then, analyzed by
9 a Luminex-manufactured flow cytometer. It is
10 a special type of cytometer that detects both
11 the inner bead fluorescence and the DNA-bound
12 fluorescence.
13 The analyses used by this
14 equipment is local, onsite. The cytometer
15 performs three specific functions. The first
16 one is shown in the top diagram, and it is
17 about resolving single beads from doublets
18 from particles in suspension, just as you
19 would do in normal flow cytometry.
20 The second step is in the bottom
21 graph. What it does is look at the inner bead
22 fluorescence to discriminate the different

1 bead species or bead types. Each one of those
2 small clouds that you see in the bottom plot
3 corresponds to one bead species.
4 And the third function of the
5 cytometer is obviously to detect the DNA-bound
6 fluorescence. What you see on the screen is
7 the raw data that comes from quantification of
8 that phycoerythrin fluorescence.
9 In the first column, you have a
10 list of samples, and in the rest of the
11 columns you have the fluorescence that
12 corresponds to each one of several bead types.
13 These values, these raw data values, are,
14 then, used by a proprietary software that
15 performs data analysis with them. And from
16 that data analysis, the software issues one of
17 three of types of calls: homozygous common,
18 heterozygous, or homozygous variant for each
19 one of the polymorphic positions.
20 Just to get a more graphical view
21 of those three calls, I have included a graph
22 that lists on the X-axis a list of samples and

1 on the Y-axis a ratio that is calculated from
2 the raw data. When this ratio is high, as in
3 these cases, the samples are assigned
4 homozygous common general-type call. When the
5 ratio is intermediate, it is a heterozygous
6 call, and when it is low, it is a homozygous
7 variant call.
8 Data interpretation by the
9 software is all based on information in the
10 scientific literature. Both steps, the
11 inference of an allele from a polymorphism
12 result and the phenotype prediction from the
13 general type, are based on that information.
14 This is what you would see as a
15 user of the platform for one of the red blood
16 cell antigens, in particular, RHCE. In this
17 column, there is a listing of all the alleles
18 that the test interrogates. In this column,
19 the particular alleles that have been detected
20 in your sample. The next column, again, is a
21 complete listing of all the interrogated
22 phenotypes. And the last column, the

1 particular phenotypes detected in your sample.
2 The process takes about four hours
3 and 10 minutes, as you see in this diagram.
4 Most of this time is taken by the PCR set,
5 which is about 50 percent or 60 percent of the
6 time.
7 The total hands-on time of the
8 process, in the bottom bar, is about 30
9 minutes, which is taken by PCR setup,
10 hybridization setup, and labeling setup steps.
11 And now, the content of the tests.
12 The red blood cell antigen test interrogates
13 all the antigens that you see listed there.
14 These antigens have been selected based on
15 both their clinical relevance and the
16 population frequency.
17 As examples, V, hrs, VS, hrv are
18 typical antigens of African-American
19 populations that are relevant in multi-
20 transfused. And another example is Mi
21 antigen, which is more typical of Asian
22 populations.

1 There is a total of nine RhC
2 antigens, six Kell, six MNS antigens, four
3 dombrock, and two apiece for the rest of blood
4 groups. For the HPA antigen product, the
5 number of antigens is 18. And the throughput
6 of both assays is the same, 48 samples per
7 run. You can do two runs in an eight-to-nine-
8 hour shift, which means about 100 samples per
9 day, which translates into approximately 2,000
10 per month.
11 The performance characteristics of
12 these products are being evaluated as I speak.
13 So, what I am going to show you in the next
14 two slides are the performance characteristics
15 of the predecessor assays, which were called
16 ID-CORE+ and ID-HPA.
17 The ID-CORE+ study made use of
18 1,000 pedigreed samples, and the agreement
19 percent was 100 percent for most of them,
20 except for RHCE and MNS antigens. RHCE,
21 highly homologous to RHD, which often leads to
22 miscalls. And the same for MNS, due to high

1 sequence homology between the different
2 glycoforms A, B, and E.
3 The platelet product reached 100
4 percent agreement for all antigens with a
5 smaller sample size.
6 Thank you.
7 (Applause.)
8 DR. WESTHOFF: Thank you, Gorka.
9 And last, but not least, Greg
10 Denomme from BloodCenter of Wisconsin, who is
11 leading their efforts to establish high-
12 throughput molecular genotyping of donors and
13 patients.
14 Greg is a clinical lab scientist
15 with training in immunohematology from the
16 Canadian Society of Medical Laboratory
17 Science, and he has a Ph.D. in microbiology
18 and immunology.
19 Greg?
20 DR. DENOMME: Well, thank you,
21 Connie, and the Scientific Advisory Committee,
22 for the opportunity to speak. I appreciate it

1 very much. So, let's get started.
2 A little bit different slant. You
3 have heard a lot about red cell genotyping.
4 So, I am going to focus on two aspects, the
5 program at BloodCenter, how it evolved,
6 including its throughput capacity and some
7 validation discussion on TaqMan technology,
8 and slow down a little bit to tell you about
9 our converting ACGT into phenotypes using a
10 rules engine, and the electronic handling that
11 wouldn't be possible to handle all the data
12 without thinking ahead and planning this out.
13 And then, I will spend a bit of
14 time identifying efficiencies that can be
15 realized because this is very rapid and very
16 high-throughput genotyping; give you our
17 experience on the uncommon and rare donors we
18 have found.
19 We are talking about a process
20 that is probably 30 hours in its total
21 turnaround time. Inventory optimization and
22 impact, a little bit there. I will give you

1 a rundown of our phenotype and genotype
2 discrepancies, which you have already heard.
3 I don't think it is going to be any different.
4 And then, something that caught our eye
5 initially, coming back to why we chose the
6 program, is the design flexibility.
7 So, awhile ago, BloodCenter
8 recognized that their phenotyping program --
9 it is prior to my arrival -- is lacking. It
10 was on an ad-hoc basis to screen donors for
11 some of the in-demand lower uncommon
12 phenotypes.
13 And the problems you are well
14 aware of. On the left is some 15 or more
15 reagents. I count five methods at
16 BloodCenter, and we were spending an awful lot
17 of labor, time, and money on trying to keep up
18 with the demand for antigen-negative units.
19 And on the right, some of the
20 trials and tribulations. Reagents are
21 expensive, as Connie outlined. Some of them
22 are not available; some of them, they are

1 available and, then, become in short supply.
2 And things like anti-S probably 10 or 12 years
3 ago, we experienced a shortage. Things like
4 anti-Jsa, dombrock A are simply difficult to
5 find.
6 And the more we phenotyped, the
7 more we put demand on the manufacturers. So,
8 if we did this, what I am presenting today, as
9 phenotyping, we wouldn't have been able to get
10 the reagents to do it, even if we could do it.
11 Hospitals, as you well know, do
12 their own phenotyping. We can't translate
13 those results back to the donor. So, that
14 information is lost and it is grossly
15 inefficient, as you can all imagine.
16 We chose the OpenArray system by
17 LTI. It is a nanofluidic chamber. It is a
18 small, stainless slide. It met our needs
19 because of the throughput capacity. There are
20 48 matrices. Each matrix has 64 wells. It is
21 an open-well system. That is why it is called
22 OpenArray.

1 In our design, with 32 SNPs, each
2 subarray can handle two donors. So, the upper
3 32 wells is committed to painting DNA for one
4 donor -- I will show you that process in a bit
5 -- and the lower 32 for another donor.
6 So, in this configuration, we are
7 able to test 91 donors. We have four controls
8 per array and one null template control.
9 We found that it was readily
10 scalable in its current design. We are
11 testing 1500 donors in an eight-hour shift,
12 with the capacity to type 4,000 or more donors
13 per week -- or sorry -- per day, if we up the
14 equipment.
15 So, the advantage of the stainless
16 steel wafer, as we call it, is it is comprised
17 of 3,072 open wells. We are dealing with nano
18 volumes. We are dealing with solution-phase
19 assays. No gels, no additional handling.
20 And really, what is happening is
21 that there is parallel testing in each well.
22 So, each well is doing something different

1 than the well beside it. A simple array with
2 its 48 submatrices is the equivalent of 384
3 well plates or 32 96 well plates. The
4 advantage here is we can template things off
5 of things like 384 and 96 well plates, as you
6 will see.
7 We call this deconvoluted testing.
8 It is that validation is by assay rather than
9 a multiplex validation. There is no
10 multiplexing. So, once we have validated an
11 assay, it is put on the chip or the array.
12 And so, it is taking up real estate or you can
13 take that off and put something else in that
14 spot that has been validated. So, it really
15 is just deciding how you want to spend your
16 real estate on the chip.
17 The proof-of-principle was
18 performed in 2008, published in early 2010,
19 427 donors, a total of 260 phenotype/genotype
20 comparisons, and we have found four
21 phenotyping errors, much like you have heard
22 in the past. A Duffy B was, in fact -- the

1 donor was Duffy B negative by serology, found
2 to be Duffy B positive; a DOA negative by
3 serology -- this was an unlicensed reagent --
4 a DOA positive by DNA, and JKB.
5 So, the Duffy B and the DOA were,
6 in fact, in error, which leaves us the
7 potential for hemolytic transfusion reaction.
8 The JK would not have -- it was the reverse of
9 what you would think ordinarily. So, it was
10 listed as JKB positive. It was, in fact, JKB
11 negative.
12 And then, we were able to
13 determine from this very small population two
14 genotypic variants, a Duffy X and a JSA MOD.
15 So, DNA, as we have been saying for the last
16 hour and a half, is a very powerful tool.
17 Currently, we are using this as a
18 screening tool. So, we screen lots of donors.
19 Eventually, everything gets phenotyped when it
20 is needed for a patient. So, nothing is a
21 test of record. And we generally, for an
22 assay development, we rely on the serological

1 controls in order to set up an assay. We
2 typically have three homozygous donors for the
3 common allele, three heterozygous, and three
4 homozygous donors for the rare allele, where
5 possible.
6 Workflow is along the lines of,
7 once a sample is barcoded, then we follow that
8 sample through template worksheets until the
9 data is output by the OpenArray instrument.
10 So, samples are barcoded. We have chosen
11 repeat samples, and we defined a repeat donor
12 as a donor who has given blood at least three
13 times in the last three years, at the time of
14 donation at least three times, and that one of
15 those donations was within the last 12 months.
16 That donor is selected, lined up
17 for DNA extraction by sample barcode into a
18 worksheet, extracted on 96 well templates, and
19 then, master-mix preparations for the
20 OpenArray are built on a 384 plate. And so,
21 96 well plates generates four open arrays.
22 The open array plate loading is

1 done by an instrument. It is not done
2 manually. And the cassettes can be loaded by
3 instrument. There is a phase of cycling, much
4 like any other PCR. And then, those arrays
5 are moved to an instrument that images the
6 wells. And we are working with a two-color
7 FAM-and-VIC system for each allele. And then,
8 the entire array also has a ROX image, so that
9 we can tell at least DNA is loaded into the
10 plates.
11 Once we do clustering, which is
12 tech-operated, so this part is not black box,
13 the techs actually review the results, look at
14 clusters, and decide on clustering, and to
15 generate an output file. That output file is,
16 then, dropped into a folder, and during the
17 night, the data, the ACGs and Ts are put
18 through an electronic, what we call, rules
19 engine that does predicted phenotypes.
20 The next morning those phenotypes
21 are reviewed. If there are no calls or there
22 are flags by the reporting system, that donor

1 can be excluded from going into the database
2 and be repeated at another time.
3 So, what is important during the
4 actual testing is the amount of operator
5 oversight that we designed. I put this into
6 three different modules.
7 The first is sample selection, as
8 I mentioned, it is templated. We extract DNA,
9 and each DNA plate can be evaluated for DNA
10 integrity, either by the concentration of DNA
11 or the 260/280 ratio. So, at any time, we can
12 look at the performance of our DNA extraction
13 prior to setup.
14 The second is the open array
15 setup. It is still templated on the sample
16 barcode and the DNA template of 96 well
17 plates. After the run, we can validate the
18 run by looking at both the FAM-and-VIC results
19 for our SNPs, and then we can evaluate the
20 entire open array for DNA painting through
21 what is called a ROX fluorescent image. We
22 can decide if that run was valid or not.

1 After the run is done, there is
2 tech evaluation of clustering set by a set of
3 rules. After clustering, then we can look at
4 DNA controls and determine whether our
5 controls are acceptable or not.
6 Once that is done, then the output
7 file is submitted to the rules engine. And
8 the next morning we look for computerized
9 exception reports of specific samples. We can
10 go in and delete samples that have
11 unacceptable calls or are flagged for other
12 reasons.
13 And then, the data is parsed and
14 it is put into the database. Part of the
15 parsing is that we look at any phenotypes that
16 are on the donor, and we also get a report if
17 there is a phenotype and a genotype
18 discrepancy.
19 So, our first pass in 2010 was to
20 try to get 30,000 donors typed. We hit around
21 28,000 early 2011, and it was a split of
22 repeat donors who had self-identified as

1 Caucasian. It is about 65 percent of all
2 donations at the BloodCenter of Wisconsin. In
3 a year, we get approximately 2,000 other
4 ethnicities donated.
5 And what we do is, using the
6 allele frequencies, we can determine whether
7 or not there are the number of, what we call,
8 screened or selected inventory. These are
9 desired types. If we count all donors, you
10 see that there is, in fact, nearly 500 donors
11 are 2R2 from this 28,000 Caucasian donors
12 type. And if you wanted to find donors that
13 were multiple antigen-negative for SKW/JKA, as
14 an example, we have 16 donors and believe that
15 this is the new O negative.
16 So, in total, if we wanted to look
17 at donors which we hold in our inventory,
18 multiple antigen-negative, they can go to a
19 patient who has one antibody or all five. We
20 are able to reduce the inventory we are
21 holding by the immunohematology reference lab,
22 and approximately 400 donors passed this

1 litmus test. A variety of donors had rare
2 types. If we count VS homozygous as a kind of
3 HRB weak or HRB negative, we had a vast number
4 of donors that qualified as HRB weak, for a
5 total of nearly 300 rare donors for almost 700
6 donors from this analysis.
7 So, what we did in 2010, starting
8 July the 17th, we typed donors using the
9 OpenArray system from end-to-end processing
10 and handling. By the end of 2010 -- sorry --
11 by September 23rd, we had typed 25,000, nearly
12 25,000 donors. And then, we went into what we
13 called a maintenance testing in which we are
14 testing approximately 12 percent of our donors
15 that are repeat donors each year -- sorry --
16 12 percent over the total database.
17 And then, in 2013, we asked the
18 question, on the basis of how many donations
19 per week, how many had a red cell genotype?
20 And we are holding steady. So, we believe our
21 maintenance typing is keeping our donor
22 database healthy at about one out of every

1 three donors having a complete genotype.
2 The impact is that, from its
3 inception after 2010, we have increased nearly
4 two-thirds the number of donors we have been
5 able to share on the ARDP program, or the
6 American Rare Donor Program. We have 293
7 different rare antigen-negative donors that we
8 have been able to recruit again: Colton,
9 Cromer, Lutheran B, Lutheran 8, little k, YTA.
10 We have not ever serologically-
11 screened donors for, say, little e neg or
12 little c negative. Again, we had 141 donors
13 that are HRB weak, HRB negative. These donors
14 can actually be used for sickle patients who
15 happen to be VS homozygous.
16 So, we are getting into those
17 areas where supply and demand are starting to
18 meet. Twenty-two dombrock variants from Holly
19 and Joseph. We expanded our O negative
20 inventory to include DOA neg and DOB neg. So,
21 we have a number of O negatives that we can
22 call on, 266 group O R2R donors, and we are

1 allowing our hospitals and our outlying blood
2 center repository to hold our 1R1 units.
3 I don't think you can see this
4 from the back, but our phenotype and genotype
5 discrepancies. Just look at the black boxes.
6 The transcription error, as Connie has talked
7 to, addressed in the past here, we are finding
8 that we have got about a 2-to-1 ratio of
9 transcription errors using a manual system to
10 phenotype donors in the past. And
11 approximately, out of every three
12 phenotype/genotype discrepancies, 1 in 3 were
13 detecting variants from other various blood
14 group systems that you would know.
15 As well, on our chip we have a
16 nucleotide that helps resolve the hybrid
17 partial C. So, it gives us a clue whether a
18 D gene is present or some kind of gene is
19 present in our Rh negative donors. To date,
20 we have 53 donors that we have to evaluate, 53
21 Rh negative donors who appear to have a D
22 gene. Seven have been confirmed to have a D

1 gene. They each had either an Rh big C or big
2 E, or both, present. And we broke down the
3 frequency amongst our donors, with the
4 African-Americans being the highest number to
5 have a gene and, then, by Rh complete
6 phenotype.
7 So, we believe that, once these
8 donors return, we don't recruit them to
9 confirm. We wait for them to return. But a
10 lot of these will be resolved on the basis of
11 nucleotide 455 of RHD.
12 The advantage of the system, as I
13 mentioned earlier, it is design flexibility.
14 We are currently using 32 SNPs. It predicts
15 43 antigens, 37 of the common sort of almost
16 direct one-to-one relationship to the SNP, and
17 then variance within Rh, Duffy, and the JK
18 system.
19 As I mentioned, we can add in a
20 SNP that can resolve the partial C, and we
21 have developed additional targets in RHCE,
22 UVAR, TC, Indian, and the HPA. These can be

1 swapped in and out, depending on the chip
2 design that you would prefer for your donor
3 population.
4 It doesn't affect validation
5 because each of these are validated
6 independently. And we can show that, no
7 matter where they are loaded, they still
8 perform as they did in the initial validation.
9 So, the flexible design has add-on
10 capabilities and expands the number of donors
11 that can be tested. So, 32 assays, 43
12 antigens, we are testing about 90 donors per
13 array. It covers everything from the
14 comprehensive antigens to uncommon and rare
15 types, and it is really used for all of our
16 donors.
17 We can trim that back to 16 SNPs
18 and still include some common antigens and Rh
19 and MNS variants or we can pare it down to
20 literally 8 SNPs, 14 antigens, and it really
21 does the yeoman's work of the antigens within
22 the Rh, Kell, Duffy, and Kidd systems.

1 So, in summary, the rapid, high-
2 throughput red cell genotyping, per day we
3 have got an end-to-end process design. It is
4 scalable to 4500 donors per day. The large-
5 volume testing in a short time created a donor
6 database that remains robust, and we are able
7 to do maintenance or residual typing at a very
8 low cost.
9 Parsing the data and comparing it
10 to historical phenotype data gives us what I
11 call quality assurance gains. We have found
12 transcription errors and variant alleles.
13 Because of its flexibility and design, as new
14 clinically-relevant SNPs are discovered, we
15 can swap them in and out of the system without
16 going back and doing a full multiplex
17 validation. So, things like JR, Lan, and Val
18 can be added on incremental without a large
19 effort. And the nucleic acid chemistry is
20 compatible with infectious disease testing.
21 I believe that is all I have. So,
22 thank you very much for your attention.

1 (Applause.)
2 DR. WESTHOFF: Thank you, Greg.
3 We are scheduled now for a 15-
4 minute break. We are about 15 minutes behind.
5 So, if we could return just a little before
6 5:00, and we have some questions to cover, but
7 we also want some discussion and questions
8 from the floor. So, I do hope you can stick
9 around and come back and join the panel in
10 about 15 minutes.
11 Thank you.
12 (Whereupon, the foregoing matter
13 went off the record at 4:44 p.m. and went back
14 on the record at 5:04 p.m.)
15 DR. WESTHOFF: We have some
16 questions first, and I will be asking the
17 panel for their opinion. And certainly, we
18 are very interested in your opinion and any
19 other questions.
20 So, if we could have the
21 questions?
22 Okay. Multiplex testing involves

1 the determination of multiple antigens and/or
2 polymorphisms. So, let's think about how
3 comprehensive a routine testing platform would
4 need to be. In other words, is there a role
5 for low-resolution, high-resolution platforms
6 in red cell testing and platelet testing, just
7 as in HLA testing? So, how comprehensive
8 should a routine platform be?
9 I would like the opinion of our
10 esteemed panel members, starting from that
11 end. Okay?
12 DR. LEFFELL: Well, I think for
13 HLA you are dealing, as I tried to point out
14 in my talk, with a very complex system. You
15 could boil the determinants you would need
16 down to an antigen level and have roughly 100.
17 But the problem is you have to deal with
18 multiple allele groups that encode those
19 different antigens. And so, it would be very
20 difficult to set a set number of sequences or
21 polymorphisms that you would need to define to
22 limit, to even reach an antigen level.

1 DR. FLEGEL: But the situation is
2 quite different for blood groups because the
3 clinical consequences are well-defined for the
4 most part. And therefore, I think one can
5 define various groups of antigens or alleles
6 that should be tested, and we have a good one
7 that for the most part -- they are still
8 developing things. D is certainly not fully
9 understood; Cc/Ee, now we have got a better
10 understanding when it comes to sickle cell and
11 thalassemia, for example. But, generally, we
12 do know the number of alleles that need to be
13 part of that set.
14 The multiplex testing, it depends
15 on how you define that. I think the model
16 approach, as, for example, represented by Greg
17 Denomme's platform in Milwaukee, is in the end
18 the better thing because it is easier to
19 handle at the validation level.
20 DR. WESTHOFF: I would just like
21 to interject your opinion about population
22 variation and differences in different regions

1 of the country, how one would deal with that.
2 DR. FLEGEL: Well, there are
3 differences, but in the end in the current
4 situation, probably one would, my approach
5 would be to provide one platform for
6 everybody. That is the answer.
7 DR. WESTHOFF: Dr. Gabriel?
8 DR. GABRIEL: Well, I think for
9 red cells you need some sort of screening
10 assay, and multiplex screening assays which
11 are rapid, cheaper than usual assays, are I
12 think in this field very important.
13 And I think it is also very
14 important to have modeler system which enables
15 different sizes and can be adjusted to
16 populations.
17 On the other hand, the trend is
18 clearly going into next-gen sequencing, and
19 this is a very different approach. It should
20 be considered that next-gen sequencing needs
21 very much more validation and very much more
22 sophisticated bioinformatics. And it takes a

1 longer time to get it into routine business.
2 So, it is also very important to
3 see what is going on in this field and maybe,
4 also, here it is a sequential approach that
5 you go for, let's say, smaller variations and
6 graft them onto the very high-resolution
7 typing.
8 DR. DENOMME: As Bill noted, what
9 we know about blood group antigens is well-
10 defined. From my experience, the modular
11 approach has been very advantageous. For
12 example, after some 25,000 donors, we had
13 found a few heterozygous JK, the
14 Polynesian/Finn Jk null. So, we removed that
15 from the chip and put something else in.
16 So, there is this need to ensure
17 that the rare types nationally are taken care
18 of through some program. You know, the ARDP
19 is doing well in that regard.
20 But, for patients, I think it is a
21 little bit different. If they were to have a
22 JK null allele, we would like to know that

1 before we think that they are JKA positive or
2 JKB positive. So, there is a little bit
3 different approach.
4 And then, if I look at what we
5 have done, this wouldn't be suitable in some
6 parts of the world because, for example, it
7 doesn't include Miltenberger, which is very
8 common in Southeast Asia.
9 So, when I look at the demand, it
10 really boils down to the common antigens we
11 all see on antigrams. There is a need to
12 ensure, because it is so incremental, that we
13 can find those recipients who should be
14 Lutheran B negative, and that would alert us
15 prior to transfusing them and would be easy to
16 provide blood.
17 And the last point is, for
18 example, the Cellano negative, we no longer
19 freeze them. We keep 10 or 12 in our
20 inventory and rotate them.
21 So, we are seeing gains where
22 something a few years ago was considered a

1 need to have, and to have nearby, to now we
2 are confident that at anytime, even a 20-unit
3 liver transplant, we would be ready with the
4 appropriate antigen-negative units.
5 DR. OCHOA: What goes into a
6 genotyping test should be a balance between
7 what that test should interrogate and what the
8 test can interrogate. What I see as what
9 should go in the test is what is clinically-
10 relevant and what has a sufficiently-high
11 population frequency.
12 What can go in the test is limited
13 by the test itself. For instance, the Luminex
14 platform I talked about is limited to 50
15 polymorphisms because each polymorphism takes
16 two beads to be interrogated.
17 Another limitation is the
18 multiplex PCR. You amplify simultaneously 20
19 amplicons, perhaps 30, but, as you increase
20 the number of amplicons above those numbers,
21 then the PCR becomes very unstable. And that
22 also places a limit to the number.

1 So, I would say make a listing of
2 antigens based on their clinical relevance and
3 on their population frequency, and then start
4 testing those antigens from the top to the
5 bottom and feed them into your test, as long
6 as the test permits and as long as your
7 amplification is sufficiently stable.
8 DR. WESTHOFF: Any other comments
9 from the floor? Just go to the microphone.
10 But Greg brings up an issue that
11 we had talked about early in this technology
12 in that, do we want a different platform for
13 patients versus donors? And do we want the
14 ability to turn something off and, then, turn
15 something else on?
16 One of the comments from the
17 manufacturers had been it doesn't really save
18 that much to turn something off and on, and
19 that streamline testing is usually the cheaper
20 way to go. But, certainly, this varies with
21 different platforms.
22 Okay. What are the benefits of

1 next-generation sequencing in blood cell
2 antigen typing for HLA or red cells? And I
3 think we have heard a little bit about it, but
4 if you would like to expound?
5 DR. LEFFELL: Again, I think the
6 biggest advantage of next-generation
7 sequencing is that it will deal with the
8 problem of the ambiguous heterozygote.
9 DR. WESTHOFF: And by that, you
10 mean linkage association?
11 DR. LEFFELL: Well, yes, it is
12 linking the different polymorphic sequences,
13 but it is setting the phase for those, so that
14 you can resolve those heterozygous
15 ambiguities.
16 And that is going to require
17 substantially longer read lengths, which we
18 can currently do with the Sanger-based,
19 sequencing-based typing, but, again, it is
20 heterozygous. And so, we have to use a group-
21 specific amplification to resolve those
22 ambiguities.

1 I think the limitations -- and
2 Christian Gabriel can speak to this much more
3 expertly than I can -- but it is going to be
4 establishment of the longer read lengths, a
5 turnaround time in bringing it to a cost-
6 effectiveness that can be applied clinically
7 that will bring HLA.
8 But I think I absolutely agree
9 with his earlier statement that it is the way
10 that the resolution of HLA is going to have to
11 go.
12 DR. FLEGEL: Yes, reading length
13 is the biggest problem because for a couple of
14 block group antigen testings we need long
15 readings. Otherwise, we cannot make any
16 prediction.
17 Also, the next-generation
18 sequencing, as great and important as it is,
19 should not prevent us from applying the
20 current technology to patient care, because
21 they actually can benefit right now from the
22 technology that is available now. We do not

1 have to wait for next generation. That is two
2 different topics.
3 DR. GABRIEL: I think there are
4 some improvements ongoing now with the read
5 lengths. We are currently now sequencing read
6 lengths up to 900 basis. So, now we have a
7 new system where we are connecting exons
8 together and have the internic areas.
9 Next-gen sequencing I think, as we
10 said, has very great advantages in this field
11 of resolving ambiguities. It will bring a
12 benefit for stem cell transplantation.
13 However, currently looking at
14 next-gen sequencing in a model that you would
15 like to see it as a high-throughput sequencing
16 system which is, let's say, some sort of other
17 multiplex where you test hundreds of patients
18 or donors on one sequencing system, I think
19 that is taking too much time. The other
20 multiplex systems like bead arrays are more
21 appropriate for that.
22 And next-gen sequencing also

1 requires a long time of preparation before you
2 can go for sequencing. So, it is somehow
3 limited to, let's say, more sophisticated
4 questions of how to resolve the genetic
5 composition of the patient or a donor, but not
6 for a screening purpose.
7 DR. DENOMME: Well, I see the
8 next-gen sequencing advantage in those blood
9 group systems that are bigenic or, say, Rh,
10 MNS, these kinds of things. Probably a longer
11 read length will make me feel a little more
12 comfortable making assumptions like someone
13 who is Duffy B homozygous and they have the
14 GATA box mutation. I know they are Duffy A-
15 neg, B-neg, but if they are Duffy A-pos, B-pos
16 at 125 and have the GATA, there is this small
17 risk in the back of my mind that they may be
18 slightly different. Sooner or later, this
19 will happen. Next-gen sequencing can resolve
20 that, and probably very useful in the Kell
21 blood group system, that kind of thing.
22 DR. OCHOA: Along the lines of a

1 comment that Greg just made, one of the
2 challenges of other genotyping technologies
3 such as the targeted genotyping of several
4 platforms, one of the challenges of these
5 platforms is the more complex blood groups,
6 ABO and RHD, because of the large extent of
7 variation within those groups. That is also,
8 I believe, a terrain where next-generation
9 sequencing could make a difference.
10 DR. WESTHOFF: And one thing I
11 would mention about next-generation
12 sequencing, though I think it would be very
13 helpful for a confirmatory or a high-level
14 resolution testing, we don't know the impact
15 of lots of SNPs that we will run into that
16 maybe aren't associated with a phenotype or a
17 potential target for an immune reaction.
18 So, I think in some systems it may
19 be a little more information without a
20 clinical picture, then we might know what to
21 do with it or how to interpret.
22 All right. Yes, Sue?

1 DR. LEFFELL: I just wanted to add
2 one other comment, which really sort of
3 elaborates on something that Christian
4 mentioned. I think one of the things that
5 really has to be considered in a multiplex
6 system that would incorporate HLA and
7 potentially with blood group antigens is what
8 level of risk are you willing to accept if you
9 ignore alleles.
10 By this, I mean for HLA, for
11 support for transfusion or in trying to type
12 donors and recipients to analyze HLA-specific
13 antibodies, you really need an antigen level.
14 And you can make some assumptions which I
15 tried to point out on the most common
16 assignments that you can get with a lower-
17 resolution typing. But you will be ignoring
18 the possibility of rare alleles.
19 And so, that is a decision I think
20 that the laboratory medicine community needs
21 to make in conjunction with the clinicians as
22 to what level of risk are they willing to

1 accept if you exclude from your test systems
2 a certain percentage of rare alleles. Just a
3 thought, food for thought.
4 DR. WESTHOFF: I think that makes
5 sense because the other contrast I see with
6 infectious disease testing that applies to
7 donors is I don't think of that so much as the
8 practice of medicine or involving the practice
9 of medicine. It is the donor center's
10 responsibility to test a unit and make sure it
11 meets the standards of purity and potency.
12 And these other assays get into the practice
13 of medicine, I think.
14 DR. FLEGEL: I would like to add
15 one sentence, that the huge polymorphism of
16 the intones, for example, is not a threat.
17 That is actually something very positive that
18 can be used for antigen prediction eventually
19 because of the association of rare variants in
20 the intones once we know all this information.
21 And next-generation, in the end
22 perhaps we will be surprised that the greatest

1 benefit of next generation is actually this
2 useless information in the intones that
3 suddenly becomes very useful, once we know the
4 association with clinically-relevant antigens.
5 DR. WESTHOFF: All right. The
6 next two are somewhat related, I think. It is
7 something we all struggle with. What
8 validation is feasible for rare red cell and
9 HLA alleles, and at the same time what QC
10 material for multiplex, what kind of synthetic
11 controls are possible, cell lines? What kinds
12 of ideas do we have to find this material?
13 DR. LEFFELL: I get to start
14 again?
15 Again, with the HLA system, which
16 is really the only thing that I can speak with
17 any expertise, you have got about 80 percent
18 of the recognized WHO alleles that are
19 extremely rare. They may have been reported
20 only once or twice, and we simply don't have
21 reference reagents.
22 You can find for probes and

1 primers, because of the sharing of motifs, you
2 could cover a lot of specificities, but there
3 is going to be a certain component for which
4 we simply don't have reference reagents.
5 DR. WESTHOFF: So, ASHI has
6 weighed-in, I am sure, on that? Or ASHA dealt
7 with synthetic controls or other material?
8 DR. LEFFELL: Not to any great
9 extent. It is something that is just a topic
10 of conversation at this point.
11 DR. FLEGEL: You know, a
12 validation is better than no validation. So,
13 we have to develop efficiency programs and
14 encourage participation in good, regular
15 proficiency programs. Of course, if there is
16 no cell line, then we have to be content with
17 synthetic controls. But I think we should
18 make a good effort to establish cell lines,
19 and for blood groups, this will be possible
20 for almost all of the prevailing alleles that
21 are out there. And it is rather getting the
22 right mix perhaps of hemi- and heterozygous

1 samples that could be a challenge for
2 validation and, quote, "control" purposes.
3 But I look forward to that. We
4 can make a very good impact to improve these
5 proficiency test systems as well as the
6 quality control materials with cell lines.
7 DR. GABRIEL: Well, I just have
8 been wondering why not the term "biobanking"
9 has been coming up so far. For instance, it
10 would be very nice to have, let's say, in the
11 blood bank some sort of biobank where you have
12 all the extracted DNA of your donors. And
13 then, you can go on for different red cell
14 antigens. So you can take care of, one set,
15 let's say of five or six antigens on the first
16 run. And then, go on further and select out
17 those samples which are more interesting and
18 go for a selected procedure.
19 But, on the other hand, also, it
20 would give the advantage to have some sort of
21 reference samples. And it would be very nice
22 to have the ISPT or WHO coming in for

1 reference samples of red cell antigens. It is
2 worldwide needed to have these samples for
3 testing in every country.
4 DR. DENOMME: Well, we are seeing,
5 as we add more SNPs to look for rare blood
6 types, that the validation or the controls
7 needed start to take up real estate. And the
8 advantage of doing multiple donors at once is
9 you can commit more of that real estate to
10 running controls, as you add on more rare
11 types.
12 So, it is a little bit of a
13 problem if you had to run them every time.
14 And I think we are learning things like, in
15 large parallel testing, that it is not just
16 that you have a positive control, but that you
17 have certain elements of clustering, certain
18 frequencies of alleles between heterozygous
19 and the homozygous common. You can then
20 predict quite reliably that, if there was a
21 homozygous rare, it would have been found.
22 And so, we are looking at things like that.

1 Of course, biobanks, and we see
2 now emerging some reference reagents in Rh
3 free fetal DNA, that kind of thing. Those are
4 all very beneficial and required.
5 But the day-to-day thing, I think
6 we have to think about it. It becomes onerous
7 to run all of the rares every time, even if
8 you went with a heterozygous.
9 DR. OCHOA: I agree with the
10 comments of other panelists on the
11 complementarity that exists between cell lines
12 and synthetic DNA for the purpose of
13 referential material. Obviously, for minor
14 antigens, developing cell lines may be a
15 sufficiently feasible approach.
16 But if genotyping is to be
17 extended in the future, it varies the ABO.
18 The number of variants is so large that the
19 panels of cell lines are going to grow
20 accordingly and may not be as feasible. In
21 that case, synthetic DNA provides a fairly-
22 good substitute.

1 With regards to the feasibility
2 aspect of the validation studies, I just want
3 to throw a comment about the fact that
4 previews, validation designs have been mostly
5 addressing simple tests based on serology
6 reagents. And what we are facing here is
7 complex tests, multiplex tests that
8 interrogate multiple anolytes simultaneously.
9 And therefore, to some extent, that the
10 designs apply to other blood typing devices
11 may have to be revisited.
12 DR. WESTHOFF: I think what you
13 might be referring to is in a multiplex
14 reaction, if there is one failure, how does it
15 impact the rest of the panel possibly? Is
16 that --
17 DR. OCHOA: Yes, and, also, the
18 fact that the various antigens in a multiplex
19 test are the ones that dictate the sample
20 size.
21 DR. WESTHOFF: That is right.
22 We successfully established a

1 number of cell lines, but at the same time it
2 has been difficult to get the rare donors into
3 established cell lines. And then, cell lines
4 don't always last as long as you had hoped
5 they would. And also, there is chromosomal
6 loss sometimes in cultures.
7 So, I am interested, too, in the
8 FDA's approach. I know a reference panel is
9 being developed. If Jason or someone could
10 comment on if that is an initiative to be
11 human DNA from a cellular source or if they
12 are also entertaining synthetic or alternative
13 cell lines or samples?
14 AUDIENCE MEMBER: I just wanted to
15 go back to sort of following up on the next-
16 generation sequencing question, making sure I
17 understand the question about what are the
18 risks we are willing to tolerate. So, as I
19 understand it, what you are articulating is
20 that, if you can actually sequence large
21 portions of genomes or large portions of the
22 HLA region, you are going to know about very,

1 very rare haplotypes. And then, as a donor,
2 you feel an obligation to make a statement
3 about whether those are likely to be antigenic
4 or deleterious in some sort of way when blood
5 is transfused.
6 I think that is very analogous to
7 the variant of unknown significance column in
8 medical genome sequencing these days because
9 what we see routinely is, when you move to
10 clinical genome sequencing, you get back
11 hundreds of thousands of variants, most of
12 which you have no idea about having any
13 functional consequence.
14 I mean, I think that the same sort
15 of ethical obligatory regimes that apply there
16 probably would be useful in your context as
17 well. But I would be interested in your
18 thoughts on that.
19 DR. LEFFELL: One of the problems
20 we have since we have been sequencing HLA
21 alleles is that we now have a vast number of
22 alleles for which we have no idea, based on

1 any kind of empirical scientific test, what
2 their antigenicity is. We can infer certain
3 things from sequences, but these don't always
4 hold up, because we have learned that
5 substitutions in other exons can affect the
6 confirmation and drastically alter the
7 antigenicity.
8 So, that is one of the issues that
9 you would have to deal with. As you said, you
10 will define thousands of possible alleles, and
11 we don't know what their clinical relevance
12 may be.
13 The other issue that we may be
14 faced with is, because the HLA system is
15 evolving, you will find new alleles that have
16 not been described before. So, there are two
17 components to that.
18 Christian, would you like to add
19 to that?
20 DR. GABRIEL: Well, we do also
21 some sequencing for hematology. And I see
22 there a very relevant problem, that there are

1 a lot of SNPs and mutations coming up in
2 different regions, and nobody knows how to
3 handle this. That is true.
4 But I don't think that is really
5 true for HLA because we are expanding more and
6 more into the intronic regions, and we are
7 more and more interested to see, also, the
8 adjacent intronic sequenced because it has
9 some effect on the expression of the HLA.
10 And on the other hand, what I
11 foresee is that we have probably to rethink
12 the databases we are using today because in
13 some occasions we came across the problem that
14 only the exon 2 of this specific Class 2 or
15 Class 1 region was sequenced, and it was put
16 in the database.
17 So, I think it is very important
18 that the whole HLA community comes together
19 and thinks about how to resequence all the
20 known alleles today and to put the whole area
21 into one pot. And that is important because
22 it as some implications. We know that there

1 are patients who are getting graft versus host
2 disease, although they are a 12 out 12 match.
3 So, some other things are still
4 ongoing. One research that we sequenced,
5 shotgun sequenced, the whole chromosome 6 in
6 such a case, and it is very interesting to see
7 that we can have a level of compliance between
8 parents and the patient which has far more
9 SNPs and deletions in comparison between the
10 donor and the patient. So, it is important to
11 have something ongoing in the HLA field to get
12 this into a big database from beginning to
13 end.
14 AUDIENCE MEMBER: Certainly,
15 thanks for that.
16 I guess your point plays well into
17 your comment earlier, a comment about the need
18 for biobanking. But if we biobank, it is
19 easier to characterize the allele diversity of
20 that biobanking, which is possibly an
21 interesting topic to consider tomorrow,
22 actually, when we have got some people from

1 the cancer sequencing community present.
2 DR. WESTHOFF: Mike?
3 DR. BUSCH: Yes, hi. Mike Busch.
4 A couple of points in this
5 context. One is I will come to the study in
6 a minute, but we have just completed a pilot
7 study looking at elution from the leuko-
8 reduction filters. You know, every donor who
9 is collected, we trash most of the white cells
10 because we have leuko-reduced them. And those
11 cells can actually be eluded and get credible
12 the NA yield and are intact cells that could
13 be frozen away up to five to ten days after
14 filtration.
15 So, if we just simply stuck a
16 label on those leuko-reduction filters, and
17 then if we turned around the characterization
18 quick enough, you would have more white cells,
19 more DNA than you could ever need for quality
20 control and for the work.
21 The reason we actually did that,
22 relevant to the last comment and something I

1 wanted to bring up anyway, we are doing a
2 large study within the group. It is called
3 the RBC-omic study. And it is trying to
4 understand the basis for poor storage of red
5 cells in a subset of donors. There is quite
6 a heterogeneity.
7 So, this study will enroll 14,000
8 donors and characterize their end-of-storage
9 hemolysis, both spontaneous and stressed. And
10 then, we will be doing targeted exon
11 sequencing. And then, a large GWAS that will
12 be enriched for over 3,000 known polymorphisms
13 that influence red cell storage or
14 functionality. Nobody has studied the impact
15 on storage.
16 But out of that study, now we are
17 recognizing that we will be identifying lots
18 of donors who are carriers. You know, they
19 are healthy, but they may have no or mild
20 manifestations of these genetic polymorphisms.
21 And so, one of the issues we are
22 having to do now is to convene a group of

1 experts to kind of guide us as to which of
2 these findings warrant donor notification.
3 So, the whole issue of, as you look, you are
4 going to find lots of polymorphisms that we
5 don't understand or we do know have some
6 potential penetrants or relevance to these
7 people's subsequent children.
8 We are also going beyond that in
9 the sense of looking at polymorphisms that
10 influence the ability of donors to give blood
11 repeatedly, that not only hemochromotosis, but
12 there are some new and what appear to be very
13 promising penetrant polymorphisms in genes
14 like TEM PER 6 that are really able to tell us
15 which donors can give six times a year and
16 which can only give two related to iron
17 absorption.
18 So, I think the whole realm of
19 work that you guys are in, red cell
20 polymorphisms or HLA, et cetera, you know, we
21 have the potential to really expand this, both
22 with relevance to our donor health, but also

1 the consequences to recipients to make better
2 blood components.
3 But the banking side and the
4 ethical side of this are really sort of just
5 beginning to emerge.
6 DR. WESTHOFF: Thank you, Mike.
7 And thanks for mentioning a great source of
8 the filters. That is where we get all our
9 storage DNA also. It is a great reserve.
10 Jason?
11 AUDIENCE MEMBER: Hi. Connie, to
12 answer your question regarding the reference
13 panels we are working on, it is going to focus
14 on blood group antigens, not IRA right now.
15 It will be cell and panels. Yes, we
16 understand your concern regarding how long we
17 can keep the cell line. We will make an
18 effort, for example, creating a working cell
19 bank and master cell banks to keep as long as
20 we can.
21 Of course, there is concern, also,
22 regarding how the power reach of this panel,

1 for example -- currently, we are focusing on
2 the significant ones, recommended by AABB,
3 but, of course, if you are looking to least of
4 the panel, I will say we have missed many D-
5 related alleles. So, in the field test, that
6 could be where we can put more work.
7 Regarding the HRA reference
8 material, I think this could be even more
9 challenge because HRA right now you have over
10 8,000 different alleles, much more than blood
11 group alleles, the number. Over 80 percent of
12 them are real ones based on the definition
13 defined by the Society as real ones is less
14 than 1 in 1,000 in many cases, maybe when
15 meeting or something.
16 So, finding all of them in 8,000
17 and in that low frequency, that could be very
18 challenging. I think we have to keep in mind
19 when we think of synthetic materials with
20 these cell panels, of course, frequency and
21 how many we need then.
22 That is my comment.

1 DR. WESTHOFF: Thank you, Jason.
2 With that, if there are no other
3 comments from the audience or the panel,
4 thanks to the panel experts for participating
5 today.
6 And thank you for staying to the
7 bitter end. Thank you. See you tomorrow.
8 (Applause.)
9 (Whereupon, at 5:38 p.m., the
10 meeting was adjourned, to reconvene the
11 following day, Thursday, April 11, 2013.)
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C E R T I F I C A T E

This is to certify that the foregoing transcript

In the matter of: Detection of Transmissible Agents in Blood Donations

Before: FDA

Date: 04-10-13

Place: Bethesda, MD

was duly recorded and accurately transcribed under my direction; further, that said transcript is a true and accurate record of the proceedings.

Court Reporter