1 available. This is similar to the same
2 situation with bioactive lipids.
3 CD40 ligand can be tested by ELISA
4 and there are kits available but they're for
5 research use only.
6 A little more specific, testing
7 for HLA antibodies, the antigens that are
8 available are usually isolated affinity
9 chromatography or they're produced by
10 recombinant technology.
11 These antigens can be adhered to
12 solid surfaces, so there's multiple different
13 types of assays out there. There's ELISA
14 kits. There's flow cytom--they put the
15 antigen on beads and use flow cytometry to
16 detect the antibody.
17 There's also a system available, a
18 modified flow cytometer, to do more rapid
19 testing.
20 So testing for HLA antibodies does
21 lend itself through high throughput testing,
22 and most--a problem, though, with this is if

1 you start testing for HLA antibodies, is most
2 of the products we find with HLA antibodies
3 won't cause TRALI. Neutrophil antibody.
4 Again neutrophils are needed. The problem
5 with this is neutrophils have a short life
6 span, so you literally have to test the cells
7 the same day you isolate them. There's a
8 variety of cellular assays that have been
9 described, agglutination, immunofluorescence
10 or flow cytometry, and monoclonal capture
11 assay and a mixed pass of hemoglutin test.
12 In general, these require all
13 fresh granulocytes. People have wanted, and
14 worked on developing solid phase assays. The
15 problem is is that one of the antigens that's
16 commonly implicated in TRALI, 3a, hasn't been
17 characterized on a molecular basis, and
18 there's not even monoclonal antibodies to
19 this antigen.
20 So antibodies to 3a can only be
21 tested with intact neutrophils at this time.
22 Bioactive lipids. Again, people

1 are assaying these using intact neutrophils.
2 Typically what they do is they incubate
3 neutrophils with plasma or a lipid an then
4 measure the respiratory burst compared to a
5 control, and if neutrophil priming is
6 present, then the respiratory burst of the
7 neutrophil should be higher.
8 One of the problems with this is
9 even if you set up the assay, a threshold for
10 causing TRALI's not known. So, really, it's
11 not been defined, how much neutrophil priming
12 would you need to cause TRALI.
13 CD40 ligand. Again, ELISA assays
14 are available but it's only available as a
15 research tool, and then the threshold again,
16 what's the threshold of CD40 ligand that
17 would cause a transfusion reaction of TRALI?
18 That's not yet well-established. So it'd be
19 difficult to start to test for this agent and
20 exclude blood products.
21 So, in summary, for HLA
22 antibodies, testing donor samples is

1 straightforward and readily available, but a
2 positive test result has a low predictive
3 value of TRALI. Testing for neutrophil
4 antibody require working with fresh
5 neutrophils but a positive result has a
6 higher predictive value for transfusion
7 reaction.
8 Testing for bioactive lipids also
9 requires working with fresh neutrophils and
10 testing of the products at the time of the
11 transfusion would be challenging.
12 And then with CD40 ligand, again,
13 it's a straightforward test, but then again
14 we'd have to test products at the time of
15 transfusion.
16 And in conclusion, donor product
17 and patient factors have all been implicated
18 in TRALI. Unfortunately, no single factor is
19 highly predictive with TRALI. Testing for
20 HLA antibodies and CD40 ligand is feasible.
21 Testing for neutrophil antibodies and
22 bioactive lipids is possible but more

1 difficult.

2 Alan did ask me to just take a
3 minute and talk about the practices at the
4 Department of Transfusion Medicine at the
5 clinical center, and we have now gone to
6 transfusing only plasma from male whole blood
7 donors.

8 We are a little bit short of AB
9 plasma, so we do have a small number of
10 females that donate AB plasma by apheresis
11 but we screen them for neutrophil antibodies
12 and HLA antibodies.

13 We know for sure we defer them if
14 they have a neutrophil antibody. We haven't
15 found any with HLA antibodies but we see what
16 the strength of the antibody is and if
17 they've been involved with transfusions
18 before we necessarily exclude them for having
19 an HLA antibody.

20 And then our policy is if we have
21 a donor that's been implicated in TRALI, if
22 we identify a antibody to a characterized

1 neutrophil antigen, then we defer them for
2 donating.
3 And then we do have a protocol
4 that's been recently approved and we're
5 investigating the incidence of transfusion
6 reaction in recipients of plasma components
7 with and without HLA antibodies. Thank you
8 for your attention.
9 DR. SIEGAL: Thank you, Dr.
10 Stroncek. Are there any questions?
11 MS. KOCHMAN: Sheryl Kochman, FDA.
12 We have cleared an HNA test within the last
13 year and you can find the details on our Web
14 site.
15 DR. STRONCEK: Is that a
16 genotyping test or a phenotyping test? Or
17 testing for an antibody screen?
18 MS. KOCHMAN: I think it's a
19 typing test for the antigen.
20 DR. SIEGAL: Given what little we
21 know about the pathogenic mechanisms, I take
22 it there is no simple pharmacological

1 approach to prevent, like giving Benedril or
2 something?
3 DR. STRONCEK: Well, I don't think
4 so. I mean, if there was a good commercial
5 solid phase test for testing for neutrophil
6 antibodies, I think all of us would be doing
7 that. There's a number of other speakers
8 that have data and thoughts on that, but HLA
9 antibodies, in my view, they're more likely
10 than a random unit to cause a transfusion
11 reaction.
12 But the problem is if we start
13 eliminating donors with HLA antibodies, that
14 we will lose a lot of donors that aren't
15 causing any problems.
16 DR. GOLDING: Can I ask a
17 question? Basil Golding, FDA. One of the
18 things you said that struck me as being
19 important is solvent detergent treated plasma
20 is not associated with TRALI and I'm not
21 aware of any IGIV products being associated
22 with TRALI. So a simple explanation to me

1 would be a dilution factor, and that if you
2 have a high titer, it gets diluted out. So
3 my next question is what do we know about
4 quantitation and is that going to be
5 important?
6 In other words, it's not just
7 knowing that there are antibodies but what is
8 the titer that is important, and do the
9 current tests assess that?
10 DR. STRONCEK: Well, I mean, not
11 really, not much is known as far as titer and
12 quantitation of antibody in causing TRALI. A
13 couple reasons is it's a rare incidence, so
14 it's difficult to study, and there have been
15 a lot of assumptions that just antibodies are
16 bad, so people haven't really thought about
17 looking at what are the important factors
18 that--what are the factors about those
19 antibodies that are important in causing
20 TRALI?
21 DR. KLEINMAN: Just a comment.
22 There has been a report of a IVIG TRALI case

1 but it was from an experimental IVIG reported
2 by Patrick Mollison, which, by the way, can
3 be found in the 11th edition. It was made by
4 a very small pool, so pooling may indeed have
5 a role.
6 DR. SIEGAL: Dr. Szymanski.
7 DR. SZYMANSKI: I have a question.
8 It seems to me--I write that you need the
9 antibody, regardless, if you have a bioactive
10 substance or not. So why to test for both,
11 if the antibody's always needed, even if you
12 need a bioactive material?
13 DR. STRONCEK: I think the groups
14 that work with--Silliman's group, that's done
15 the work with the bioactive substance, would
16 say that you don't need an antibody
17 necessarily to cause TRALI in some cases.
18 That the CD40 ligand or the bioactive lipid
19 would be sufficient on its own.
20 DR. SZYMANSKI: But bioactive
21 liquid is coming from granulocytes or
22 lymphocytes or whatever, white cells?

1 DR. STRONCEK: They are thought to
2 be released by stored platelets and red
3 cells, so then when they're transfused, they
4 react with the granulocytes circulating in
5 the patient.
6 DR. FINNEGAN: I was delighted to
7 see that you actually looked at the patient
8 component, because in reading the materials
9 that we have, there seemed to be very little
10 assessment of the comorbidities of the
11 patients who develop TRALI. And one of my
12 concerns is if we have a national disaster
13 and we not allowing females to give plasma,
14 we're going to run into some problems.
15 Do you have a sense, other than
16 the hematological or immunocompromised
17 patients, are there patients who really did
18 not seem to develop TRALI if they were given
19 the granulocyte? I'm sure there are because,
20 obviously, the number of TRALI cases versus
21 the number of multiparous women who donated,
22 do not match up, so there have to be patients

1 who can get it and it doesn't bother them.
2 Do you have any sense of, is that
3 younger? is that trauma patients? Do you
4 have any sort of sense of that?
5 DR. STRONCEK: Not really. It's
6 really--again, there's been mostly single
7 institution studies and we all have our, you
8 know, different patient mix. But almost all
9 patients have been described to have TRALI,
10 even patients without antibodies who, you
11 know, if you really believe it's all
12 neutrophil-mediated, that they shouldn't have
13 TRALI, but some of those patients do too.
14 DR. SZYMANSKI: I also wanted to
15 bring out the idea that there are many, many
16 female donors who have donated platelets
17 many, many times, and plasma many, many time,
18 and they have never been associated with any
19 TRALI event. So they're sort of biologically
20 proven to be safe females.
21 What do you think about that? I
22 mean, why do you have to even test them?

1 DR. STRONCEK: Excuse me but I
2 couldn't hear the question.
3 DR. SZYMANSKI: The question is
4 that let's say in my institution we have many
5 females who donate repeatedly platelets, you
6 know, many times a year, and they have never
7 been associated with any TRALI kind of
8 incidents. To me, they are biologically
9 proven safe donors. I haven't seen where
10 they can just be accepted as such, without
11 any kind of testing.
12 DR. STRONCEK: I think that's a
13 good point. I think the problem is is that
14 it's hard to know if they're completely safe.
15 Again, I agree that there's many women that
16 have HLA antibodies and when you transfuse
17 their products they don't cause TRALI. But
18 it's unclear if there's a subset of people
19 with HLA antibodies that will cause TRALI,
20 but it's just a real low incidence, and then
21 other donors are completely, have HLA
22 antibodies but will never cause TRALI.

1 So I think that's one of the
2 important questions. You know, we and other
3 groups are trying to address that by having
4 prospective studies, to actually start
5 comparing the incidence of transfusion
6 reactions and the type on people with HLA
7 antibodies and without, and try and get an
8 idea, is titer important? Is it only certain
9 specificities?
10 Is there something about the
11 patient that's really the critical factor,
12 and it's not the donor?
13 DR. SZYMANSKI: Man of the females
14 have not even been pregnant, they're young
15 women, they cannot have any antibodies--
16 DR. STRONCEK: Sorry?
17 DR. SZYMANSKI: There are many of
18 these biologically safe women, have not even
19 been pregnant once.
20 DR. STRONCEK: Yes. I think even
21 in multiparous donors, women, the incidence
22 of HLA antibodies is--well, if they've had

1 three or more pregnancies it's about 20
2 percent. But there's a number of women that
3 don't have HLA antibodies.
4 DR. SIEGAL: Dr. Di Bisceglie.
5 DR. DI BISCEGLIE: I guess you've
6 identified the series of risk factors
7 associated with TRALI but what might be
8 useful is some quantitation, some relative
9 quantitation of the risk, and maybe the
10 incidence is so low that you have--it's not
11 possible to calculate, say, an odds ratio, or
12 a relative risk or a rate per thousand or
13 something like that, that would allow a
14 prioritization of the risk factors. Are such
15 numbers available?
16 DR. STRONCEK: Not yet but, again,
17 you know, the Red Cross has begun looking at
18 this in a systematic way, and the REDS group
19 will be able to look at large cases of
20 numbers of TRALI over several institutions.
21 So I think that data will be coming but it's
22 not available at this time.

1 DR. SIEGAL: The next speaker,
2 please. This will be Dr. Sarode. Current
3 use of transfusable plasma.
4 DR. SARODE: Good morning. You
5 know, when I got this e-mail about
6 transfusable plasma, I had no idea what I was
7 supposed to talk, but I figured out, since
8 it's TRALI, you basically want to know how
9 much plasma we are misusing. So that's why
10 the topic of my talk is misuse of plasma in
11 clinical practice.
12 Now in United States we transfuse
13 more than 3 million units annually, and this
14 is very old figure from 2001, and I can
15 assure you, it's more than that now, because
16 there are seven changes in the way
17 laboratories, they are doing testing for
18 correlation factors.
19 Now most of these plasmas, they
20 are transfused before any procedure is
21 performed, a surgical procedure is performed,
22 or for patients who have an abnormal

1 coagulation test in ICU setting, and which is
2 very common.
3 Unfortunately, the pre-surgical
4 hemostatic assessment is mostly performed by
5 using laboratory tests, rather than good old-
6 fashioned clinical testing.
7 So if we don't take a good
8 clinical history about bleeding, rapidly we
9 are doing screening tests for coag and not
10 bothering about the history.
11 So many times you get abnormal
12 coagulation test and we act on these lab
13 results.
14 Now this particular slide shows
15 the ratio of red cells to FFP transfusion in
16 different countries. If you look at United
17 States and Germany, we are transfusing one
18 unit of plasma for every three red cells, as
19 opposed to Finland, U.K. and France, where
20 they're transfusing only one unit for six
21 units of red cells.
22 So we are transfusing almost twice

1 more plasma. Dr. Dzik from MGH, he published
2 this study about request for plasma in their
3 hospital. So they wanted to know why
4 physicians order FFP, and guess what? Most
5 of the time they ordered plasma to correct an
6 abnormal test result, that's INR, before any
7 procedure, and there's about 15 percent of
8 patients, they get plasma to reverse warfarin
9 effect.
10 So almost 50 percent of plasma
11 used to correct an abnormal coagulation test.
12 Now you should look at literature. Not a
13 single study had shown any good correlation
14 between prolonged coagulation test results
15 and efficacy of plasma in treating those
16 numbers, or having an effect on hemostatic
17 efficacy when the coagulation tests are only
18 mild to moderately abnormal.
19 This study from Cleveland Metro
20 Hospital showed that when they had 80
21 patients of cirrhosis, who had slightly
22 elevated INR, they gave them plasma, 41

1 percent received for prophylaxis, and 60
2 percent who were already having some bleed,
3 they got anywhere between two to four units,
4 and actually 25 percent, they got more than
5 four units of plasma.
6 That's more than a liter of volume
7 in a patient who is cirrhotic, and, I mean,
8 you can imagine, pouring one liter of plasma
9 in two to four hours. It can be a disaster.
10 And what were the results? 90
11 percent of patients failed to achieve the
12 goal the had. So they wanted all these
13 clotting tests to be within three seconds of
14 upper limit of normal and 90 percent of these
15 patients who got plasma, even more than four
16 units, they did not achieve the goal.
17 So their conclusion was very
18 simple. FFP frequently failed to correct
19 INR. This is another study which was done a
20 long time back. It looked at bleeding time
21 at the site of liver biopsy, and compared
22 with prothrombin times. And you can

1 see that the bleeding times, they're all
2 over, even when the patients, they had near
3 normal PT, that longer bleeding times as
4 opposed to when they had longer prothrombin
5 time, and they had no bleeding.
6 So this again shows that even in
7 1981, we knew that there was no good
8 correlation between prothrombin time and
9 bleeding tendencies.
10 Now this table, which is crowded,
11 basically has the same message.
12 Unfortunately, there's not a single, well-
13 designed, randomized, control study in the
14 literature which has looked at efficacy of
15 plasma in either correcting an abnormal PT
16 and PTT and its efficacy in preventing
17 bleeds.
18 So these various studies, they
19 have looked at--of course these are
20 retrospective and observational studies--but
21 none of these studies found any predictive
22 value for either PT or PTT as regards

1 bleeding, post procedure.
2 Now what happens when we have
3 hemoglobin of 11 grams. We don't transfuse
4 red cells. When the platelet count is mildly
5 reduced, we don't transfuse platelets.
6 When creatinine is 2.1, we don't
7 do dialysis. You tell a nephrologist to do a
8 dialysis at 2.1, you're going to get yelled
9 at.
10 So why do we transfuse plasma,
11 when now PT is one or two seconds prolonged,
12 and we want absolutely normal PT? Is there
13 any evidence for that?
14 Now if you look at evaluation in
15 hemostasis, in our body hemostasis is a very
16 complex phenomenon. If you look at it the way
17 normal hemostasis works, you have
18 procoagulant factors, factor I through XIII,
19 except VI and XII, which are not important.
20 These clotting factors, they are kept under
21 check by natural anticoagulants,
22 antithrombin, protein C, protein S, and now

1 TFPI or tissue factor pathway inhibitor, and
2 then once you have clot in the system, you
3 have very efficient fibrinolytic system which
4 dissolves the clot, and of course you have
5 platelets and endothelial cells, which are
6 important players in maintaining normal
7 hemostasis.
8 Now not a single test assesses
9 this whole hemostasis.
10 Now PT and PTT. These tests, they
11 were done to diagnose patients who had a
12 bleeding disorder, meaning when patients had
13 hemophilia A and B, people really want to
14 know why they bleed. So they came up with a
15 test called PTT.
16 Similarly when cows in Wisconsin,
17 they were bleeding after eating plants which
18 had warfarin, they wanted to know why they
19 were bleeding, and they found out--PT.
20 So these tests, they were
21 developed in patients, or cows who have
22 bleeding tendencies. So these tests, if you

1 perform in patients who have bleeding
2 tendency, you have very high test--probably
3 you are finding them abnormal. They have
4 never been shown to assess bleeding risk in
5 nonbleeding patients.
6 If you take 100 patients in your
7 hospital and you do PT and PTT, and you find
8 slightly abnormal PT and PTT in 10 percent,
9 there's no guarantee that all these ten
10 patients, they are going to bleed after
11 surgery or during surgery.
12 Now most of the plasmas, they are
13 given for abnormal PT. Now let's look at
14 what PT is. It's a very simple test. You
15 take patient plasma and you add a reagent
16 which has tissue thromboplastin and calcium
17 chloride.
18 Now in the past, the sources of
19 tissue thromboplastin included brains, which
20 were human brain, rabbit or goat. We use
21 human placenta because they are all rich in
22 thromboplastin.

1 Now we have recombinant tissue
2 thromboplastin that is used.
3 However, in clinical practice, you
4 will hear the term INR, and if you ask what
5 is INR, most clinicians, they don't know what
6 INR is. They know this is a nice number,
7 which is associated with PT. Now this INR,
8 or international normalized ratio, was
9 developed to monitor patients who were on
10 anti-coagulation or warfarin.
11 So INR is the prothrombin time
12 ratio that, by calculation, would have been
13 obtained, though regional WHO-referenced
14 thromboplastin had been used to perform the
15 PT.
16 Now the reason we needed INR was
17 that if you have tissue thromboplastin coming
18 out of one brain and used in one lab, it can
19 last only maybe up to six months. But after
20 that you will prepare another reagent, and
21 therefore the sensitivity of each brain for
22 different, Vitamin K, different factors, was

1 different. So you had highly variable PTs.
2 And this was even worse when you had patient
3 going from one hospital to another one. The
4 reagent was totally different.
5 And most of these tests, they were
6 done in '80s by using manual or semiautomatic
7 techniques. So changing one or two seconds
8 really created havoc. So we needed a method
9 which then was provided. That's INR.
10 So the way INR is calculated is
11 you need a prothrombin time ratio where
12 patient's PT is divided by control, so, for
13 example, if PT is 24, control 12, you get a
14 prothrombin time ratio of two, and to
15 calculate INR, you raise that prothrombin
16 time ratio by ISI.
17 Now this is the key. ISI, the
18 International Sensitivity Index, which is
19 given to each tissue thromboplastin reagent,
20 when it is compared and standardized and
21 calibrated against WHO standard.
22 So in this case, if the ISI, the

1 reagent is two, your INR will be four. So
2 that's how you get INR. Now the problem with
3 tissue thromboplastin used in '80s and '90s
4 was that they had very high ISI. So to
5 maintain INR between two and three, your PT
6 was between 16 and 18. Now that's where we
7 all got trained, is '80s and early '90s, that
8 if the PT as 16 or 17, patient was
9 coagulopathic, because that was the--INR was
10 very high.
11 But the problem was if you had PT
12 of 15, patient was subtherapeutic and if PT
13 was 19 or one second above, was
14 supratherapeutic.
15 So it was giving nightmares to the
16 clinician to manage anti-coagulation
17 treatment for the patient. They had to
18 adjust doses every now and then. So we did
19 slightly better by improving the sensitivity
20 of tissue thromboplastin to two and to
21 maintain INR between two and three, now we
22 had slightly wider safety range, between 17

1 and 21. But again, it wasn't ideal. So we
2 went to recombinant tissue thromboplastin or
3 some of the other tissue thromboplastin which
4 have ISI close to one, and to maintain INR
5 between two and three, now we have PT between
6 23 and 35.
7 But what happened? We got stuck
8 on these PT values. And that's what still
9 happens. So surgeons who are used to having
10 bleeds when patients had PT of 16 and 17, in
11 '80s and '90s, they still want 16 and 18
12 here, which is actually equivalent of
13 somewhere here--28. So the problem here is
14 we have not communicated with the clinicians
15 all these changes that have happened in the
16 clinical labs.
17 And this is one of the reagents
18 that we use, it has very sensitive PT
19 reagent, and it causes very short, PT's
20 normal, but it's too sensitive to Vitamin K-
21 dependent factors, especially VII. So what
22 happens now is even slight decrease in factor

1 VII leads to prolongation of PT/INR, and we
2 react to those by giving plasma.
3 Now if you look at the hemostatic
4 levels for various factors, or important
5 factors, you basically need anywhere between
6 15 to 30 percent or 40 percent, and you can
7 perform any surgery.
8 When we did sensitivity of our PT
9 reagent for all these factors, we found that
10 when the factor VII was 49 or less, PT got
11 prolonged. But you need only 15 percent for
12 major surgery. So coming to indications for
13 plasma, the absolute indication is still TTP
14 until we find recombinant Lnt is thirteen.
15 Patients who have coagulopathy with bleeding,
16 patients who have coagulopathy during
17 surgery, and a reversal of warfarin overdose.
18 However, the ideal product would be to use
19 prothrombin complex concentrate, which is
20 recommended by American chest physicians,
21 British hematologists, Australian and
22 European societies.

1 However, we don't have a
2 prothrombin complex concentrate in this
3 country licensed for this particular effect,
4 and of course for real congenital factor
5 deficiency for which we don't have beautifed
6 concentrates.
7 However, plasma is often misused to
8 correct mild to moderate abnormal PT/INR,
9 without bleeding, and volume expanded.
10 Now the published data provide no
11 evidence-based guidance for use of pre-
12 procedure SFP, or even platelet transfusion
13 among patients with mild to moderate abnormal
14 results.
15 So what we did, we talked to our
16 neurosurgeons, and when they wanted some
17 plasma for slightly prolonged PTT, we did a
18 study. We looked at, we stored their
19 patients' plasma who had slightly prolonged
20 INR, we gave them the plasma at that time and
21 looked at hemostatically important clotting
22 factors.

1 And if you look at the results the
2 medium PT was slightly prolonged as compared
3 to upper limit of normal. Similarly, INR was
4 slightly prolonged. PTTs were normal.
5 Factor VII was normal, and Factor VIII
6 actually was very high, which is shown in the
7 next slide.
8 These are certain selected cases.
9 Now whenever we do PT and PTT, everyone
10 looks at PT/INR, and others, they ignore PTT,
11 and I don't know why. Look at these
12 highlighted PTTs. They have very high levels
13 of Factor H. A PTT of 90. Even though the
14 INR is 1.4, which surgeons thinks has risk
15 for bleeding. If you look at the Factor
16 VIII, 547. Factor VIII is a known risk
17 factor for clotting. It's a known
18 prothrombotic risk factor.
19 So by looking at PT and PTT, this
20 patient didn't need any plasma. So I
21 presented this particular study at
22 Neurosurgery Grand Rounds, and there was a

1 drastic reduction in FFP orders for
2 prophylactic plasma use, 85 percent. We
3 followed up after a year and it still remains
4 75 percent less than pre study, and I still
5 keep working with these surgeons to remind
6 them.
7 Now in Parkland, we have a very
8 active transfusion medicine practice. It's a
9 county hospital. Blood Utilization Review
10 Committee, or so-called Transfusion
11 Committee, has established evidence-based
12 transfusion criteria, and since 2003,
13 transfusion medicine residents, they are
14 involved in each and every request for plasma
15 and platelets that do not meet the guidance.
16 And the goals were to reduce
17 unnecessary transfusion and advise
18 appropriate products, because we always try
19 to treat everything with plasma and forget
20 about cryo or other products like DDVP,
21 prothrombin complex concentrates. So that's
22 what we do because all these factor

1 derivatives, they are kept in blood bank and
2 we like it.
3 And of course to reduce the
4 incidence of TRALI. I won't go into details
5 but this is a triage sheet for blood bank
6 technician to call a resident when to give
7 plasma, and similarly for platelets.
8 Now since 2000, when I came there
9 in the end of 2000, I started taking part
10 actively in 2001, you can see that the number
11 of red cell transfusions has remained the
12 same because we haven't intervened in that
13 particular practice yet.
14 But if you look at plasma, it has
15 started around 11,000 and now it's dropped to
16 less than 4800. So there's a 60 percent
17 reduction in plasma use in four years.
18 A similar thing happens for
19 platelets, decreased from close to 5000 to
20 less than 2500.
21 However, the admissions, they have
22 remained the same, surgeries, they have

1 remained the same, and trauma has remained
2 the same.
3 Now we have a master transfusion
4 protocol established for our trauma center,
5 and the goals were to provide rapid blood
6 component therapy, to prevent or treat
7 coagulopathy earlier, than waiting for it to
8 happen, and reduce blood wastage.
9 And this is our shipment protocol
10 for different products. Whenever a protocol
11 is initiated, we send five red cells to
12 plasma, in the first shipment followed by
13 five red cells to plasma, those are platelet
14 and small dose of NOVA 7, and the third
15 shipment goes with car.
16 And if you look at the blood
17 products used, pre and post MTP, we have
18 reduced the use of red cells by seven per
19 incident, so instead of using 24 we use now
20 seven. Seventeen red cells. But look at the
21 plasma. It has decreased by 50 percent, and
22 same with platelets.

1 So what's happening is we are
2 trying to prevent or treat the coagulopathy
3 earlier rather than waiting till it develops
4 and try to pull all these factors.
5 So it seems that appropriate
6 products utilization is the goal. We should
7 recommend when the cryoprecipitate is
8 indicated. All these patients who are
9 getting plasma, they may have low fibrinogen,
10 they may have dysfibrinogen anemia, which is
11 common in patients with chronic liver disease
12 or cirrhosis.
13 Use of antifibrinolytic agents is
14 encouraged. Patients with mild gum bleeds
15 and all, and they need platelets, we
16 recommend Amicar. Similarly, a reversal of
17 heparin-induced bleeding, many surgeons, they
18 still want to give FFP, which is actually
19 contraindicated, in my opinion, because it
20 supplies antithrombin and makes the bleeding
21 worse.
22 We have started using prothrombin

1 complex concentrate, which is not an ideal
2 one, but still, it has reduced our plasma use
3 by 50 percent.
4 So the take-home message is that
5 there's still significant misuse of plasma in
6 clinical practice. Blood banks should
7 practice transfusion medicine, rather than
8 function as simply blood-dispensing units.
9 That's what's happening in almost 99 percent
10 of hospitals in the country.
11 Whenever there is a request for
12 any blood products, they are dispensed
13 without any question.
14 There's a greater need for
15 clinicians and medical students education in
16 transfusion medicine and hemostasis. And
17 most importantly, they like it. If you tell
18 them what's the real stuff, they like it and
19 they respond to it. Thank you very much.
20 DR. SIEGAL: Thank you very much.
21 Are there any questions?
22 DR. SZYMANSKI: I just want to

1 commend you on this wonderful presentation
2 because it's really a great problem in this
3 country, and the smaller the hospital, it
4 seems to be even greater. I recently
5 inspected a blood bank at a relatively small
6 hospital and they use plasma for slight
7 increase in PT. They never check if the PT
8 changes after plasma transfusion, you know,
9 prior to surgery, and they really don't have
10 proper guidelines for any of the blood
11 components, and I think if you can reduce
12 plasma and limit infusions, you know, by
13 having good guidelines, like you have
14 presented, it would also reduce the incidence
15 of TRALI.
16 And I really liked your talk very
17 much. Thank you.
18 DR. SARODE: I mean, that's my
19 goal. I mean, I have been going to different
20 departments, giving Grand Rounds, and they
21 are responding very positive because these
22 changes in the laboratories, they have not

1 been communicated to clinicians and they
2 still think that, you know, a PT of 16 is as
3 bad, you know, as it used to be. But that's
4 not true. We have changed practices in
5 radiology, intervention radiology,
6 hepatology, neurosurgery,
7 [No response]
8 DR. SZYMANSKI: Yes. The surgeons
9 particularly need a lot of education in this
10 area.
11 DR. MANNO: I have a question
12 about your recommendation for a PCC as
13 opposed to FFP for reversal of coumadin. Is
14 that based on ease of use, because you don't
15 have to wait for the plasma to thaw, or
16 efficacy?
17 DR. SARODE: I think both. The
18 first thing--
19 DR. MANNO: Are there data to
20 demonstrate this?
21 DR. SARODE: Pardon?
22 DR. MANNO: Are there data, have

1 there been studies of this, or is this your
2 preference?
3 DR. SARODE: Yes. European data
4 is there, and we have our data coming out
5 soon. The advantage of PCC--now there are
6 two types of PCCs. People get confused. One
7 is--
8 DR. MANNO: I don't.
9 DR. SARODE: I know. But there
10 are many hematologists who are still
11 confused.
12 There is one called activated PCC
13 or autoplex, which is not available in U.S.,
14 and FEIBA. And there are PCCs which are
15 nonactivated clotting factors, so it has
16 purified factors II, VII, IX, and X, and
17 these PCCs, they are used to treat hemophilia
18 B. The advantage of four factor PCC, where
19 you have all Vitamin K, different factors,
20 actually some of the products in Europe, they
21 also have protein C and protein S. So it's
22 much more complete PCC.

1 And you can transfuse PCC within
2 five minutes, the dose, 15 -- for the worst
3 INR with bleed, and you will find correction
4 of INR within five minutes, as opposed to
5 FFP, when you are trying to give FFP, you are
6 thawing the plasma, it takes half an hour to
7 thaw the plasma, and maximum you can
8 transfuse is one unit every 15 to 30 minutes,
9 and that leads to a volume overload, because
10 the majority of the patients who come with
11 warfarin-related bleed, they are elderly, on
12 warfarin for atrial fibrillation or cardiac
13 condition.
14 So both safety reason, I think
15 it's very good, and rapidity is excellent.
16 And the current PCC that's available in U.S.,
17 called Profile 9, has II, IX, and X. It
18 doesn't have factor VII, and that's why we
19 had to use FFP to correct that, because INR
20 doesn't get corrected and surgeon really
21 wants INR.
22 DR. MANNO: Some of course would

1 argue about the expense of a PCC as well as
2 the old reports of thrombosis, particularly
3 in patients who have preexisting liver
4 disease, or in the neonate.
5 DR. SARODE: But most of these
6 thrombotic complications, they were seen in
7 patients with hemophilia B and who were
8 getting PCCs at regular interval for a long
9 time. So if you give daily PCCs to
10 hemophilia B with liver disease, you are
11 replacing factor IX by PCC, but they still
12 have their own factor II, VII, and X, and by
13 giving PCCs every day, you are raising their
14 other clotting factors.
15 So they had prothrombotic state,
16 and that's why they had more complication,
17 not significantly more but they thrombotic
18 complications. But when you treat PCC for
19 warfarin reversal, you give only one dose,
20 and you give all these factors, so risk of
21 thrombosis is significantly less, and the
22 European studies have shown there's no

1 evidence that it's thrombogenic.
2 DR. SIEGAL: We're going to have
3 to move on. Thank you, Dr. Sarode.
4 Dr. Steven Kleinman from the
5 University of British Columbia will talk
6 about the REDS LAPS study on HLA and
7 granulocyte antibody prevalence in blood
8 donors.
9 DR. KLEINMAN: Thank you. So I'm
10 going to address, give a preliminary report
11 on the Leukocyte Antibody Prevalence Study,
12 which the acronym is LAPS, and since we have
13 a second study planned, the acronym is LAPS
14 I.
15 This is part of the retrovirus
16 epidemiology donor study, second iteration,
17 so REDS I was a 15 year study. REDS II is
18 now a different study. I'm one of the
19 members of the REDS II group and I'm
20 presenting this on behalf of many other
21 investigators. They actually have an old
22 slide projector control here and an old slide

1 projector. I haven't seen that for a long
2 time. It'd be a page down. Yes.
3 So the structure of REDS II. This
4 is a study that was funded beginning in 2004
5 by NHLBI as a five year study. It has
6 multiple project areas. I'll just be talking
7 about the leukocyte antibody prevalence study
8 today.
9 There are six blood centers that
10 are participating in this study. They are
11 the blood center of Southeast Wisconsin, the
12 American Red Cross New England Region, along
13 with the American Red Cross Southeast Region,
14 and Emory University, Hoxworth Blood Center
15 from Cincinnati, Institute for Transfusion
16 Medicine from Pittsburgh, and the blood
17 centers of the Pacific, San Francisco.
18 The coordinating center for this
19 study is Westat, located locally, and the
20 central laboratory is Blood Systems Research
21 Institute in San Francisco.
22 The objectives of this leukocyte

1 antibody prevalence study. There are several
2 objectives. The main objective is to
3 determine the HLA class I and II antibody
4 prevalence in blood donors, and correlate
5 this with the number of pregnancies, the
6 lifetime history of transfusion, the time
7 from the last immunizing event, and to
8 compare these alloexposures with a baseline
9 group that has no evident alloexposure, so
10 never pregnant or never transfused.
11 So in addition to determining the
12 prevalence of these antibodies, another goal
13 is to actually identify the HLA antibody
14 specificities, and the third goal is to
15 determine the presence of antibodies to
16 neutrophils, and because neutrophil antibody
17 testing is complex and expensive, our goals
18 are a little more modest here. We don't
19 think we can test the whole cohort for
20 neutrophil antibodies, and so we don't know
21 if we'll have the power to relate neutrophil
22 antibody prevalence to these epidemiological

1 factors, pregnancies and transfusion, but we
2 are planning to do some neutrophil antibody
3 testing, first in those with HLA antibodies
4 because we think those will be higher rates
5 of neutrophil antibodies as compared to some
6 of our donors without HLA antibodies.
7 So in doing a statistical power
8 calculation as we began this study, we
9 estimated from existing data in the
10 literature, and there are some papers
11 indicating the rate of HLA antibody
12 prevalence in donor populations, that we
13 would need to enroll about 5100 female donors
14 to have greater than 90 percent power to
15 detect differences in HLA antibody
16 prevalence, both by number of pregnancies and
17 the interval from the last pregnancy.
18 So that was our sample size
19 calculation for female donors. With regard
20 to male donors, the preliminary data from the
21 literature suggests that the--and in very
22 small numbers--that the rate in transfused

1 male donors and untransfused male donors of
2 HLA antibody is not every different, and so
3 we could not power the study to actually find
4 a difference in rate, and we decided that we
5 would look at a thousand donors, a thousand
6 male donors with a lifetime history of
7 transfusion, a thousand never-transfused
8 males, and the purpose here was to try to
9 tighten the confidence intervals around the
10 prevalence estimates that are currently in
11 the literature.
12 So a little bit about the
13 protocol. We give everybody enrolled into
14 LAPS a questionnaire that--well, it includes
15 both transfusion history, and pregnancy
16 history for the female donors. The questions
17 for pregnancy history are taken from the
18 National Health and Nutrition Examination
19 Survey, NHANES. These have already been
20 validated. It's a series of six questions.
21 They ask the donor whether they've
22 ever been pregnant, and if so, the number of

1 pregnancies, and divide this between number
2 of live births, still births, miscarriages or
3 abortions, and then we ask for the date of
4 the last pregnancy and the transfusion
5 history consists of a question, Have you ever
6 received someone else's blood? and if yes,
7 then the date of the last transfusion.
8 One of our first tests was to
9 decide what assay we would use to measure HLA
10 antibody. The potential assays that are out
11 there are flow cytometry assays, both flow
12 PRA, and Luminex, which I'll talk about in a
13 minute. There's also, as Dr. Stroncek
14 mentioned, solid phase ELISA assays, and then
15 there are the classical methods of
16 lymphocytotoxicity, usually now done with
17 human, anti-human immunoglobulin enhancement.
18 We decided that in this research
19 study we should use the tests that had the
20 highest sensitivity for HLA antibodies and
21 based on the high sensitivity of Luminex and
22 flow PRA, and the high throughput that we

1 could achieve with Luminex, since we did have
2 such a big study, the assay chosen was the--
3 we chose the Luminex platform which is a
4 specialized flow cytometer, and we used the
5 LABScreen reagent from One Lambda, a company
6 in California.
7 We decided we would perform both
8 supplementary screening--I'm sorry. In
9 addition to screening assays, we would
10 perform supplementary testing with their
11 single antigen assay to confirm the screening
12 results, and I'll get to that a little bit
13 more specifically in a few slides..
14 So the LABScreen reagent that we
15 used is called the LABScreen mixed reagent.
16 It's a screening assay that uses multiple
17 beads coated with purified HLA antigens.
18 Each bead contains purified antigens from
19 five to six cell lines. The assay consists
20 of five beads that have class I antigens,
21 three beads that have class II antigens.
22 There are a total of 54 different HLA class I

1 antigens and 32 class II antigens involved in
2 the initial screening assay.
3 The company has also developed a
4 couple of beads for a new antigen system
5 that's been discovered, about five to ten
6 years ago, called MICA, or MICA--I'm not sure
7 how it's pronounced--which stands for MHC
8 Class I related antigen.
9 This antigen is present on
10 endothelial--this antigen system, which
11 actually has multiple specificities, just as
12 HLA does, is present on endothelial cells but
13 is present on a number of other cells in the
14 body as well, and we thought this would be of
15 interest to look at because endothelial cells
16 have been implicated in TRALI.
17 So we use the Luminex flow
18 cytometer and the assay measures, laser-based
19 light emission, through the binding of
20 reagents that are conjugated to antibody,
21 which is, in turn, bound to the class HLA 1
22 and 2 antigens.

1 And here is a schematic provided
2 by the company. There are, as I said,
3 multiple beads here that fluoresce at a
4 difference intensity and then each bead is
5 covered with different purified HLA proteins,
6 and basically, the principle of the assay is
7 the Luminex bead has a bound antigen to it,
8 or a series of antigens. If the patient has
9 alloantibody it will bind to the antigen, and
10 then there'll be a photochemically-tagged
11 anti-lgG. If that binds, then there'll be
12 emission of light and you can see that in the
13 flow cytomer, and you can actually know which
14 bead it's coming from, and therefore you can
15 decide which antigens are implicated, whether
16 they're class 1 or class 2.
17 So this is a screening assay. One
18 could go beyond this to actually try to
19 identify the antibody specificity. Their
20 common way of identifying the antibody
21 specificity is similar to the old ways, and
22 that is that you would use a sort of panel

1 reactive antibody. You'd have multiple beads
2 and you'd have to try to dissect what was
3 happening.
4 But for purposes of this study,
5 the company also has a single antigen assay
6 which isn't used as frequently because it's
7 more expensive, but we are using it for this
8 study.
9 So in this assay, each bead
10 contains one and only one recombinant HLA
11 antigen and that's characterized well out on
12 the molecular level. So, for example, you
13 can have an A2 but we have multiple A2s on
14 different beads.
15 So this mix contains 94 class 1
16 and 52 class 2 antigens, each on a single
17 bead. The specificities included are within
18 class 1 are the AB, BW, and C loci, and
19 within class 2 cover DR, DQ and DP. All of
20 the common antigens are represented.
21 Obviously, there are some rare
22 antigens that are not included on the panel.

1 And so the idea here is if you get
2 reactivity, you can be much more sure of the
3 specificity or the multiple specificities
4 that might be present in a given donor.
5 But because HLA antibody
6 interpretation is complex, even with this
7 system, we've adopted, and most of the
8 investigators in REDS II are not HLA experts,
9 we've established a review panel of three HLA
10 experts, and each confirmatory testing result
11 will undergo review by at least one expert
12 and any problematic case will be reviewed by
13 a panel of experts in order to assign both
14 interpretation of the confirmatory test and
15 the specificities.
16 So that's a little bit about the
17 lab study. I wanted to give you a little bit
18 of background about the data that we have
19 overall in REDS II, cause I'm going to show
20 some of that in a moment.
21 And so we have accumulated in a
22 central location, all of the donation and

1 donor data that are accumulated by the six
2 blood centers. So we have one complete year
3 of data for 2006. This represents 1.2
4 million successful donations. So it probably
5 constitutes about 7, 8 percent of donations
6 in the U.S.
7 So we have all the basic
8 demographic information on each donation made
9 by each donor. We know the gender, the age
10 of the donor, that sort of thing.
11 We know what type of donation was
12 made, whether it's whole blood of apheresis.
13 And in addition, we have two additional
14 pieces of data that aren't usually obtained
15 by blood centers.
16 We've added questions at these six
17 centers to the usual donor questionnaire. So
18 we have the pregnancy history. We ask a
19 simpler question of all donors than we do to
20 the LAPS enrollees. So we ask, Have you ever
21 been pregnant, and if so, how many times?
22 And we give them a option of one, two, I

1 think up to six, actually. And then we ask a
2 question, if they're ever been transfused.
3 So I want to show you some data
4 from the donation database here. This may
5 have some implication for thinking about male
6 versus female plasma issues. So you can see
7 here, for whole blood donors, whole blood
8 donations, it's basically a 50/50 mix between
9 donations coming from male donors and female
10 donors.
11 On the other hand, if you look at
12 platelet pheresis donors or donors from whom
13 we collect platelets and another component at
14 the time of apheresis, you can see that these
15 are skewed to be greater than 60 percent male
16 and less than 40 percent females. So we have
17 a lot more male platelet pheresis donors.
18 Plasma pheresis donors, same thing, but about
19 55 percent male and I just threw this in,
20 it's not particularly relevant, but for red
21 cells collected by automated apheresis, for
22 which you have to have higher hematocrits, in

1 general, almost 90 percent of those
2 procedures are done in male donors.
3 Now as I said, we collect the
4 transfusion history data from all of these
5 donors and it's summarized here. This is on
6 a per donor basis rather than on a per
7 donation basis.
8 So you can see that amongst our
9 male donors, about 2.6 percent indicated that
10 they had a previous lifetime history of
11 transfusion, and just to remind the
12 committee, we generally ask all donors if
13 they've been transfused in the last 12
14 months, and if they had, they're not allowed
15 to donate until those 12 months are over.
16 But if they've been transfused
17 greater than 12 months ago, they are eligible
18 as a donor. So these are people who were not
19 transfused in the last year because they're
20 eligible as donors but had a previous
21 transfusion.
22 And in female donors, you can see

1 that the number is about 4 percent.
2 Interestingly, between 2.5 to 4 percent of
3 the donors, depending on gender, answer that
4 question as "not sure," whether they've ever
5 had a lifetime transfusion.
6 If we were to do this per donation
7 rather than per donor, based on our
8 experience in REDS I, some of these donors
9 turn out to be multiple time donors and at
10 least in REDS I, where we had a similar
11 percentage of about 4 percent of donors who
12 had a previous history of transfusion, they
13 accounted for about 5 percent of donations.
14 So that's the background on
15 history of transfusion.
16 Now returning back to the specific
17 protocol of LAPS, here's our enrollment to
18 date, and our enrollment is almost complete.
19 Rather than 5100 female donors, we've been
20 so aggressive, that we have almost 6000
21 female donors who have been enrolled in this
22 study.

1 We have 1241 males who have not
2 been transfused, they're easy to enroll, but
3 we have, so far, only 749 males who have been
4 transfused, and as you can see, if only 2
5 percent of our, 3 percent of our males have
6 been transfused, it's hard to find these
7 people when donors come to mobiles.
8 So we've had to do recruitment
9 through special efforts to find male donors
10 with a previous history of transfusion.
11 That's why the enrollment is ongoing.
12 Whereas the enrollment of the first two
13 groups, we just approached donors coming in
14 at multiple collection sites determined by
15 the individual blood centers, and these were
16 based on ease of logistics as well as trying
17 to get a representative demographic mix of
18 donors that do visit those particular blood
19 centers.
20 Now we've analyzed the
21 questionnaires from these enrollees and I
22 just want to go through the parity history in

1 the female donors that we've enrolled.
2 So, again, close to six thousand
3 donors. And what I've represented here is--
4 the green bar represents the parity history
5 of all the female donors, not the LAPS
6 enrollees, but the greater than 400--I think
7 300,000 female donors in the database for
8 2006.
9 And this is coming off the
10 question that we ask each female donor at the
11 time that they come in for their donation.
12 The blue bar is also from the
13 REDS donation database. It represents the
14 apheresis female donors and then the gray bar
15 represents the enrollees in LAPS.
16 A couple of things to point out
17 there. Number one, the number of never
18 pregnant females, this is on a donor basis,
19 so of our female donors, about 44 percent
20 have never been pregnant, which is actually
21 much higher than what has been reported in
22 previous papers in the literature about the

1 U.S. donor base, and of course these papers
2 are ten to fifteen years old, and think what
3 we might be finding is the demographics of
4 pregnancy have changed, at least in blood
5 donors, if not in the population, in general,
6 and so we have a lot more females who have
7 never been pregnant.
8 We haven't completed the
9 demographic analysis, but preliminarily, it
10 looks like this is, as you would expect,
11 related to age, that the younger donors are
12 less frequently pregnant than women who are
13 older.
14 You can see, there's a bit of a
15 discrepancy between apheresis donors, in that
16 there are fewer never-pregnant women donors,
17 and then we have the LAPS donors who happen
18 to be mostly whole blood donors but their
19 pregnancy history reflects a little bit more
20 that of the apheresis donors.
21 And then we have about 11 percent
22 of donors who've been pregnant once, about 20

1 to 22 percent that have been pregnant twice,
2 and then anywhere in the general population
3 of donors, about 25 percent that have been
4 pregnant three times or more, and then even
5 more in apheresis donors.
6 And so this is important when we
7 actually get HLA antibody prevalence and our
8 enrollees will have to do a weighted average
9 to say what it is would be in the overall
10 database.
11 So I want to give you the status
12 of the actual testing now. We've completed
13 the screening assay, HLA screening assay on
14 about 4700 samples. The supplementary single
15 antigen assay testing is in progress. We
16 only have very few results on that and the
17 data analysis is still in progress.
18 But I do want to give you some
19 preliminary observations. It's interesting,
20 the package insert for the One Lambda
21 LABScreen test has a statement, that they
22 suggest using the cutoff, their normalized

1 cutoff ratio of 2.2, but at the same time,
2 they say that each individual laboratory may
3 need to determine their own cutoff.
4 It's also interesting that the
5 data from previous studies, which comes from
6 generally in-house validations, and they go
7 to a population, they expect not to have any
8 HLA antibodies, so a non-alloimmunized
9 population and get the cutoff value, btu
10 those populations are much, much smaller than
11 the group we've screened from, from LAPS.
12 And so another thing to recognize
13 is the cutoff, as recommended by the
14 manufacturer, was developed in the context of
15 using this assay system to maximize
16 sensitivity, particularly in the organ
17 transplant setting.
18 So they would screen patient
19 sera, and they were looking for patients who
20 had HLA antibody, and clearly, if a patient
21 who had HLA antibody was then given an organ
22 with the corresponding HLA antigen, you would

1 be worried about an anamnestic response, so
2 that any indication of previous immunization
3 would lead to the potential for organ
4 rejection.
5 But I want to point out that this
6 is very different from the way we might be
7 using an assay to determine the safety of a
8 donor in a TRALI situation, because here,
9 we're talking about passively transfused
10 antibody causing the pathology.
11 And so it may not be important to
12 detect antibody at very low tiers, cause it's
13 all going to get diluted out in the person's
14 own bloodstream. So here's all I can say
15 about the data right now. Using an NBG ratio
16 of 2.2, we're finding HLA antibody in a
17 significant proportion of apparently non-
18 alloexposed persons. So non-transfused males
19 and never- pregnant females. And
20 interestingly, if you go back to the one
21 paper that everybody quotes about this
22 phenomenon, which was published in about 2000

1 out of St. Louis, in apheresis donors, they
2 used lymphocytotoxicity and they showed a 7.8
3 percent incidence of HLA antibodies in never-
4 pregnant females, and our findings at this
5 lower NBG ratio are in that order of
6 magnitude.
7 However, we haven't yet done
8 confirmatory testing, and as I said, if we
9 choose a higher NBG ratio, so set the cutoff
10 higher, than the rate significantly
11 decreases, and we're doing some analyses to
12 see where the cutoff really should be set,
13 and this is highly dependent on the
14 confirmatory assay results.
15 And same thing as you would expect
16 with any assay, that again, depending on
17 where we set the cutoff, we'll also see a
18 different rate of HLA antibodies in females,
19 depending on their level of pregnancy, and we
20 are seeing a correlation, as we would expect,
21 of the greater the number of pregnancies, the
22 greater the amount of HLA antibody,

1 regardless of whether the donor is--
2 regardless of the cutoff we use.
3 So our thoughts now are that the
4 same concerns about assay sensitivity, which
5 we're demonstrating in the Luminex system,
6 which is how sensitive do we want to be, will
7 also apply to other HLA detection systems.
8 And so as of now, it's unclear if
9 antibody with low signal strength is of
10 significance for the safety of transfusion
11 recipients, and it's unclear how such
12 information should be used to make donor
13 deferral decisions, even if we're adopting
14 the precautionary TRALI risk reduction policy
15 that says we want to minimize transfusion
16 from HLA-immunized or leukocyte-immunized
17 donors.
18 One other aspect of this study I
19 wanted to mention is we are establishing a
20 repository. We have anywhere from four to
21 six aliquots of plasma frozen, two aliquots
22 of whole blood, and in many cases two

1 aliquots of serum. These are stored at minus
2 70 degrees Centigrade. We'll be able to
3 access these for neutrophil antibody testing
4 and maybe neutrophil antigen or HLA antigen
5 testing, if we decide to do it.
6 We can also access these for HLA
7 antibody testing using other test systems,
8 provided we get the funding to do that, and
9 we can actually recall donors if we want to.
10 And then my final slide is to tell
11 you what we're planning to do. In addition
12 to analyzing the HLA antibody data, we will
13 do neutrophil antibody testing. We are
14 planning to do some HLA titering studies to
15 see how strong some of these antibodies are,
16 we'll look at the MICA data, and we may do
17 alternate HLA tests.
18 And finally, we are far advanced
19 in a planning stage of what we're calling
20 LAPS II, which is a clinical study in which
21 we will identify previous products donated by
22 these persons with HLA antibody, and then go

1 back to recipients of high plasma volume
2 components from these donors, well, not to
3 the recipients but to their charts, over the
4 last few years, and through our triage
5 protocol of looking at their x-rays first,
6 decide whose medical record to review, and
7 see if we can document whether people with
8 leukocyte antibodies resulted in the
9 production of more TRALI than donors who did
10 not have leukocyte antibodies. So that's in
11 the planning stage currently. Thank you.
12 DR. SIEGAL: Thank you. Are there
13 any questions?
14 DR. KLEIN: Steve, thank you for
15 sharing the data with us. I'm not surprised
16 about the frequency of the female, nonparous
17 women with this sensitive Luminex assay. In
18 fact, I'm surprised it was as low as it is.
19 But we know that history is sometimes
20 misleading and spontaneous abortions occur.
21 Do you have any data to share with
22 us about male screening at this point,

1 because clearly, that's a big issue,
2 especially for apheresis platelets?
3 DR. KLEINMAN: Yes. A couple of
4 preliminary observations. If you look at any
5 specific cutoff right now, we've looked at
6 two, a ratio of 2.2 and a ratio of 10, and if
7 you take a preliminary look at the screening
8 data and look at the frequency of antibodies
9 in never-transfused females, never-pregnant
10 males, never-transfused males, and transfused
11 males--now we only have data on about 250
12 transfused males--the numbers in those three
13 groups are, at a particular cutoff are fairly
14 comparable to one another.
15 So it doesn't look like
16 transfusions--again this is preliminary--but
17 it doesn't look like transfusions in the male
18 donors has caused much additional HLA
19 antibody to the background rate of
20 transfusions of antibody in never-transfused
21 males.
22 So it doesn't look like

1 transfusion's a very powerful stimulus, at
2 least in the amount that our donors have been
3 transfused. But this is preliminary, Harvey.
4 DR. KLEIN: But it looks like
5 about 5 percent of your males are positive by
6 this assay transfusion in this; correct?
7 DR. KLEINMAN: Well, it depends
8 where we set the cutoff. If we set the
9 cutoff down at 2.2, then it looks like again,
10 without confirmatory data, somewhere about 7
11 to 8 percent. But, again, confirmatory data
12 will probably bring that down, and we haven't
13 looked at the strength of antibody. If you
14 go up to a much higher ratio, in the ten
15 range, then we're down to 1 to 2 percent.
16 DR. KATZ: Steve, do you have the
17 year of transfusion in these histories, or--
18 DR. KLEINMAN: We do have--
19 DR. KATZ: --whether they got LR,
20 or what they were transfused--
21 DR. KLEINMAN: We do have the year
22 of transfusion. We haven't analyzed on it

1 yet. And again, we only have data on 250
2 transfused males. We have 750 enrolled,.
3 We're continuing with enrollment and hope to
4 get to a thousand. So, again, it's a very
5 partial data set and, you know, we hope to
6 have a lot more data in the next few months.
7 DR. SIEGAL: Are you looking at
8 the women in terms of how many sexual
9 partners they've had as--
10 DR. KLEINMAN: No; there's no
11 history of sexual partners. We're not taking
12 a history of sexual partners. It brings up
13 an interesting question that we've tried to
14 ask the experts, and if you really haven't
15 been alloimmunized by pregnancy or
16 transfusion, is it possible to have HLA
17 antibody based on immunization, based on
18 sexual contact with partners? I mean, is
19 that an immunizing event for HLA antibodies
20 that could explain this background rate?
21 Apparently nobody knows the answer to that.
22 Or could this background rate be

1 cross-reactivity against other antibodies,
2 perhaps through exposure to vaccines that
3 might have been grown in cell lines,
4 etcetera, and, you know, we know that's
5 possible, people have reported that as
6 possible, but we really don't have an expla--
7 or is it just, you know, false positive stuff
8 that nobody, as with many assays, that
9 nobody's ever able to identify the cause?
10 So we really don't know at this
11 point. But we don't have that data to be
12 able to evaluate it.
13 DR. NELSON: This is really a
14 fantastic study. It seems, though, that the
15 sample size is going to be too small,
16 probably, if the rates are one in a thousand
17 and one in ten thousand, and you've got 5000
18 people to actually identify, link antibodies
19 with TRALI cases, and I wondered if there was
20 thought about doing a case control study in
21 the REDS, where you might be able to save or
22 collect, since the event occurs within a few

1 hours of the transfusion, and actually link
2 the antibodies with the TRALI event?
3 DR. KLEINMAN: Yes. Two comments
4 on that, Ken. With regard to the sample size
5 and the look-back study, depending on the
6 assumptions you make, and obviously that's
7 always true for sample size calculations, we
8 think that we might have an adequate sample
9 size to do the study, if you think that TRALI
10 actually occurs at some of the higher rates
11 that people are predicting.
12 With regard to doing a case
13 control study, going forward, there is an
14 NHLVI-funded SCCR, you know, Specialized
15 Clinical Center of Research, or something
16 like that, that Pearl Toy's group has at
17 UCSF, in conjunction with Mayo Clinic, and
18 that is one of their protocols.
19 They have, I think, gone through a
20 year of enrollment of TRALI patients, and
21 controls, where they're actually collecting
22 ever donor unit and analyzing it, and I think

1 the reason they're not here is because it's
2 just too early to have enough data to be
3 meaningful.
4 But I think that's the stud that
5 will look at it in the appropriate scientific
6 control fashion. The look-back is the best
7 we think we can do within the REDS context.
8 DR. GLYNN: Steve, could you give
9 us the estimates for the women who were
10 pregnant, who have been pregnant before?
11 DR. KLEINMAN: Well, you know,
12 it's a dilemma on how much data to present
13 because we really haven't, you know, it's
14 really an analysis in progress, and if this
15 meeting was two months from now, I think we'd
16 feel much more confident about presenting
17 actual numbers.
18 But, again, depending on where you
19 set the cutoff--I guess a few things I can
20 say. It's more common to find HLA class 1
21 antibodies than HLA class 2 antibodies by
22 about threefold, and it's actually not that

1 uncommon to have both HLA class 1 and class 2
2 in the same person.
3 With regard to numbers, they
4 certainly are comparable, if not higher to
5 what's been reported in the literature, and
6 that has been about 15 percent in people with
7 one to two pregnancies, and greater than 25
8 percent in people with three or more
9 pregnancies.
10 So, again, if we use the lower
11 cutoff value, we're finding numbers that are
12 as high, probably higher than that. But we
13 really would like to have our confirmatory
14 data analyzed, because I think if somebody
15 has antibody on the screening system and
16 then, quotes, it's positive on the
17 supplemental test, while it doesn't
18 necessarily confirm it, it tells us that
19 there's reactivity against a specific HLA
20 antigen and not other HLA antigens, and
21 whether that makes it HLA antibody or cross-
22 reactivity, we still don't know, but I think

1 that's really the crux of the data that'll be
2 more solid.
3 DR. WILLIAMS: Alan Williams, FDA.
4 Two quick donor management questions.
5 What has been the experience in
6 administering the NHANES pregnancy question?
7 Put differently, is it feasible in a blood
8 donor setting, and secondly, are donors being
9 given the results of the antibody testing?
10 DR. KLEINMAN: So the first
11 question about administering the
12 questionnaire, I really can't answer. I
13 don't think it's been a problem in--well, it
14 has been easy to administer in the research
15 setting but whether that would be practical
16 to ask six questions in the normal donor
17 setting, I don't know, because they're
18 actually given a questionnaire that they fill
19 out, a separate questionnaire.
20 Clearly asking the two additional
21 questions at the time of donor interview,
22 which we've done in REDS I and REDS II, that

1 I think has been a very transparent and easy
2 process.
3 So if you just add a question,
4 have you ever been pregnant?, and then, if
5 so, how many times? But that doesn't go into
6 sort of the details between miscarriages,
7 abortions, and the advantage of NHANES is
8 you're telling people that you're really
9 interested in not only live births but, you
10 know, any other pregnancy episodes, and we're
11 not doing that on the short form. So I don't
12 know how feasible that is.
13 With regard to giving results back
14 to donors, that was sort of an issue of great
15 debate at the time of taking the protocol
16 through to IRBs and the decision was left to
17 each center, but I think at the time that the
18 research study was approved, most people felt
19 that HLA antibodies results would not be
20 meaningful to give back to donors because
21 they really had no health significance, and
22 at that time they had no consequence on your

1 donation eligibility.
2 But, on the other hand, if we
3 found donors with granulocyte antibodies,
4 especially certain specificities, most people
5 felt that donors should be told that, so the
6 consent that donors sign has said you may be
7 given your test results if deemed to be of
8 medical significance.
9 Thus far, since we don't have
10 confirmatory data back, except on a handful
11 of people, we haven't actually given any
12 donors' results yet.
13 DR. SIEGAL: Ms. Baker.
14 MS. BAKER: So the questions on
15 pregnancy history in the REDS database, they
16 are or are they not the six NHANES validated
17 questions?
18 DR. KLEINMAN: No. The NHANES
19 validated questions are only for the 6000
20 women who enrolled in the LAPS study. The
21 other, the three to 400,000 women who've been
22 asked the pregnancy questions, I don't

1 remember the exact questions but I think
2 they're essentially, number one, have you
3 ever been pregnant? and number two, how many
4 pregnancies have you had? And I think they
5 give either an oral answer or fill in
6 something. So it's less substantial in the
7 NHANES.
8 DR. SIEGAL: Dr. Kleinman, thank
9 you very much. I think perhaps the other
10 questions could be private. We're running an
11 hour behind.
12 DR. NELSON: Just one quick. Did
13 you ask about the mode of delivery, C-section
14 versus--
15 DR. KLEINMAN: No.
16 DR. NELSON: Cause that might be
17 interesting as to when sensitization might
18 occur.
19 DR. KLEINMAN: Sure. Well, I
20 mean, it's the same issue, you know, how many
21 questions can you ask people in a quick
22 intake interview and we just tried to hit the

1 highlights.
2 DR. SIEGAL: I'd like at this
3 moment to suggest that we take a break since
4 we're a half an hour behind and continue. We
5 don't, at the moment, have any open public
6 session speakers, so we may be able to make
7 up some time. But we are an hour behind, so
8 let's make this break quick. Are there any
9 questions to that?
10 [No response]
11 DR. SIEGAL: Okay. Let's do it.
12 [A recess was taken from 10:59
13 a.m. to 11:13 a.m.]
14 DR. SIEGAL: All right. All right.
15 So to restart, we have Dr. Richard Benjamin
16 from the American Red Cross, talking about
17 their experience with TRALI.
18 Dr. Benjamin.
19 DR. BENJAMIN: Good morning, and
20 thank you to the committee for the
21 opportunity to present the American Red
22 Cross's experience with TRALI. In the

1 beginning of 2003, the Red Cross instituted a
2 nationwide hemavigilance program that we call
3 the Donor and Recipient Complication Program.
4 The single most common adverse
5 event reported to this program is TRALI. In
6 the period from 2003 to 2005, there were some
7 550 reports of TRALI, with increasing
8 incidence during this period. This data is
9 now published in Transfusion this month by
10 Dr. Anne Eder and most of my presentation is
11 taken from this publication.
12 Of those 550 reported TRALIs over
13 three years, there were 72 fatalities, and we
14 can see here that in 2003 we had 17 fatal,
15 suspected TRALI fatalities reported. It rose
16 to 33 in 2005, and we had another 33 in 2006.
17 We did an analysis of these fatal
18 cases. We focused only on the fatal cases
19 because the data tended to be more complete
20 and the investigations were better in the
21 fatal cases.
22 We did a retrospective review

1 using three of our transfusion medicine
2 specialists to go through the 72 cases, and
3 basically they classified them as probable
4 TRALI or unrelated cases.
5 They decided, without looking at
6 the antibody results, that about 38 of them
7 were probably TRALI. We then looked at the
8 antibody data and about 75 percent of these
9 probable TRALI cases, there had been a
10 antibody-positive female donor involved in
11 the case.
12 I should point out that the
13 antibody testing in the study was not
14 standardized. It was performed in multiple
15 labs, in multiple hospitals, and, in fact,
16 only in a minority of cases were the
17 recipients actually typed.
18 So only about in 20 to 25 percent
19 of cases did we actually have cognate
20 recognition. So I would not use this data to
21 prove that antibodies cause TRALI. That's my
22 point here.

1 Anyway, 75 percent did have
2 antibodies, and we document that here.
3 When we looked at the components
4 that were involved in the TRALI cases, and I
5 think about a third of them, there was a
6 single unit transfused within six hours of
7 the onset of TRALI, so a good third, that
8 clearly we could say that unit caused the
9 TRALI.
10 The units most commonly involved
11 were plasma units, 24 cases out of the 38,
12 and 18 of those cases had a antibody positive
13 female donor involved.
14 That's where we get our
15 conclusion, that if we intervene with respect
16 to plasma, we could prevent six fatal cases a
17 year from the 18 cases shown in this figure.
18 There were five fatalities
19 implicating apheresis platelets and seven red
20 cells, or with red cells.
21 Clearly, we transfuse many more
22 red cells than we do plasma and platelets, so

1 we converted these data into rates, and the
2 rate, clearly, for plasma, was much higher
3 than any other component. But one in 200,000
4 distributed units of plasma was implicated in
5 a TRALI fatality.
6 About one in 300,000 apheresis
7 platelets and for the other components, it
8 was in the range of one in two million
9 components implicated in a fatality.
10 We looked at the odds ratios
11 comparing plasma to red cells with an odds
12 ratio, highly significant, of 12.5.
13 Apheresis platelets versus red cells, odds
14 ration of 7.9. The difference between plasma
15 and pheresis platelets was not significant
16 but clearly a strong trend.
17 We then correlated that with the
18 amount of plasma in these components and
19 plasma and apheresis platelets both contained
20 about 250 or 300 mls of plasma. The other
21 components are clearly less than 50 mls, on
22 average.

1 We also looked at the proportion
2 of female donors in these components and most
3 of our components have about half and half
4 male and female, except for apheresis
5 platelets, that are 65 percent from male
6 donors and 35 percent from female donors. A
7 conclusion from these data was that fatality
8 appeared to be correlated with the amount of
9 female plasma exposure in the system.
10 The Red Cross felt that they
11 needed to intravene and do something about
12 these fatalities, and clearly, we wanted to
13 focus on the plasma and apheresis based on
14 these data.
15 We're also very aware that we
16 transfuse way more red cells than we do
17 plasma and platelets, and that we had no way
18 of intervening to do anything about red cells
19 at this point in time. So if we intervened
20 on plasma, at best, we could address about 60
21 percent of the problem. We are not going to
22 completely avoid TRALI by any intervention

1 around plasma or platelets but we could get
2 to the majority.
3 So it's going to be a partial
4 solution, whatever we do.
5 What were the possible solutions?
6 I think you've seen this slide before.
7 Well, clearly, Dr. Sarode talked about the
8 appropriate use of products. We've spoken
9 about the selective use of male products,
10 donor histories, testing, pool and store.
11 All blood-derived platelets have been
12 apparently less frequently involved in TRALI.
13 We're talking to manufacturers about
14 platelet additive solutions to reduce the
15 amount of plasma in apheresis platelets, and
16 we recognize that solvent detergent plasma
17 could be attractive from the TRALI point of
18 view.
19 I do want to emphasize what Dr.
20 Sarode said about appropriate use of blood
21 clots. Of the 24 fatalities that implicated
22 plasma, twelve of them, the plasma was

1 transfused for reversal of coumadin, and in
2 six of those twelve, there was no indication
3 that the patient was bleeding at the time.
4 So when we did a clinical
5 correlate, we tried to look at the clinical
6 correlates of these fatalities, the only
7 thing that stuck out was coumadin reversal.
8 So I do want to emphasize that that is
9 probably the single best intervention to
10 reduce TRALI. Maybe to reeducate our
11 physicians about the use of plasma for
12 coumadin reversal.
13 Having said that, we embarked upon
14 asking the question for plasma, at least,
15 could we selectively use male products?
16 Within the Red Cross system, we produce just
17 over 5 million units of plasma from whole
18 blood. We transfuse about a million and a
19 half as transfusable plasma and the rest are
20 sent to fractionation, either as less than 24
21 hours or greater than 24 hours plasma.
22 We have this vast pool of plasma

1 for fractionation less than 24 hours, and it
2 occurred to us that we could start simply
3 swapping out the male plasma that was sent to
4 frac, at least initially with the FP24, the
5 24-hour plasma that we were making, and later
6 on, with the FFP.
7 So we undertook a pilot, where, in
8 October of last year, in three of our
9 divisions, where we attempted to make
10 predominantly male plasma, FP24, plasma
11 frozen within 24 hours, and we did this
12 really to assess the manufacturing issues,
13 not the clinical efficacy.
14 Essentially, we labeled the
15 products at donation with a blue sticker or a
16 pink sticker, or an M or an F, you know, a
17 very crude system, and when they came into
18 manufacturing, we triaged them for
19 fractionation versus useful transfusion. It
20 was instituted as a business practice. The
21 SOP was one page long. The final product was
22 not labeled, nobody was notified, and if we

1 had shortages of plasma, which did occur
2 around the West Nile season, and around AB
3 plasma, we would use female plasma.
4 We did succeed in getting to
5 greater than 95 percent male plasma in the
6 divisions where we tried this, quite
7 successfully, and we continue to do that for
8 FP24. The system is feasible in the Red
9 Cross system.
10 So with the discussions that we've
11 had with the AABB and the ABC, and the
12 bulletin that came out last November, we are
13 moving to address the issue of plasma in the
14 Red Cross system. Right now, 95 percent of
15 our plasma comes from whole blood donations
16 and about 5 percent from apheresis.
17 For the whole blood plasma, that
18 includes FFP, FP24, and cryo pool plasma. It
19 is our intention to move to greater than 95
20 percent male by November of this year. We
21 would like to get to the goal of a 100
22 percent but we recognize that availability is

1 going to be an issue, and, in fact, right
2 now, we only collect enough AB plasma to get
3 to 92 percent male.
4 So we are certainly going to have
5 to do some selective recruitment and some
6 active intervention to get to 95 percent.
7 It is our intention to move to
8 more FB24 as opposed to FFP. We could,
9 indeed, make male-only FFP but there are
10 significant costs involved in doing that and
11 one of the drivers from the start here, has
12 been to do this at minimal cost to our
13 hospitals.
14 So in our hands, in fact, FFP will
15 become a specialty product. We do not intend
16 labeling any products with donor gender. For
17 apheresis plasma or the Auto-C, we will be,
18 we have a few regions that produce quite a
19 lot of Auto-C plasma, and they will be going
20 forward with a donor history and testing
21 strategy for apheresis platelets and the
22 concurrent plasma, which is a very small part

1 of our plasma.
2 We will be looking at history and
3 testing, but, again, for November of next
4 year, not this year. So that's the strategy
5 we're taking at this point.
6 I want to emphasize this change of
7 moving from FFP to FP24. Right now, we're at
8 about 55 percent FFP, 45 percent FP24. We
9 have shifted, over the last five years, from
10 27 percent FP24.
11 We are going, by November of this
12 year, to have to go to the majority of our
13 plasma to be FP24. I want to point out that
14 of our 35 regions, seven regions at this
15 point in time, distribute a 100 percent FP24,
16 including our second largest plasma
17 distributor, which is our Greater Chesapeake
18 and Potomac Region, which is Baltimore and
19 Washington, only distribute FP24 at this
20 point in time.
21 So some major institutions, such
22 as Johns Hopkins, have found this perfectly

1 acceptable.
2 What about apheresis platelets?
3 Well, this shows all of our apheresis
4 procedures with platelets, platelet products
5 and platelet plasma products. We are at
6 about 67 percent male on our apheresis
7 platelet donors at this point, so we like
8 to think we're already at predominantly male
9 platelets, but clearly, we could like to do
10 something about the female donors here and it
11 is our plan to go to a history and testing
12 strategy for platelets.
13 So for apheresis products, for
14 platelets, free storage AquaDose, whole
15 blood-derived platelets have been piloted and
16 the pilot is being successful, and is coming
17 to an end as we speak, so we will be
18 developing that as an alternative to
19 apheresis platelets.
20 As we lose donors, we may have to
21 fill up the gap with AquaDose platelets. For
22 apheresis platelets, we assume we must test

1 and we assume history will play a role. But
2 having been involved in the lab study, we
3 really do believe we need this data before we
4 make decisions around what to do, concerning
5 transfusion or pregnancy. I'm particularly
6 concerned about any data that might suggest
7 that untransfused males or females may have a
8 background of antibody. If so, would that
9 mean, ultimately, we need to test everybody?
10 I don't know. I think this needs to be
11 resolved and REDS II, the lab study is very
12 timely and we will wait for that data before
13 making firm decisions, and I do hope the
14 committee will too.
15 In terms of testing, we are faced
16 with the issue that there are no licensed
17 tests available for donor screening, that are
18 licensed for donor screening for HLA or HNA
19 antibodies.
20 In fact I'm not aware of any
21 suitable technology for routine screening for
22 neutrophil antibodies on a large scale. We

1 are working with manufacturers for HLA
2 antibodies. We are aware of at least two
3 that are developing automated systems for
4 this, and that may facilitate the HLA
5 screening.
6 But there will be uncertainties.
7 What is the appropriate screening strategy?
8 How do we define a relevant positive? Are
9 low titer, low, verity antibodies relevant or
10 not?
11 What's the appropriate donor
12 deferral? Right now, we intend switching
13 donors that are positive to red cells and
14 fractionated plasma. We don't intend turning
15 anybody away and we're certainly not going to
16 evoke the wrath of female donors by, in any
17 way, turning them away. In fact, we're
18 encouraging them to donate as much as
19 possible.
20 If you find an antibody-positive
21 unit, what's the appropriate recall or
22 withdrawal strategy? Again, we don't know.

1 And final slide, in summary, we do believe,
2 at this point, that prudent measures to
3 reduce patient exposure to alloantibodies may
4 reduce risk and are wise.
5 We do recognize that availability
6 issues around AB plasma and apheresis
7 platelets argue against gender labeling on
8 the use of only male products and we want to
9 emphasize that there are uncertainties still
10 around etiology of TRALI, the lack of
11 antibody screening technology and the
12 understanding of specific antibodies, that
13 really does argue against universal history
14 or antibody screening at this time.
15 The interventions we're putting
16 forward are only going to intervene for 60
17 percent of the fatal TRALIs that we've seen,
18 since we are not yet addressing the red cell
19 issue. So we need to be aware that we can't
20 get to zero, we need to do the best we can
21 with what we've got. Thank you. I'm happy
22 to take questions.

1 DR. SIEGAL: Thank you, Dr.
2 Benjamin. Any questions?
3 DR. KLEIN: Richard, I know for
4 many members of the committee, the concept of
5 FP24 is probably a normal one. So could you
6 tell us whether there are clinical
7 implications of switching to FP24 from FFP.
8 DR. BENJAMIN: FP24 essentially--
9 let's first understand what the difference
10 is. To make FFP, you have to get the blood
11 back from a drive and frozen within eight
12 hours, which means that if you're running a
13 blood drive, you have to shuttle the blood
14 back to your manufacturing center, and you
15 have to have people waiting on that box of
16 blood to process it immediately and freeze
17 it. There are costs involved in that.
18 Blood centers prefer to wait till
19 the end of the drive, get the whole box of
20 blood back into the second or third shift of
21 the day, and process everything in one go,
22 and generally the blood is frozen within 16

1 hours of collection.
2 We know from studies, that the
3 only factor that is decreased in FP24 is
4 Factor VIII. From the time of collection to
5 eight hours, you lose about 20 percent of
6 your Factor VIII. By 24 hours, you lose
7 about another 17 percent.
8 So there's about a 17 percent
9 difference in Factor VIII levels between FFP
10 and FP24. We still have greater than 60
11 percent Factor VIII. According to the circle
12 of information, they are used
13 interchangeably, except for the replacement
14 of Factor V and Factor VIII. Well, we don't
15 use plasma to replace Factor VIII, and I
16 think the Factor V description is antiquated.
17 It's quite clear that there's no difference
18 in Factor V levels in FFP and FP24.
19 Many centers use 24-hour plasma
20 interchangeably with FFP, and some cities, as
21 I said, Baltimore, are at a 100 percent and
22 see no difference at this point in time.

1 DR. KATZ: I think there's one
2 other thing, particularly for the clinicians
3 to understand, that some of us were waiting
4 till January to switch to FP24, which my
5 center has done, because we were waiting for
6 the data on Adams PS and TTP. For many of
7 us, the really clear indication for plasma
8 transfusion has been TTP.
9 The confirmation that Adams PS13
10 levels are preserved in FP24 was critical.
11 DR. SIEGAL: Okay. Is there a
12 comment in the back?
13 DR. SARODE: At what temperature
14 do you store whole blood when you're
15 transporting for FP? Is it 4 degrees?
16 DR. BENJAMIN: Excuse me?
17 DR. SARODE: Whole blood when
18 you're transporting from collection site to
19 the blood center.
20 DR. BENJAMIN: We are required to
21 place the blood in a container, so that it
22 moves progressively towards storage

1 temperatures which are, you know, four to
2 ten. So it's placed on ice. How long the
3 blood actually takes to get down to 4
4 degrees, I don't know that.
5 DR. SARODE: So are you going to
6 collect pooled platelets from that particular
7 product, because for that you cannot keep
8 your whole blood on ice.
9 DR. BENJAMIN: Right. So if
10 you're making random whole blood-derived
11 platelets, you'll have to treat them
12 differently. You absolutely have to shuttle
13 those back. But then you're using some of
14 the plasma to make the random go in a
15 platelet. So that's not the ideal product
16 from to make FFP.
17 DR. SARODE: So basically you'll
18 get two products out of whole blood.
19 DR. BENJAMIN: You are, but you're
20 taking some of your plasma for the random
21 donor platelet.
22 DR. SARODE: And your comment

1 about decreasing Factor VIII, I mean, we had
2 done the study that showed that Factor VIII
3 is the only factor which reduces for, you
4 know, 4 degrees, significantly. Factor V is
5 stable for up to five days. So it's not an
6 issue.
7 And most of these patients who
8 need, actually, plasma, they have very high
9 levels of Factor VIII because it's an acute
10 phase reactant. So all these questions with
11 cirrhosis and all, they have Factor VIII, 200
12 and 300 percent. So that's how we convince
13 our clinician to use plasma.
14 DR. BENJAMIN: Thank you for the
15 slide you showed this morning of a number of
16 patients that had such high Factor VIII
17 levels. I will be asking your permission to
18 use that slide because it's a wonderful
19 demonstration that Factor VIII is an acute
20 phase reactant, that it goes up in ill
21 patients, it doesn't go down.
22 DR. WILLIAMS: A comment and a

1 question. The comment is I believe that most
2 of the plasma frozen within 24 hours, that
3 was being referred to as whole blood-derived,
4 which is mentioned in the circular of
5 information, can also be prepared from
6 automated or apheresis procedures, but this
7 currently is not a licensed product, and we
8 encourage submission of data to help validate
9 this, and have it become a licensed product
10 in the future.
11 The question is looking at the
12 differences in the incidence of TRALI between
13 single donor platelets and plasma, where the
14 amount of plasma is similar, most likely the
15 gender differences play a role, but I don't
16 have a good sense for the deferral of donors
17 with prior, implication in prior TRALI cases,
18 given that the apheresis platelet donors are
19 a very multi repeat donor population.
20 Could that possibly be a factor as
21 well, or is it probably too small?
22 DR. BENJAMIN: I haven't looked at

1 the data but my gut feeling is it's such a
2 small number, that it would have no impact.
3 DR. WEINSTEIN: I was wondering,
4 do you know if the von Willebrand factor is
5 also affected by being in contact with cells
6 for up to 24 hours?
7 As I understand it, you do not
8 separate the plasma from cellular material,
9 potentially up to 24 hours. It's not only
10 the Factor VIII that can be affected. It's
11 also von Willebrand factor multimers, and
12 this can potentially have an effect on the
13 manufactured product that you're making from
14 this material.
15 So has that been examined?
16 DR. BENJAMIN: I'd have to go back
17 and see which assays they actually used in
18 the published work, whether they were
19 actually measuring Factor VIII or von
20 Willebrand, but I'm assuming that it would go
21 down together with Factor VIII.
22 DR. WEINSTEIN: [off-mike] and

1 affecting multimer size, in particular You
2 may find that that's a factor.
3 DR. EPSTEIN: Thank you, Dr.
4 Benjamin. You mentioned the potential use of
5 screening for HLA or HNA antibodies for the
6 subset of donors for whom you need an
7 apheresis platelet, and you flagged that you
8 might make that decision, or at least look at
9 it based on the result of the REDS II LAPS
10 study.
11 So this is really a question for
12 Steve Kleinman, which is in that study, if I
13 understood it correctly, you're only going to
14 be looking for the anti-neutrophil antibodies
15 in subjects who already have a positive HLA.
16 And those two things may not
17 correlate very well, and based on what we
18 heard from Dr. Stroncek, the "bad actor" is
19 more likely to be the anti-neutrophil
20 antibody.
21 So my question to you is whether
22 there's been any consideration within REDS of

1 adding testing for anti-neutrophil antibodies
2 and, again, I would mention that Sheryl
3 Kochman pointed out that FDA has cleared at
4 least one assay for HNA antibodies, including
5 HNA 1, 2 and 3. So there's at least one
6 approved assay that could be used and I'm
7 sure there are other experimental assays.
8 So I'm just concerned that we
9 "might be missing the boat," you know, that
10 we're going to learn a lot about HLA from
11 REDS II LAPS, but it's relevance to TRALI
12 might be constrained for lack of studying the
13 anti-neutrophil antibody, and then given the
14 Red Cross interest, you know, maybe there's
15 role for cooperation.
16 DR. KLEINMAN: Yes. Jay, just a
17 couple comments on that. That is really a
18 question of active debate within the REDS
19 group and it really just, it comes down to
20 money. You know, with the budget--and I
21 don't know if the NHLBI people are still
22 here, but if they are, that'd be great,