1 So, I think that VEGF may be playing some
2 role in capillary leak.
3 [Slide.]
4 The other candidates, biological response
5 modifier that Chris has been looking at most
6 recently, and this is his very latest data, is
7 interleukin 18. This is an interesting cytokine
8 that has been getting recent attention. It is
9 produced by monocytes and macrophages.
10 Interestingly, it is present in the
11 bronchial alveolar lavage fluid in patients with
12 ARDS. It primes the neutrophil oxidase and
13 activates other neutrophil functions.
14 You can see that, and this is unpublished
15 data of Chris, that it does accumulate during
16 product storage, and leukodepletion will abrogate
17 it, and here is evidence that it primes the
18 neutrophil oxidase in the two-hit model.
19 DR. NELSON: Dr. Bushkov, we are about a
20 half-hour behind, and the problem is we can't stay
21 here until 8:30 tonight.
22 DR. BUSHKOV: I will just summarize then.
23 I want to show you what leukodepletion does to
24 these products, which I think is material, and then
25 I will finish.

1 How do we stop this priming activity? We
2 can use the fresh products, red cells under 14
3 days, platelets under 2, we can wash them, we can
4 pre-storage, leukoreduce.
5 You can see that washing will get rid of
6 some of the priming activity, most of it, not too
7 practical.
8 [Slide.]
9 This is priming activity in red cells and
10 leukodepletion will significantly decrease this.
11 The dark bars are the leukodepletion. However, in
12 whole blood platelets, it does not do this.
13 [Slide.]
14 This is just to show you that the priming
15 activity, if you leukodeplete, there is less injury
16 in the pulmonary edema model.
17 [Slide.]
18 In summary, I think there is substantial
19 clinical epidemiological and lab evidence and
20 animal data to suggest that many cases of TRALI are
21 associated with the infusion of biological response
22 modifiers with neutrophil priming activity, that
23 the implicated products in this model are whole
24 blood platelets and red cells.
25 There is much less priming and apheresis

1 platelets and frozen plasma. The priming activity,
2 at least in the red cells, appears to be generated
3 by leukocytes, and it is significantly reduced by
4 pre-storage leukodepletion, however, this doesn't
5 appear to be the case for whole blood platelets.

6 [Slide.]

7 Other biological response modifiers may be
8 playing a role like VEGF and IL-18, and recipients,
9 predisposition does seem to be important.

10 In summary, I think this is a
11 multifactorial complication with recipients and
12 product factors playing a role. I think a lot of
13 TRALI is subclinical and that what we are seeing
14 very often and recognizing is simply the tip of the
15 iceberg.

16 Thank you.

17 DR. NELSON: Thanks.

18 Are there questions or comments?

19 Thank you very much.

20 Dr. Finlayson from the FDA is next.

21 **John Finlayson, Ph.D.**

22 DR. FINLAYSON: Very brief because unlike
23 the other speakers, I have not been working on
24 TRALI. As a matter of fact, until Dr. Holness
25 asked me to speak, I didn't even know how to spell

1 it.

2 [Slide.]

3 I am going to talk about two papers which

4 interestingly enough came out in successive months

5 in Transfusion, one, the Palfi study you have

6 already heard referred to at least twice. The one

7 that I want to concentrate on, which I will simply

8 call Reference 1, is Rizk, et al.,

9 Transfusion-Relate Acute Lung Injury After the

10 Infusion of IGIV. This is a report of a single

11 case, and I shall endeavor to suppress my

12 unsuppressable desire to talk about risk factors.

13 [Slide.]

14 This, as I have said, was a report of a

15 single case, but it was very nicely worked up. The

16 patient was a 23-year-old male with multifocal

17 motor neuropathy. It was decided that he should

18 receive IGIV at a dose of 2 grams per kilogram of

19 body mass.

20 I should point out this is an off-label

21 use, and the dosage, however, is similar to that

22 that is sometimes used in the treatment of ITP,

23 immune thrombocytopenic purpura. It was decided

24 that he should receive this dose on two successive

25 days, which is again often used in the treatment of

1 ITP.

2 So, he was given, because he had a body

3 mass of 91 kilograms, he was given 90 grams of

4 IGIV, which would amount to a dose of very close to

5 1 gram per kilogram over a period of three hours.

6 He was sent home, but within an hour after

7 completion of the infusion, he began to have a

8 series of symptoms which I have listed here - dull

9 chest pressure, shortness of breath, dyspnea,

10 frothy pink sputum.

11 He came back into the hospital. The chest

12 x-ray showed bilateral interstitial and alveolar

13 infiltrates. The laboratory workup indicated,

14 among other things, surface-bound IgG on

15 granulocytes. The conclusion from this was that it

16 was TRALI, and they offered as a possible cause

17 rapid infusion of granulocyte antibody.

18 I would like to now move on to Reference

19 2.

20 [Slide.]

21 Since time is short, I will just go to one

22 sentence from the Palfi reference, which happens to

23 be the very last sentence, which says, "Thus"--this

24 is the one about multiparous donors--"Thus, an

25 optimal approach"--optimal, you know, not just

1 good, optimal approach--"to consider would be the
2 use of plasma from multiparous donors only for
3 fractionation into plasma protein derivatives."
4 Okay. So what I want to do is offer a
5 precaution and then a precaution about that
6 precaution, sort of like if you were here yesterday
7 to hear Elliot Cowan, who said, "On the one hand,
8 and then on the other hand, and then on the other,
9 other hand."
10 Maybe we could just sort of use one of
11 those pieces of paper and look at these one line at
12 a time.
13 [Slide.]
14 TRALI may be under-reported, as several
15 people have indicated, and it may be under-reported
16 as a side effect of IGIV. The converse to that is
17 that additional reports may follow in the wake of
18 the report by Rizk, et al.
19 Now, this may be a good thing because, as
20 I indicated here, perhaps it is an under-reported
21 side effect of IGIV. On the other hand, some of
22 the symptoms, in fact, probably all of the symptoms
23 with the exception of the frothy pink sputum have
24 been reported in the sequelae of IGIV
25 administration in the past, and certainly if it is

1 administered too rapidly.

2 The other possibility, of course, is, as

3 we have heard before, fluid overload. The authors

4 of the Rizk paper described their workup of two

5 lots of IGIV from the same manufacturer, but it was

6 not clear which, if either, of these lots had been

7 given to the patient.

8 One of these was a 5 percent protein

9 solution, the other was a 10 percent protein

10 solution. The manufacturer makes both. If it was

11 a 10 percent solution, it seems to have been

12 administered at exactly the rate that the package

13 insert indicated.

14 So, rapid is a somewhat relative term.

15 So, we have both possibilities, that we may have

16 under-reporting, but we may see a little bit of

17 over-reporting subsequent to this.

18 [Slide.]

19 If TRALI is a true side effect of IGIV,

20 and if TRALI is precipitated by antibodies, be they

21 antibodies to granulocytes or HLA, or something

22 else, or indeed if it is precipitated by other

23 substances in IGIV, then, use of plasma from

24 multiparous donors for fractionation into IGIV,

25 which you will remember was part of the

1 recommendation of Palfi, et al., should not be
2 encouraged because not only could one capture these
3 substances in the IGIV product, but indeed might
4 end up enriching them, that is to say,
5 concentrating them there.
6 That is the end of my presentation.
7 DR. NELSON: Maybe we can take a break now
8 since we are a little bit late for 15 minutes.
9 [Recess.]
10 DR. NELSON: We are now ready for the open
11 public hearing. I think Kay Gregory is going to
12 make a comment from the AABB.
13 **Open Public Hearing**
14 MS. GREGORY: I just thought I would say
15 this morning I am here as myself. In the interest
16 of time, you have already heard a description of
17 the American Association of Blood Banks yesterday,
18 I will just remind you that we represent not only
19 blood collection facilities, but also transfusion
20 services.
21 We appreciate this opportunity to discuss
22 TRALI. The AABB agrees with the FDA that TRALI is
23 a significant transfusion concern and we need to
24 increase awareness of TRALI through broad physician
25 education.

1 We believe it is critical that increased
2 attention be paid to education and to
3 non-infectious hazards of transfusion including
4 TRALI. The AABB has just issued an association
5 bulletin to our members specifically addressing
6 non-infectious serious hazards of transfusion.
7 In this bulletin, we outline the existing
8 state of knowledge regarding a variety of
9 non-infectious transfusion risk including TRALI,
10 and recommends certain courses of action including
11 increased research to reduce their incidence.
12 The AABB strongly urges that steps be
13 taken to better understand the incidence and causes
14 of TRALI, so that we can take appropriate measures
15 to protect patients from this potentially fatal
16 transfusion reaction, however, we believe there are
17 insufficient data available at this time to
18 contemplate large-scale interventions for donors.
19 First, AABB recommends research to define
20 the real scope of the problem. The AABB proposes
21 that a prospective epidemiologic study to establish
22 the incidence of TRALI be undertaken. For example,
23 we propose a multicenter study of acute lung
24 problems in the transfusion setting to assess,
25 evaluate, and analyze all pulmonary reactions using

1 a standardized protocol.
2 We believe that the scientific community
3 can and should define the magnitude and severity of
4 TRALI.
5 Second, we need to understand more about
6 the mechanisms that cause TRALI. The AABB proposes
7 that the NHLBI establish a multicenter study
8 utilizing controlled trials methodology. Clinical
9 researchers should establish the importance of
10 leukocyte antibodies, lipid generation, and other
11 potential causative mechanisms.
12 Such studies should provide the scientific
13 basis for therapeutic or preventive measures. For
14 example, it may not be appropriate to defer all
15 multiparous women. While a high proportion of
16 multiparous women become alloimmunized through
17 leukocyte antigens, often these antibodies are
18 transient and their clinical significance is
19 unclear.
20 Furthermore, controversy surrounds the
21 selection of screening tests, the sensitivity and
22 specificity of such tests in this setting, and
23 their clinical interpretation.
24 In the research setting, it should be
25 possible to evaluate the clinical significance of

1 HLA and granulocyte antibodies, as well as other
2 proposed screening methods.
3 Once the mechanism of TRALI are better
4 understood, the risk factors in donors and
5 recipients may become apparent. When a severe
6 clinical reaction has occurred, an antibody has
7 been identified in the donor, and the recipient has
8 the corresponding antigen, the preventive measure
9 is relatively clear. In such cases, it is
10 generally agreed that blood from that donor should
11 not ever be again transfused to the same recipient.
12 However, it is not so clear that such a donor
13 should be permanently deferred from donating any
14 blood component.
15 The appropriate preventive measures are
16 even less obvious for the majority of pulmonary
17 reactions that occur in the transfusion setting.
18 Finally, more data are needed before
19 establishing additional comprehensive donor
20 deferral for TRALI. We need to understand what
21 proportion of the donor population would be
22 affected by the proposed deferral criteria, so that
23 the potential impact on the blood supply can be
24 evaluated.
25 These data are especially critical today

1 as we face an already shrinking blood supply and
2 potential shortages due, among other causes, to new
3 donor deferrals for individuals who have traveled
4 to the UK and other European countries.
5 Today's blood supply is fragile. The AABB
6 strongly urges the committee and the FDA to
7 consider the risk and benefits of widespread donor
8 deferrals before taking steps that could
9 unnecessarily hinder patient access to the blood
10 they need.
11 Thank you.
12 DR. NELSON: Thank you very much.
13 Questions? Paul.
14 DR. McCURDY: This is more of a comment
15 than a question. Several years ago, I believe it
16 was shortly before I officially retired from the
17 NHLBI, which would have made it about 1997 or 1998,
18 the Division of Lung Diseases had an initiative
19 that was focusing on acute respiratory distress
20 syndromes.
21 I was able to ascertain that one of the
22 examples of an ARDS that could be studied in this
23 was TRALI. What I don't know is whether any of the
24 successful competitors are studying that, and I
25 have essentially lost track of it.

1 On the other hand, I have discussed it
2 briefly with Dr. Nemo, and we will try and see what
3 might be happening along that line.
4 DR. NELSON: Dr. Nemo is here, I saw him.
5 Yes. Could you tell us anything, George?
6 DR. NEMO: [Off mike.]
7 DR. NELSON: I will repeat. Dr. Nemo
8 doesn't know anything at the moment, but he will
9 find out for us. All right?
10 DR. NEMO: Right.
11 DR. NELSON: Thanks.
12 DR. McCURDY: That is what I meant to say,
13 but obviously didn't.
14 [Laughter.]
15 DR. NELSON: Dr. Bianco has a refreshingly
16 brief statement from the America's Blood Centers.
17 DR. BIANCO: We are trying to contribute
18 to the schedule here. It is brief, but ABC members
19 share FDA concerns about TRALI. While rare, this
20 is a serious and sometimes fatal
21 transfusion-associated event.
22 We agree that FDA should consider
23 interventions to address the issue of TRALI. Thus,
24 the answer to the first question posed to the
25 committee is yes.

1 ABC members also believe that at the
2 present time, and with the present knowledge,
3 further action should be restricted to donors
4 implicated in TRALI episodes as stated in the third
5 option for Question 2.
6 FDA recommendation that blood centers
7 identify donors implicated in a single unit TRALI
8 case or in more than one multiple unit TRALI case
9 seems reasonable. However, ABC members also
10 believe that allowance should be made for clinical
11 judgment about the diagnosis of a TRALI case,
12 because patients who receive transfusions are often
13 very sick, and we heard very good presentations
14 about it today.
15 Many clinical events initially interpreted
16 as TRALI turn out to have other etiologies. Those
17 events should not lead to donor deferral even if
18 the donor happens to have an HLA antibody. To
19 illustrate, a recent report in Transfusion
20 suggested that about one-third of platelet donors
21 are multiparous women and that about one-fourth of
22 these have HLA antibodies. Yet, there were no
23 clinical cases of TRALI associated with 9,000
24 transfusions using apheresis products from these
25 donors.

1 Thus, plasma from very few donors with
2 antibodies to HLA cause TRALI.
3 Regarding Question 2b, if yes for donors
4 with risk factors, what would be appropriate to do,
5 ABC members believe that in most cases donors who
6 are associated with a TRALI event, and have
7 antibodies to HLA, or granulocytes, should be
8 deferred. However, they would like to have the
9 flexibility to use their plasma for non-injectable
10 products or washed with suspended cells.
11 ABC members would not recommend the use of
12 apheresis platelets from these donors because they
13 contain at least 200 milliliters of plasma, and
14 even small amounts of plasma have been reported
15 associated with TRALI.
16 We thank FDA for the opportunity to
17 comment.
18 DR. NELSON: Thank you.
19 Questions or comments?
20 Thanks, Celso.
21 Is there anybody else that wanted to make
22 a comment?
23 [No response.]
24 DR. NELSON: I wonder if we could revisit
25 the questions.

1 **Committee Discussion**

2 DR. HOLNESS: The questions are:

3 1. Should FDA consider interventions at
4 this time to identify donors and/or donations with
5 an increased risk for producing TRALI in a
6 recipient?

7 Shall I read them all or one at a time?

8 DR. NELSON: The second question, which
9 sort of relates, this is: If yes, would it be
10 appropriate to identify blood donors with a history
11 of multiparity, three or more pregnancies;

12 ii. History of allogeneic transfusion,
13 two or more donor exposures;

14 iii. History of implication in a single
15 unit TRALI case or more than one multiple unit
16 TRALI cases.

17 So, the two questions are somewhat
18 related.

19 Question 1 says interventions to identify
20 donors and/or donations with an increased risk for
21 producing TRALI. This is a research question, I
22 guess. It is not so much that such donors would
23 automatically necessarily be deferred or prevented
24 from donating for blood for other uses.

25 DR. SIMON: I think you are hitting at it,

1 that probably there has been sense from the
2 presentations that we need more research and more
3 data, and I at least would be reluctant to
4 recommend that the FDA go forward with guidance or
5 some regulatory action to intervene or preclude
6 certain donations or set up a particular schema.
7 I think, to me, the presentations suggest
8 that while the clinical syndrome per se I think is
9 fairly well defined, it almost seems like an
10 Edmundson at the Mayo Clinic, we had two different
11 phenomenons occurring because one seemed to be
12 related to platelets from what we call random donor
13 platelets or whole blood derived platelets, and the
14 other seemed to be related to a variety of products
15 in both Sacramento and Mayo and the Red Cross
16 series.
17 So, it is not even clear where this is
18 coming from, and then it gets more confused as to
19 granulocytes and different kinds of antibodies, and
20 at least my opinion would be that at the current
21 time, until we can get more research definition, I
22 would not want to advise the FDA to move to any
23 particular set of interventions of regulatory
24 actions.
25 DR. NELSON: I think really what is

1 needed, I think is more and better data
2 particularly since this committee is concentrating
3 on donors, more data with regard to the donors.
4 Now, obviously, this is a complex reaction
5 that may involve donors and recipients, and a
6 match, and, you know, a pathogenesis may--it is an
7 agent/host sort of thing, but this committee really
8 can't, it would be hard for us to make a
9 recommendation that was logical with regard to the
10 recipient, because that is a decision made by the
11 physician as to who should receive the transfusion,
12 but we could sort of make some recommendations with
13 regard to the donor.
14 We have heard also that the Red Cross and
15 ABC currently defers a donor who has been involved
16 in a single unit transfusion, is that right,
17 Celso--and the American Red Cross, as well, so that
18 is pretty much most of the blood collection system
19 in the U.S.
20 DR. SIMON: I think if individual
21 organizations want to proceed with deferrals, I
22 would think the difference between that and between
23 a specific FDA recommendation--
24 DR. NELSON: But given that policy, there
25 is still apparently quite a number of cases that

1 may occur, and the condition is probably
2 under-recognized, and we really don't know.
3 DR. SIMON: It is under-recognized, and it
4 overlaps with other causes of pulmonary distress.
5 DR. NELSON: Exactly. Yes.
6 DR. SCHMIDT: I would like to state that a
7 little differently and say what is needed is more
8 data on patients, and that is not really something
9 the FDA can do.
10 DR. NELSON: I mean the FDA could
11 recommend a careful study that involved the donor,
12 the recipient, and the situation, and I guess
13 rigorously define what TRALI is and excludes cases
14 that may be from something else, you know, maybe an
15 ARDS from an infection.
16 DR. McCURDY: I agree totally that more
17 research is needed. I think the FDA should always
18 consider whether they need to do something, but I
19 wouldn't have a clue as to what they should do at
20 this point.
21 My major recommendation I think is that
22 there ought to be, for this kind of study,
23 partnering between transfusion medicine sections
24 and pulmonologists, because it is the lung people
25 that are going to see the cases, or that they

1 partner with the transfusion medicine or at least a
2 part of it, and I think you are more likely to get
3 data that are going to be useful.
4 DR. NELSON: It reminds me of Pogo by Walt
5 Kelly, where he was saying there is an earthquake,
6 run, but, you know, in which direction. That is
7 the issue here, I think.
8 DR. CHAMBERLAND: I just wanted a
9 clarification from ABC, from Celso. Your
10 statement, as I understood it, you were proposing a
11 recommendation. I was wondering if you had
12 actually surveyed the ABC membership to determine
13 currently what is happening in practice in terms of
14 donors who have been associated with one or more
15 TRALI episodes.
16 DR. BIANCO: We have discussed this
17 extensively in what we call our Scientific
18 Medical/Technical Committee before preparing this
19 statement.
20 All or I would say the vast majority of
21 ABC Centers have standard operating procedure and
22 approach when they receive a report of TRALI, but
23 that is something that is initiated by the
24 hospital, by the transfusion service that will
25 communicate to a blood center that they had a

1 transfusion reaction. That is where the
2 under-reporting is. That is where the diagnostic
3 difficulties is.
4 The blood centers vary in how much
5 information they request from the hospital in order
6 to make sure that this was a diagnosis that they
7 are comfortable with. As a routine, most of them
8 then will try to obtain testing for HLS antibodies,
9 get the donor back, and granulocyte antibodies, and
10 make a decision about the deferral or not.
11 But it is more of a clinical type of
12 approach to investigation, handled by the medical
13 director, then, a procedure that is normal would be
14 used for handling a well-established with the
15 62-page guidance on testing that we just got from
16 Dr. Ruta. It is a clinical approach.
17 DR. CHAMBERLAND: But does ABC have in
18 place something similar to Red Cross?
19 DR. BIANCO: It is blood center-specific.
20 If you recall, ABC is an association, but the vast
21 majority of the blood centers will have standard
22 operating procedures on how to investigate those
23 cases and how to proceed.
24 DR. NELSON: Celso, but I was more
25 impressed with the Mayo Clinic approach where there

1 was really careful and prospective evaluation by
2 transfusion medical specialists as to the
3 administration of the transfusion and probably the
4 data. I mean we really need more data like that I
5 think.

6 DR. BIANCO: I agree, but we need more
7 data in several things and, for instance, Mayo
8 Clinic has a very low rate of incidence related to
9 the wrong blood unit to the wrong patient exactly
10 because of that approach, but if we look at the FDA
11 data, there were 18 deaths a year in this period
12 '90 to '98. They were simply hemolytic reactions
13 that were fatal.

14 So, that is more of the structure of the
15 hospital system and the way we approach that and
16 actually of us dealing with the quality of the
17 product. Process is not necessarily product.

18 DR. BOYLE: From the standpoint of
19 information, there clearly is a problem in the data
20 coming in on transfusion-related incidents because
21 the people themselves are sick and have other
22 comorbidities at the same time, but since we have
23 seen IVIG implicated in a particular case, here is
24 a product who is received, by and large, by
25 otherwise healthy individuals for maintenance.

1 We have no mechanism at the present time
2 to know how common are certainly the less severe
3 forms that we were seeing, that were associated
4 with the same donations that the more severe were,
5 and I certainly would like to know if there is some
6 way we can get some kind of surveillance data, so
7 that we have some sense from a normal population
8 using plasma products of the types and frequency of
9 side effects that are associated with them, so we
10 would know whether we have got a problem there,
11 because I have seen things happen where people have
12 collapsed right after taking their product and have
13 some of these severe things, and the reaction is
14 that is just rate related, you know, in an hour
15 they will be fine, and it doesn't get reported.
16 So, unless you put something in place, you
17 are never going to know whether you have got
18 something boiling out there.
19 DR. NELSON: What could we recommend to
20 the FDA since this is an FDA advisory committee,
21 that would improve that surveillance?
22 DR. BOYLE: Well, I could tell you what
23 would be nice, and that is to set up some kind of
24 sentinel surveillance system where you select your
25 units and get reporting at least over a year

1 period, you know, from each infused case about
2 whether or not certain types of things had
3 happened in a uniform fashion, not leave it up to
4 people to decide what is serious and not, just
5 basically provide the symptoms that follow and have
6 people report in when they get their next infusion
7 whether or not something has developed afterwards.
8 It is not terribly complicated.
9 Potentially, it is not terribly expensive and it is
10 not something--I mean ideally you would have it
11 running on an ongoing basis, but even a one-year
12 period would give you more base rate information
13 than we have right now, and not rely on one
14 anecdotal case that suddenly we are going to change
15 policy.
16 DR. MACIK: I realize that some of the
17 questions I asked earlier were premature because I
18 got a little more information from the talks that
19 went along, but I am still caught with this idea
20 that I think there is something here, I think it is
21 something that is not recognized very often, but I
22 don't feel as a clinician myself that I have enough
23 information to know what is going on.
24 The other problem with this, and talking
25 about getting more data, is there is always the

1 disconnect. It is kind of like the computer is
2 only as good as your input. The transfusion
3 services are only as good as the clinicians up on
4 the floor reporting to them the incidents, and the
5 fact that on the Hem-Onc services, almost everybody
6 is febrile almost every day, and they are getting
7 blood products every day. We do not report all of
8 these to the transfusion service, so, you know, we
9 don't recognize this as being the same problem, and
10 the internist's literature, this isn't something
11 that is frequently talked about. Perhaps it is
12 somewhat more in the pulmonary literature, but, you
13 know, a good number of these patients never got to
14 the intensive care unit. A pulmonologist isn't
15 consulted because it is a transient phenomena.
16 So, I think there is a lot of problem here
17 that we see only the very worst cases and we are
18 making some decisions based on the worst cases.
19 You know, if this is an antibody-mediated problem,
20 unless it is not an IgG antibody, then, why aren't
21 people with IVIG having this all the time, because
22 this is a concentrate from thousands of donors,
23 very concentrated antibody that is given frequently
24 and yet we only had the one case report or probably
25 a few other case reports of this causing a problem.

1 So, you know, in the way I look at it, I
2 think this is something that clearly we need some
3 more information about, we need to understand, are
4 we underestimating is the clinician writing off
5 simply febrile reactions or mild reactions, and not
6 reporting these appropriately to transfusion
7 service, and get a little bit more information
8 about what is going on.
9 I don't think at this point the committee
10 has enough information to make a recommendation to
11 the FDA about deferring people or doing this. If
12 individual blood centers are doing that, then, I
13 think, you know, maybe they are already ahead of us
14 there, but we just need a lot more information on
15 the subject.
16 DR. NELSON: I would think of three type
17 of studies that could provide useful information.
18 One is a good surveillance of people receiving
19 IVIG, but that one, we wouldn't be able to link to
20 the donor or the specific antigen response.
21 Second would be careful evaluation of a
22 case and lookback, such as being done in Sacramento
23 where there was a reaction from a single donor or
24 you could be sure linking a donor with a reaction,
25 and then, third, a prospective study in one or more

1 transfusion services, Mayo Clinic or others, where
2 we could have data collected where the donor's
3 plasma is stored, and you know about the recipient
4 and you can prospectively identify cases that might
5 go unreported or with a standard definition.
6 I think those three things together at
7 least might move the field forward a little bit.
8 So, that is an intervention in the sense it is a
9 research plan, but it would require some funding,
10 and I would suspect that it would require staff to
11 help follow each patient that is receiving
12 transfusion, get the appropriate specimens.
13 I mean when we did this looking at cardiac
14 surgery patients in the FACT study at Hopkins and
15 two hospitals in Houston, we had staff in each
16 hospital. I mean we couldn't just ask the
17 transfusion service or the physicians to do it.
18 This will require funding.
19 DR. SIMON: I think the only one--and I
20 might get some knifes thrown at me from the
21 audience--but the only one I can think of that
22 could be done fairly quickly is the one Dr. Boyle
23 suggested, perhaps the idea of a group in some kind
24 of connection with industry could just determine
25 whether this is happening with any frequency in

1 immune deficiency patients.

2 That at least would direct us to some idea
3 of just whether this IVIG is very isolated or is
4 very significantly under-reported, but I agree with
5 you, the others we are talking about, the
6 controlled clinical trial, the funded study from
7 NIH.

8 DR. CHAMBERLAND: Ken, to add to your list
9 there, I think implied there would be the real need
10 for case controlled studies in this arena. There
11 has really been a paucity of that to date, and I
12 think it is really critical to be done, and I think
13 the AABB statement really also can be used to
14 advise the FDA, that they have put together I think
15 a well thought-out outline of what a research plan
16 might be.

17 DR. NELSON: Yes.

18 DR. HOLLINGER: Also, along these same
19 lines, the dichotomy that is seen between the two
20 presentations or the several presentations before
21 and with the group at Edmonton, I think needs to be
22 resolved. To me it is quite distinct. One, at one
23 point, plasma is the culprit, at another point it
24 is not. That kind of thing will require some
25 definitions and a variety of other things to

1 resolve that issue, too.

2 It could be two different things causing
3 the same disease. Certainly, that is a
4 possibility.

5 DR. NELSON: Is that a sufficient answer
6 to Question 1? Could we move on to Question 2.

7 DR. HOLNESS: If the answer to Question 1
8 is no, then, actually there is no need for Question
9 2. It is only if the answer to the question is
10 yes--

11 DR. NELSON: I think the intervention that
12 we are talking about is research, not excluding
13 certain donors other than perhaps we could talk
14 about whether we endorse, and I guess I would, of a
15 well-identified, single unit case that the Red
16 Cross and the American Blood Centers are currently
17 following, but even that, I think that is a prudent
18 policy, but if we found that, in fact, that this
19 reaction in a mild form was far higher than we now
20 recognize we might have to rethink that.

21 I have talked to some transfusion people
22 and they said, yes, we see it, but it is pretty
23 rare, the people at Hopkins, and it may be rare or
24 it may be unrecognized, I don't know.

25 DR. BIANCO: To the point, the fact that

1 many of us are using those procedures doesn't mean
2 that we need regulation.
3 DR. NELSON: Sure.
4 DR. BIANCO: I would like to support the
5 research approach that is being discussed.
6 DR. NELSON: I am not proposing a
7 regulation, but I think it may be a prudent policy,
8 but still I don't think it has necessarily dealt
9 with the whole issue. We need to know a lot more.
10 DR. SCHMIDT: Mr. Chairman, couldn't we
11 vote on No. 1 and then move on, and not try to
12 design a research study? If we vote on No. 1 and
13 the answer is no, we are not doing anything here.
14 DR. NELSON: Okay. Let's vote.
15 DR. STUVER: Can we get a clarification?
16 I mean I am still confused about this intervention.
17 If we are thinking to do more studies is an
18 intervention, then, we would vote yes, but if the
19 FDA is meaning intervention as do something to
20 identify donors, then, I think we would want to
21 vote no from the comments that I am hearing, so I
22 am confused, given what I am think we are saying,
23 which way we should vote.
24 DR. NELSON: Yes, because it says,
25 "interventions to identify donors and/or donations

1 with an increased risk," but it doesn't say to
2 defer donors.
3 Now, research could identify those donors,
4 and the donor characteristics could be simple, you
5 know, multiparous women only or something, or it
6 could be very complex, but that still would be a
7 research intervention.
8 What do you mean by this question,
9 interventions to identify donors who should be
10 deferred?
11 DR. HOLNESS: Yes, regulatory
12 intervention, not research intervention.
13 DR. NELSON: Okay. Let's vote on the
14 regulatory intervention.
15 If you vote yes, you mean the current
16 information is sufficient, that we could intervene
17 and recommend consideration of a policy to defer
18 certain donors.
19 DR. HOLLINGER: Or, Ken, just as you
20 mentioned, it goes along the same way, you just
21 change the question should FDA consider regulatory
22 interventions.
23 DR. NELSON: Okay.
24 DR. HOLLINGER: I would propose that that
25 is what we would change the question to say should

1 FDA consider regulatory intervention at this time
2 to identify, et cetera, and then vote on that, if
3 you would.
4 DR. NELSON: Would the FDA agree to insert
5 the word "regulatory"?
6 DR. HOLNESS: Yes, we would.
7 DR. NELSON: All right. So, then, let's
8 vote on that question.
9 All in favor of recommending regulatory
10 intervention, yes?
11 [One hand raised.]
12 DR. NELSON: All opposed?
13 [Show of hands.]
14 DR. NELSON: The Consumer--
15 MS. KNOWLES: No.
16 DR. SIMON: No.
17 DR. SMALLWOOD: The results of voting on
18 Question No. 1. I am going to read the question as
19 it was modified.
20 Should FDA consider regulatory
21 interventions at this time to identify donors
22 and/or donations with an increased risk for
23 producing TRALI in a recipient?
24 The results of voting: 1 YES vote. 13 NO
25 votes. No abstentions. Both the consumer and

1 industry representative agreed with the NO vote.
2 Normally, there would be 15 members
3 eligible to vote. Dr. Michael Fitzpatrick has
4 left.
5 DR. NELSON: Maybe I would like to ask Dr.
6 Linden, since she is one of the most knowledgeable
7 transfusion medicine specialists on the panel, and
8 I would like you to tell us your opinion.
9 DR. LINDEN: Thank you for the opportunity
10 to clarify. I certainly agree completely with my
11 colleagues that we do not have enough information
12 to do much here, and clearly further research is
13 needed to identify donor risks, recipient risks.
14 My concern is that it seems fairly clear
15 that there are donor attributes. There are I think
16 cases where a donor is implicated in multiple
17 cases, and I therefore think it is very prudent to
18 defer donors that are clearly implicated. So, that
19 was one of the measures that was offered to us, and
20 I think it is prudent to be on record saying that
21 that is a good idea.
22 But by regulatory intervention, I
23 certainly agree with Dr. Bianco, we are not talking
24 about a regulation, but just, you know, that is my
25 opinion, and I think a lot of people would agree

1 with that. That is the way I interpreted the
2 question.
3 DR. NELSON: Well, we have heard that that
4 is happening by the two major blood collection
5 centers at present as their policy, so I guess we
6 can be reassured that there is a prudent policy
7 being followed, but we still need more information.
8 You have a comment?
9 MR. RICE: Yes. I am assuming that even
10 though I voted in the majority on the no on
11 Question 1, that actually, those things suggested
12 in Question 2 are going to go on anyway, I mean as
13 kind of an ex officio, non-regulatory
14 implementation, but that I would imagine everyone
15 is doing these kinds of data collection which
16 should speak clearly to your concerns.
17 DR. NELSON: No, I don't think so. I
18 think the most controversial one is the
19 multiparity, and if we routinely excluded donors
20 even that had more than five pregnancies if we
21 include miscarriages in that number, you know, I
22 don't know how it would affect the donor
23 population, and I don't know what it would do to
24 the incidence of TRALI. I mean that is one thing
25 that we need to know.

1 I know that blood donors are actually
2 asked how many pregnancies they have, so the data
3 are available.
4 DR. SIMON: They are not routinely asked.
5 DR. NELSON: They are in the Red Cross
6 questionnaire that is used in Baltimore.
7 DR. SIMON: Not the standard AABB one.
8 DR. NELSON: They are not excluded based
9 on any number of pregnancies, but the data are
10 available.
11 DR. HALEY: We ask in certain regions if
12 women are multiparous, particularly in the REDS
13 regions, and this is a historical hangover from the
14 time when we used to look for multiparous women, so
15 that we could do HLA typing sera, so now we don't
16 need multiparous women for the HLA typing sera
17 because the methods have changed tremendously, but
18 that question is sort of left over from times past.
19 We investigate TRALI so frequently it is
20 kind of convenient to have it on the donor
21 questionnaire, so in Baltimore, our medical
22 director there uses it very frequently.
23 I would like to say something about the
24 cases that Mark was talking about, that we collect
25 in the Red Cross. These are the bad cases. These

1 are not, you know, going out and collecting at a
2 hospital level. They are the ones that were
3 serious enough to be reported back to the center,
4 and then we do the investigation and then tell the
5 hospital what we did. So, it is not meant to be
6 any kind of a wide net. These are the worst.
7 DR. HOLLINGER: Just a point of
8 clarification. If there is no regulation--I am
9 asking really the organizations here--if there is
10 no regulation, the organization of the blood banks,
11 the individual blood banks have a right to defer a
12 donor from donating from a legal standpoint. I
13 mean you could tell me if I were sort of associated
14 with it, it may not be causally or otherwise, they
15 could say we are not going to accept your blood, if
16 there is no regulation for that.
17 Could you clarify that, Celso?
18 DR. BIANCO: Yes. It is ultimately for
19 issues that are not covered either by federal or
20 local state regulations. It is a decision of the
21 medical director of the blood center to accept or
22 defer a donor based on whatever criteria the blood
23 center has.
24 DR. SIMON: I think that Dr. Hollinger was
25 getting at is, is there people who would claim a

1 legal right, and we do see that in the plasma
2 industry, because we pay, and we do periodically
3 get people who threaten to sue under ADA or
4 something because they have been rejected.
5 We have always taken the position that
6 donation is not a right and that the organizations
7 have to medically and scientifically make the
8 decision on who is acceptable, but it would be
9 interesting to see that tested someday, but that
10 has been the position we have taken.
11 DR. BIANCO: That is correct. That is the
12 same thing we see donation as a privilege, not a
13 right.
14 DR. HOLLINGER: Thank you.
15 DR. NELSON: I think we have discussed
16 this. Have we satisfactorily answered the
17 questions posed?
18 DR. HOLNESS: Yes, I think so.
19 DR. NELSON: Martin.
20 DR. RUTA: Thank you. The FDA is actually
21 working on a regulation dealing with donor
22 suitability that I hope we will publish some time
23 next year, and I think what the FDA is asking right
24 now is, you know, should we take the step as
25 issuing guidance for some immediate action in this

1 area, which I think your committee was saying that
2 you don't think there is enough data to take action
3 immediately, but in coming out with the proposed
4 rule, in the future, we can sort of raise these
5 issues to see if there is data that would warrant
6 additional actions.
7 DR. NELSON: I think we will take a break
8 for lunch now to 12:45, if we can. We will shoot
9 for that.
10 [Whereupon, at 12:01 p.m., the proceedings
11 were recessed, to be resumed at 12:45 p.m.]

1 A F T E R N O O N P R O C E E D I N G S

2 [1:00 p.m.]

3 DR. NELSON: The next topic is Studies on
4 Leukoreduction Filtration Failures.

5 The first talk will be given by Betsy
6 Poindexter from FDA.

7 **V. Studies on Leukoreduction Filtration Failures**

8 **Introduction and Background**

9 **Betsy Poindexter**

10 MS. POINDEXTER: Good afternoon.

11 [Slide.]

12 As on overview, in September 1998, the
13 Blood Products Advisory Panel advised the FDA that
14 the risk-to-benefit ratio associated with leukocyte
15 reduction is sufficient to justify universal
16 leukocyte reduction of blood components for
17 transfusion.

18 In January of 2001, the FDA issued draft
19 guidance entitled "Pre-Storage Leukoreduction of
20 Whole Blood and Blood Components Intended for
21 Transfusion" to update the regulatory standards for
22 leukocyte reduced products.

23 [Slide.]

24 Also, in January of 2001, the Public
25 Health Service's Committee for Blood Safety and

1 Availability endorsed requiring universal
2 leukoreduction, however, rulemaking is still
3 pending.
4 Now, we are at the present time. FDA is
5 currently reviewing public comments to the January
6 2001 draft guidance document. Several of these
7 comments question FDA's approach to assuring
8 success of leukoreduction.
9 The draft guidance also suggests routine
10 donor screening for sickle cell trait or the use of
11 a validated alternative method should be considered
12 for all donors.
13 [Slide.]
14 The initial reports of these
15 leukoreduction failures do to sickle cell trait
16 particularly came to our attention at the November
17 AABB meeting in 1999. To save some time, I am not
18 going to go over the scientific presentations that
19 were made, but will tell you that the reported
20 difficulties were with whole blood products and
21 with red blood cell products.
22 [Slide.]
23 The reported difficulties included filter
24 clogging, and on occasion, the filtration would
25 appear to be complete, but the leukocyte reduction

1 had not been performed, and these products were
2 filtered both a room temperature, at 20 to 24 C, as
3 well as 4 degrees Centigrade.
4 The current criteria for leukoreduced
5 products is that they recover 85 percent of the
6 transfusion product, the therapeutic product,
7 whether it was red blood cells or platelets, with a
8 residual white blood cell count of less than 5 x
9 10^5 per unit.
10 [Slide.]
11 The conclusions from the initial reports
12 were that successful leukocyte reduction from whole
13 blood from confirmed sickle cell trait donors did
14 not appear to correlate with blood temperature at
15 the time of filtration; that centers needed to be
16 aware of the variable effectiveness of
17 leukoreduction in subsets of donors; and that a
18 high percentage of the products collected from
19 donors with sickle cell trait will have filter
20 failures during the red blood cell component
21 preparations.
22 [Slide.]
23 They also concluded that autologous units
24 from sickle cell trait patients should not be
25 filtered for fear that the product would not make

it through the filter, so that it would not be available for the patient upon their elective transfusion or that if it did make it through the product, it would not be leukocyte reduced.

The effectiveness of leukodepleted products should be further evaluated.

[Slide.]

The filter failures that have been reported, at least one-third of the products appear to be from sickle cell trait donors, and two-thirds of the products appear to be from unknown causes, for which we need definition.

The definitions of filter failures as described currently by the blood centers vary, from the time to filtrations, vary anywhere from 40 to 60 minutes upwards to 8 to 10 hours or even 24 hours. That varies from blood center to blood center. There is no consistency.

[Slide.]

We had some areas where there may be donor issues that might cause or may potentially cause filtration failures. These include cholesterol, triglycerides, cold agglutinins, diabetes, G6PD deficiencies, iron deficiencies, medications that the donor might be one at the time of donation,

1 their initial platelet count or white blood cell
2 count.
3 [Slide.]
4 In addition, there are blood collection
5 practices that might create some filtration
6 problems including donor screening, the choice of
7 anticoagulants used, the proper mixing during blood
8 collection, the preparing or not preparing of
9 random donor platelets from the whole blood. We
10 have had varying views. One center says that if
11 they make platelets from their products, they
12 appear to filter more successfully. Another
13 reported that if they did not make platelets, they
14 had more filter failures.
15 And the bleeding times, we have had a
16 creeping of the bleeding times for donors from 5 to
17 10 minutes outwards to 15 and 20 minutes, and
18 perhaps even longer, again being a blood center to
19 blood center variable.
20 Also at issue might be the core
21 temperature of the blood during filtration.
22 [Slide.]
23 Today, we will hear a variety of topics,
24 the first one being presented by Connie Noguchi
25 from the National Institutes of Health, who will

1 talk about the pathophysiology of sickle cell
2 anemia, hemoglobin S polymerization.
3 We will also have blood center
4 presentations from the Canadian blood services,
5 from the American Red Cross, and from two blood
6 centers in the United States.
7 Our first speaker is Dr. Connie Noguchi.

8 **Constance Noguchi, Ph.D.**

9 DR. NOGUCHI: Thank you.
10 [Slide.]
11 What I have on this slide is what is
12 commonly associated with sickle cell anemia, and
13 that is the marked change in cell morphology upon
14 deoxygenation. However, my focus today is not on
15 cell morphology, but rather what goes inside the
16 cell as oxygen is removed. In particular, it is
17 polymerization of sickle hemoglobin, which is the
18 fundamental cause of pathophysiology in the
19 disease.
20 The two important take-home points that I
21 hope to convey is the importance of oxygen
22 saturation and perhaps more importantly in the
23 context that we are going to discuss, hemoglobin
24 concentration.
25 [Slide.]

1 In terms of oxygen, normal hemoglobin
2 undergoes a very subtle but important confirmation
3 shift when oxygen is removed. The alpha/beta dimer
4 is illustrated here, shifts apart, forming a large
5 cavity within the hemoglobin, providing binding for
6 2,3-DPG.
7 Now, the importance here in addition to
8 binding oxygen is the physical size of the
9 hemoglobin molecule. It is rather bulky and
10 generally around 64 angstroms in diameter.
11 [Slide.]
12 The consequence is illustrated here in the
13 red cell where there is significant crowding
14 between the hemoglobin molecules. In fact, at
15 concentrations about 32 or 34 grams per deciliter
16 associated with concentrations of hemoglobin within
17 the red cell, the molecular distance between
18 hemoglobin molecules is less than 1 molecular
19 diameter.
20 As a result, the hemoglobin molecules
21 behave as though they are 50 times more
22 concentrated than they would be if they were point
23 particles.
24 Before I leave this slide, I just want to
25 mention that the sickle mutation is on the surface

1 of the hemoglobin molecule, and there is 1 per
2 betaglobin chain.
3 [Slide.]
4 Now, the pathophysiology of the disease in
5 terms of biochemistry is summarized on this slide.
6 I am going to make a few important points that one
7 doesn't usually associate with sickle cell anemia.
8 The first is that hemoglobin is encoded in
9 two chromosomal loci, the alpha and the beta. The
10 betaglobin is the mutation, but the alpha plays an
11 equally important role depending on the context.
12 On the alphaglobin cluster in chromosome 16, there
13 are actually two alphaglobin genes encoded as
14 opposed to just one for the adult beta on the
15 chromosome 11.
16 The consequence of the mutation is to
17 alter the surface charge of the hemoglobin molecule
18 because you have a substitution of a valine in the
19 6 position for glutamic acid. As a result, when
20 oxygen is removed, the hemoglobin molecules
21 assemble, making a long fibrous liquid-like, liquid
22 crystal-like fiber.
23 In the extreme case, upon complete
24 deoxygenation, you can have extensive fiber
25 formation and eventually alteration in cell

1 morphology.

2 [Slide.]

3 Now, electron microscopy and x-ray

4 crystallography studies, we know the structure of

5 the fiber, illustrated here, its 14 strands of

6 hemoglobin molecules with a slight helical twist.

7 These 14 strands assemble as pairs of

8 half-staggered molecules, illustrated here, and the

9 important consequence of the mutation is that the

10 valine 6 mutation on one molecule fits into a

11 hydrophobic pocket of an adjacent molecule,

12 illustrated here. These are both in the betaglobin

13 chain.

14 With the normal glutamic acid, in addition

15 to being bulky, illustrated here, there is a charge

16 which prevents the normal glutamic acid in the beta

17 6 position to fit into the assembly process,

18 illustrated here.

19 Now, from the crystalline structure,

20 another important feature is that only one of the

21 two betaglobin mutation sites is in molecular

22 contact. This means that if you had a hybrid

23 hemoglobin molecule with half sickle and half

24 normal hemoglobin, you have a probability of about

25 1/2 going into the polymer phase compared to an SS

1 molecule, and that become relevant as we continue
2 the discussion.
3 [Slide.]
4 Now, as a consequence of the
5 polymerization of sickle hemoglobin, you have a
6 marked reduction in the solubility of hemoglobin
7 within the red cell. In fact, deoxyhemoglobin S,
8 at physiologic conditions, has a solubility of
9 about 16 G/dl compared to the intracellular
10 concentration which is about twice that of 32 to
11 34.
12 As a result of the low solubility and the
13 crowding in the red cell, you have extensive
14 polymerization when oxygen is completely removed.
15 However, even as you add oxygen back at 70 percent,
16 for example, where the amount of deoxyhemoglobin is
17 substantially less than 16 G/dl, you can still
18 detect significant amounts of polymer, and this is
19 direct attributed to the physical size of the
20 hemoglobin molecules.
21 As a result, the important determinants of
22 hemoglobin polymerization in the red cell are
23 hemoglobin composition, because it is only sickle
24 hemoglobin that polymerizes, hemoglobin
25 concentration because of the crowding and oxygen

1 saturation, because it is only the deoxyhemoglobin
2 that fits into the polymer phase.
3 Now, we were able to use solid-state
4 nuclear magnetic resonance to quantitate how much
5 polymer was in a sickle erythrocyte, in an intact
6 sickle erythrocyte at physiologic conditions, and
7 this is illustrated here.
8 What we see is not only is there maximal
9 polymer at complete deoxygenation, but polymer
10 fraction gradually decreases, so that even at high
11 oxygen saturations, usually associated with the
12 arteriolar circulation, we have significant amounts
13 of polymer.
14 Now, this has a much more dramatic effect
15 in populations of sickle erythrocytes because in
16 addition to having the potential to form polymer,
17 sickle erythrocytes also have a very broad
18 distribution in intracellular hemoglobin
19 concentration. In fact, well, this may be the
20 profile of a whole sample of sickle erythrocytes.
21 If we fractionate according to cell density or
22 concentration. We have different populations,
23 concentration ranging from below 30 G/dl to well
24 over 40 G/dl.
25 What you can appreciate is as the

1 hemoglobin concentration increases, so does the
2 potential to form polymer, and this has particular
3 manifestations at very high oxygen saturations, so
4 that even above 90 percent in the most dense SSL
5 fraction, you have the potential for
6 polymerization.
7 This is illustrated on the next slide
8 where we have done some filtration studies.
9 [Slide.]
10 I should mention that filtration, for
11 those of us study red cells, is not the same as
12 filtration for those of you who do blood banking.
13 Filtration here is usually done with a lot
14 hematocrit, about 3 to 8 percent, and we are
15 looking at filtration through 5 micron pores.
16 When we completely saturate hemoglobin by
17 adding carbon monoxide, so you have no potential
18 for forming polymer, we see that even in the dense
19 cell fraction, there is some residual impairment to
20 flow. This is related primarily to the fact that
21 you have some irreversible membrane damage, called
22 "irreversibly sickled cells" in this population.
23 However, at room air, what you also see is
24 a marked increase in impairment to flow as the
25 proportion of dense cells increases, and this

1 begins to approximate a completely deoxygenated
2 sickle cell population, whole cell population.
3 This is primarily a result of the disproportionate
4 contribution of the dense cell fraction.
5 So, while the average parameter of the
6 bulk population would predict that you should not
7 see any polymer, because of the presence of these
8 dense cells with very high hemoglobin
9 concentration, you see a disproportionate effect on
10 cell reallergy [?] infiltration.
11 Now, in terms of hemoglobin composition,
12 since sickle hemoglobin is required for
13 polymerization, we can dilute out the percentage of
14 sickle hemoglobin by adding combinations of non-S
15 hemoglobin, and this includes fetal hemoglobin,
16 hemoglobin A2, hemoglobin A, and for individuals
17 with SC disease, hemoglobin C.
18 What we see is a marked increase in
19 deoxyhemoglobin solubility, so that at
20 concentrations or proportions of sickle hemoglobin
21 about 30 to 40 percent, associated with individuals
22 with sickle trait, the solubility now increases to
23 about 24 or 25 percent oxygen saturation compared
24 to the 16.
25 Now, also illustrated on this slide, for

1 those of us looking for therapeutic strategies, you
2 see an even greater increase in solubility because
3 of fetal hemoglobin and hemoglobin A2. This
4 relates somewhat to the crystal structure that I
5 had mentioned earlier, because the hybrid molecules
6 between hemoglobin F and hemoglobin S are not able
7 to go into the polymer phase, whereas, the hybrid
8 molecules between hemoglobin A and hemoglobin S, or
9 hemoglobin C and hemoglobin S, because of their
10 similarity to each other, are able to go with half
11 the probability.
12 [Slide.]
13 Now, the consequence of this is
14 illustrated on the next slide, where these are
15 polymer fractions measured in cell populations
16 taken from individuals from sickle trait. The
17 important feature here is that in sickle trait,
18 polymerization potential is not eliminated, but
19 rather markedly reduced, so that you don't begin to
20 see any polymer until oxygen saturation begins to
21 fall below 50 percent or venous concentrations in
22 lower.
23 As a result, there is no clinical
24 manifestation associated with sickle trait,
25 although under extreme circumstances of low oxygen

1 or extreme dehydration, you can get the cells to
2 polymerize in the laboratory--or not polymerize,
3 but the hemoglobin to polymerize in the laboratory.
4 [Slide.]
5 One of the consequences as a result, was
6 the ability for us to measure filtration in the
7 sickle trait samples. Because sickle trait is a
8 more uniform population, we generally don't have to
9 worry about the dense cell fraction. So, this
10 gives us a way of looking directly at the
11 relationship between polymer formation and cell
12 filtration. Again, these are low hematocrit
13 filtrations through 5 micro pores.
14 What we see--I would like to focus on this
15 part of the slide here--was that impairment of
16 filtration in sickle trait erythrocytes correlates
17 directly with the extent of polymer formed within
18 those cells. It confirms two things. One is that
19 without the contribution of dense cells, we can
20 look directly at polymer; and, second, that our
21 hypothesis that polymer formation was indeed the
22 primary defect in causing abnormal reallergy [?]
23 was indeed shown by these results.
24 I mention here just to show that again,
25 you don't see polymer formation or impairment to

1 flow until you drop well below 60 percent oxygen
2 saturation or lower compared to SS or SS with alpha
3 thalassemia where you begin to see impairment to
4 flow at very high oxygen saturations.
5 Another example of where we can begin to
6 see abnormal flow or manifestations of sickle trait
7 is in the kidney, and the reason is because of the
8 hyperosmolarity in the renal medulla, as well as
9 the relatively low oxygen saturation.
10 [Slide.]
11 We were able to do a study with Marty
12 Steinberg in Jackson, Mississippi, where we looked
13 at a large number of individuals with sickle trait,
14 and what we observed was that indeed there was a
15 urinary concentration defect or a renal
16 concentration defect resulting in decrease in
17 urinary osmolarity, and that is illustrated here.
18 We look at the decrease or the inability
19 of the kidney to concentrate urine as a function of
20 percent of hemoglobin S. Now, since the sickle
21 trait erythrocytes are a more uniform population,
22 we can directly correlate the percentage of sickle
23 hemoglobin with polymer formation.
24 What we see is a very good correlation
25 between renal function and percent sickle

1 hemoglobin.
2 The other important feature of this slide
3 shows you the variation of sickle hemoglobin is due
4 much to the alphaglobin gene status. For
5 individuals with alpha thalassemia, homozygote
6 alpha thalassemia, you have the lowest level of
7 sickle hemoglobin, of only about 30 percent.
8 In contrast, for sickle trait individuals
9 with the full complement of four alphoglobin genes,
10 you have the greatest amount of sickle hemoglobin
11 in their erythrocytes, ranging between 40 and 45
12 percent.
13 So, while there is no clinical
14 manifestation, we begin to see some renal effects
15 due to sickle hemoglobin polymerization. Now,
16 although rare, the incidence of renal medullary
17 carcinoma is elevated in individuals with sickle
18 trait, likely related to hemoglobin polymerization
19 itself.
20 [Slide.]
21 Now, to summarize what we know now about
22 sickle trait polymerization, is that, first, while
23 it is markedly reduced and there are no clinical
24 manifestations, the potential for polymerization
25 still remains in these cells, and for those of us

1 who are doing clinical diagnosis in the laboratory,
2 at the bench, for example, we can take advantage of
3 that by looking for sickle cells upon complete
4 deoxygenation.
5 The second is that the parameters that
6 determine polymerization in the SS erythrocyte also
7 determine polymerization in sickle trait. That is,
8 the proportion of sickle hemoglobin or hemoglobin
9 composition, the concentration of hemoglobin within
10 the red cell, and the oxygen saturation.
11 Third, that the kidney provides sort of a
12 test system, if you will, to demonstrate that
13 polymerization can indeed exist in the sickle trait
14 population, giving you the renal concentration
15 defect.
16 [Slide.]
17 Now, in terms of therapy for those of us
18 interested in looking for therapeutic strategies
19 for sickle cell anemia, one of the targets is to
20 reduce polymerization. Here, I list a couple of
21 them. One of them is to increase fetal hemoglobin
22 or alter hemoglobin composition, and the second is
23 to prevent cell hydration or alter hemoglobin
24 concentration.
25 What I left off this slide, although there

1 have been other strategies designed to change
2 oxygen affinity, as well, I think this provides
3 some clues as to what might be going on in the
4 Leukotraps with sickle trait, and that is that if
5 the defects are related to polymerization, there
6 are three things that can affect that.
7 One is alphaglobin status. The second,
8 more importantly, is the osmolarity during blood
9 collection, because extreme osmolarities will
10 indeed markedly increase hemoglobin concentration,
11 and therefore, markedly increase the potential for
12 even a small population of cells to undergo
13 polymerization.
14 Third, in order to test the effect of
15 polymerization, a simple or a straightforward
16 laboratory test would be to add that carbon
17 monoxide and see if you are able to restore
18 filtration.
19 Thank you.
20 DR. NELSON: Thank you for a very elegant
21 presentation. That was a Nobel Prize lecture, I
22 guess, from Dr. Pauling.
23 Any questions or comments? Yes.
24 DR. RUTA: When we introduced
25 leukoreduction, we started running into these bumps

1 along the road. One of the problems that has been
2 reported in the literature is failure to
3 leukoreduce with sickle trait, others are clots
4 that we are going to hear about.
5 I am wondering, it is very nice, but is
6 there any hope for a simple solution that you can
7 see right now?
8 DR. NOGUCHI: I believe so. I think that
9 for myself, the most important experiment would be
10 to see if you eliminate the potential for
11 polymerization if that does restore the ability to
12 filter. Once you know that, then, it is easier to
13 then go back and look. The second candidate would
14 be if it is polymerization, do you markedly affect
15 osmolality during collection, so that you create a
16 subpopulation of very dense cells.
17 DR. RUTA: Might there be conditions of
18 collection that you can see that might affect the
19 oxygen concentration or other parameters that you
20 think might then lead to prevention of the
21 sickling?
22 DR. NOGUCHI: Polymerization, yes. I
23 think the second most important thing to look at
24 would be osmolarity while you are collecting blood.
25 DR. JOHNSON: Johnson, Los Angeles. With

1 respect to the polymerization of ASLs, would not
2 the potential for polymerization related to
3 osmolarity be balanced somewhat by the beneficial
4 effect on polymerization of temperature, the
5 decreasing temperature at which most of these cells
6 are filtered?
7 DR. NOGUCHI: Yes. I should mention that
8 our experiments are all done at physiologic
9 temperatures, which is 37 degrees, and as you
10 decrease temperature, you see a marked increase in
11 hemoglobin solubility, so that at 4 degrees,
12 solubility increases to 32 G/dl. However if the
13 collection process creates a small population of
14 very dense cells, those are the cells that would
15 still have the potential to polymerize. But yes,
16 temperature should affect it.
17 DR. BIANCO: Dr. Noguchi, Celso Bianco,
18 America's Blood Centers. There are many or several
19 reports of alterations in adhesiveness of cells
20 with sickle cell hemoglobin, but they are
21 restricted to the SS cells.
22 Are you aware of changes in surface
23 adhesiveness of cells with sickle trait?
24 DR. NOGUCHI: No, I am not, and I guess
25 that is why my bias has been that the effects that

1 were seen may be related to polymerization.

2 DR. HOLLINGER: Along the same lines, most
3 of the studies that you showed were with extremes
4 of deoxygenation and dehydration. You talked about
5 less than 50 percent, I think, of oxygen before you
6 see things, and so on.

7 So, can you explain to me a little bit
8 about why, in sickle cell trait, you think there is
9 this filtration problem, because under those
10 conditions you probably don't have those as the
11 blood is being drawn, and so on?

12 DR. NOGUCHI: You are talking about the
13 leukopack filtration?

14 DR. HOLLINGER: Yes.

15 DR. NOGUCHI: The real question is the
16 process of collection, are the cells maintained at
17 290 or 300 milliosmos throughout, and that is the
18 real question, because once you change the
19 hemoglobin concentration, you markedly alter its
20 ability to polymerize.

21 DR. STRONCEK: We are filtering venous
22 blood, and the bags are fairly small surface area,
23 so the question has come up is if the white cells
24 metabolize enough oxygen in those bags to drop the
25 O_2 content low enough to be a problem.

1 With us, we filter at room temperature,
2 too, so, you know, some places do filter at 4
3 degrees, but many of us filter room temperature
4 blood, too, so it could be a problem sickling.
5 DR. NELSON: Ron.
6 DR. GILCHER: Ron Gilcher, Oklahoma. We
7 have also attempted to leukocyte reduce apheresis,
8 collected red cells, and there, you are metering
9 the anticoagulants, so you would tend to obviate
10 the effect of a high concentration of anticoagulant
11 for the first 50 or 30 cc of red cells that are in
12 the bag, but we were unsuccessful with that, as
13 well.
14 DR. NOGUCHI: So, gradual introduction of
15 anticoagulant doesn't improve filtration.
16 DR. GILCHER: No, at least in the
17 experiments that we did, that is, metering the
18 anticoagulant, so that you wouldn't have any change
19 in the osmolality, it didn't work.
20 DR. NELSON: Thank you.
21 Next is Dr. Thomas Walker from Canadian
22 Blood Services.
23 **Thomas Walker, M.D.**
24 DR. WALKER: Thank you, Mr. Chairman. I
25 would like to thank the FDA for the opportunity to

1 come here this afternoon. We find we plagiarize so
2 many ideas from the FDA, AABB, and ABC, we welcome
3 the opportunity to perhaps pay back to some degree.
4 [Slide.]
5 What I am going to offer to you this
6 afternoon starts with a brief outline of our
7 history in Canada with universal pre-storage
8 leukoreduction; a bit of feedback on the learning
9 curve we rode as we went through the process; our
10 experience with blocked filters and the
11 contribution of both clotting and sickle trait;
12 want to wave a bit of a red flag about some
13 ergonomic concerns that blind-sided us along the
14 way.
15 Of course, the whole reason for the
16 exercise is to get residual leukocyte levels down,
17 so I will share our experience in that regard with
18 you. Finally, I hope to leave you with some food
19 for thought.
20 [Slide.]
21 The history in Canada goes back to March
22 of 1998 when the Canadian Red Cross Society, who
23 was operating our blood system at that time,
24 implemented universal, prestorage leukoreduction of
25 platelets and platelets apheresis.

1 This was implemented across the country at
2 16 centers. Interestingly, the TRALI epidemic on
3 which Dr. Boshkov reported this morning was one of
4 the precipitating factors for the decision, not by
5 our regulator at the time, but by the funding
6 agencies to introduce leukoreduction of platelet
7 concentrates.
8 In June of 1999, the Canadian Blood
9 Services and Hema-Quebec, who had taken over the
10 blood system from CRCS, added prestorage
11 leukoreduction of red cells to the mix. CBS at our
12 14 centers, and Hema-Quebec at their two.
13 My presentation this afternoon is going to
14 focus on experiences in CRCS and CBS. I am afraid
15 I don't have any data from Hema-Quebec.
16 [Slide.]
17 Once we had trained our staff and allowed
18 them to demonstrate their competence performing the
19 process, we have had very few problems in either
20 red cell recovery or in residual leukocyte counts
21 in either red cells or in platelets. Now, we are
22 dealing with the 5×10^6 limit for the residual
23 leukocytes. That may be a contributing factor.
24 Our big headache was achieving consistent
25 platelet yields. Our specification is 55×10^9

1 platelets per unit of platelets from whole blood.
2 We make the measurement in individual units, and 75
3 percent of the units we test must meet the
4 specification.
5 Well, during implementation, some centers
6 could only achieve 30 percent, and as recently as
7 November of last year, we had centers experiencing
8 transient dips down to 50 percent. Looking back, we
9 validated this process in two centers. We didn't
10 optimize it, and we did not enforce an optimum
11 process in all of our locations.
12 As a result, small deviations in the
13 process, for example, a center using a bucket with
14 a liner instead of an unlined bucket, changing the
15 weight of the rotor, changing the acceleration and
16 deceleration times had a big effect on our platelet
17 yield.
18 Also, this process takes longer than the
19 old process did. We found ourselves pushed to make
20 the 8-hour limit from production of platelets. The
21 staff were hurrying, they weren't as careful as
22 they needed to be, not to disturb the platelet
23 interface, and that had a bigger effect than we
24 thought it would.
25 [Slide.]

1 Regarding blocked filters, the most recent
2 data I have is over the period from April 2000 to
3 March of this year, and we have seen what we call
4 blocked filters, i.e., the product will not pass
5 through the filter, in 0.08 percent of filtrations.
6 Unfortunately, our production records are
7 manual, so we are not able routinely to check
8 whether the unit we are having a problem with now
9 came from a donor whose previous unit also gave
10 problems. One of our centers, however, put in the
11 effort to look at it, and I will report on their
12 results later.
13 In approximately 65 percent of those
14 cases, however, we did see clotting.
15 [Slide.]
16 The clots are sometimes seen in the
17 collection containers, less frequently in the
18 filter, and the strongest association as far as
19 causes of the clotting seems to be prolonged bleed
20 times, i.e., often longer than 20 minutes.
21 Thinking this through, prior to the
22 implementation of leukoreduction, these clots would
23 have been seen by the hospitals, and, in fact, it
24 wasn't unusual for us to get complaints from the
25 hospitals about a red cell unit that contained a

1 clot.

2 With the change in the process, we are
3 taking these clots out in-house now, so I don't
4 believe that there has been a change in the process
5 that is causing the clotting. It is just that the
6 clotting is now getting in the way of the process.

7 [Slide.]

8 Regarding sickle trait, this is data that
9 was generated in our Toronto Centre, January to May
10 of 2000, and it was reported by Toronto Centre
11 staff at last year's AABB.

12 They looked at nearly 60,000 collections;
13 40 of those filter blocked. That is a rate of
14 about 0.07 percent. In 14 of those 40 cases, the
15 donors were sickledex positive; 7 of those 14
16 donors had donated previously, and for 5 of those
17 7, all previous donations had also failed to
18 filter.

19 We, too, are looking at what we should do
20 regarding deferral of sickledex positive donors.
21 We do not have any reports relating sickle cell
22 trait to a residual white cell failure. I don't
23 know that we are not seeing it, but we have not had
24 any reports.

25 [Slide.]

1 On the ergonomic front, the introduction
2 of leukoreduction was a big change for our staff.
3 Perhaps we didn't manage it as well as we might.
4 Again, perhaps we didn't analyze the process well
5 enough because we have received numerous reports of
6 various problems including reaching up to put the
7 bag on the hanger, bending down to seal the bag
8 after filtration is complete.
9 We use Pall systems. I believe this is
10 reserved to the WB system. When you get the set,
11 there is air in the filter housing. That gets
12 pushed into the bag as the blood filters. The
13 hospitals don't like air bubbles in the bags, so we
14 have got to get it out and put it somewhere, and
15 the squeezing of the air out of the unit takes a
16 surprising amount of force, and our staff reported
17 repetitive stress injuries as a result of this -
18 almost shut down a center one day.
19 [Slide.]
20 Residual leukocytes. I don't know whether
21 you will find this good news or bad news, but
22 looking at platelets--and this is experience going
23 back to the 1998 implementation by Canadian Red
24 Cross--7,600 units tested, 99.3 percent of those
25 met the equivalent of the 5×10^6 limit, 97.4

ajh

1 percent met the equivalent of the 1 x 10^6 limit.

2 When filtering red cells initially as

3 whole blood, 1,655 units, 99.9 percent met the 5 x

4 10^5 and 96.8 percent met the 1 x 10^6 limit.

5 [Slide.]

6 Looking at red cells filtered as red

7 cells, i.e., after removal of the platelet-rich

8 plasma, 3,100 units, 99.9 percent success at

9 meeting the 5 x 10^6 limit; 98.7 percent success in

10 meeting the 1 x 10^6 limit.

11 However, beginning this year, we started

12 receiving a surprising number of reports of

13 failures to meet the 5 x 10^6 limit. In five

14 months, we got 19 reports. All of last year we got

15 13.

16 [Slide.]

17 In investigating it, we found instances in

18 which the filter matrix had popped out of the

19 housing, leaving a large space. Now, the worst of

20 these were in cases where we had an RC filter,

21 which is supposed to be given a soft spin in

22 reduction of platelets, and somebody decided to try

23 and make cryo instead, so they gave it a hard spin.

24 You can call that abuse of the filter.

25 I brought some photos along of what these

1 looked like, is somebody would like to see the gory
2 details.
3 [Slide.]
4 The bottom line, however, is that (a) we
5 cannot say that that was the cause.
6 [Slide.]
7 We cannot link this observation to failed
8 units because we were catching these before the
9 units went on to production, but it does make one
10 wonder if perhaps there are instances when the
11 displacement is not as severe, and the blood is
12 just bypassing the filter.
13 We took this up with Pall, and do not
14 really have a good cause-effect relationship in
15 this, but they have devised some strategies for
16 reducing the probability of this happening, and we
17 were advised that they are working to put those
18 into place now.
19 [Slide.]
20 Finally, a few points to consider. One,
21 don't underestimate how much impact this process is
22 going to have on your staff, the ergonomic effects.
23 Processing is going to take longer, so
24 make sure you schedule your production, so that the
25 staff aren't going to rush.

ajh

1 An aside here, our regulator allowed us to
2 filter all units warm, i.e., they suspended the
3 requirement to refrigerate units immediately on the
4 condition that we get all the processing done
5 within 8 hours. This allows us to filter
6 everything warm. The flow is much quicker when the
7 blood is warm. I think this is something that
8 should also be looked at in the U.S.
9 Finally, thorough process validation.
10 Know the process, know what even the slightest
11 variation in your key parameters is going to do to
12 the result, and optimize the process before you
13 roll it in.
14 [Slide.]
15 Donors with sickle cell trait. What we
16 are looking for allogeneic blood donors is to test
17 those donors whose units failed to filter and defer
18 those who test positive on sickledex from blood
19 donation.
20 For autologous, test African-Canadian
21 donors for sickle cell trait in advance, and if
22 they are positive, don't filter the units.
23 Mr. Chairman, I would be happy to try and
24 answer any questions.
25 DR. SCHMIDT: I have two questions. In

1 the Toronto study, you said that 35 percent of the
2 donors had sickle trait. You didn't mention what
3 was with the rest of them. We presume it was
4 clotting, but the 35 percent just balances the 65
5 percent before that you had said was a clotting
6 problem.
7 Was that accidental? It adds up to 100.
8 I mean did they look at the non-sickle in the
9 Toronto study?
10 DR. WALKER: I am afraid that was not
11 answered in the data that Toronto provided me. We
12 could make an inquiry of Dr. Tinmouth and his staff
13 and attempt to get an answer back.
14 DR. SCHMIDT: The other question has to do
15 with the filtration of autologous units, which Dr.
16 Poindexter mentioned before. This doesn't sound
17 like a reasonable practice. Is this done
18 routinely, autologous units, do leukocyte
19 reduction, and if so, why?
20 DR. WALKER: Yes, it is our routine
21 practice, and it was just done for simplicity.
22 This way, we have one process for all the
23 donations.
24 DR. SCHMIDT: But if it means you are
25 going to eliminate some patients because they have

1 sickle trait, and they can't do autologous, the end
2 result is a real negative.
3 DR. WALKER: We are handling them as the
4 exception, and if we identify the sickle trait in
5 advance, then, we do collect them into a CPDA1
6 unfiltered set and handle it that way. We have a
7 comparatively small population at risk for sickle.
8 DR. NELSON: Toby.
9 DR. SIMON: I had a question, an add-on to
10 Paul's. I guess you could have some indication in
11 autologous in that the white cells could release
12 cytokines, and so forth, and so you might still see
13 some reactions.
14 But I guess I wanted to clarify--I may be
15 the only person who doesn't have this straight--but
16 do I understand correctly that we have two concerns
17 here vis-a-vis the sickle cell trait units, number
18 one, that they will clot up the filter, you can't
19 filter them, and therefore you lose the red cells,
20 or have we managed to get them through the filter,
21 but we don't have leukodepletion? Is it both
22 problems that we are dealing with? Okay. So, we
23 have both loss of red cells and units that aren't
24 truly leukocyte reduced, I guess is the right word.
25 DR. WALKER: We do not have any data

1 regarding the residual white cell failure related
2 to sickling, but we can't say it isn't happening
3 here.
4 DR. RUTA: You seem to have a lower
5 frequency of clots up in Canada. We have been
6 having reports of 1/2 to 1 percent of clots, and I
7 would wonder if you would comment on why you think
8 you are having a lower level of a problem with
9 clots.
10 DR. WALKER: I am sorry, I didn't quite
11 catch.
12 DR. RUTA: We seem to be having a higher
13 level of clots in this country, reports of 1/2 to 1
14 percent, and you are reporting much lower than
15 that, and I was wondering if you might want to
16 comment on why you are seeing fewer clots up there.
17 DR. WALKER: Possible differences. We do
18 have an enforced 15-minute limit for the bleed time
19 over which units would not be used for production
20 of platelets or FFP, and since we have a good
21 demand for platelets and FFP, we try to make sure
22 we don't lose units from that cause.
23 Also, for as long as I can remember, we
24 have used an automated blood shaker system, so that
25 our units are continuously agitated during

1 collection. I don't know whether that has an
2 effect or not. The manufacturers of the units
3 would say it does, but it is a difference.
4 DR. RUTA: One other question for you or
5 maybe any of the other speakers who are going to
6 come up. One of the concerns with the clots is not
7 just that, well, you lose a unit, it will block,
8 and the units gets lost, but I guess it would be a
9 question as to could there be small clots that
10 would actually allow the unit to filter, but
11 interfere with the leukoreduction.
12 I was wondering if you could comment on
13 that or maybe some of the other speakers as they
14 come up.
15 DR. WALKER: If I could just go back to
16 your question about the incidence of clotting, we
17 did have a problem a few years ago where we had a
18 very high rate of clotting in the segment lines on
19 our red cell units. I think it is common practice
20 in both countries to leave segments attached to the
21 bag for cross-match purposes. We were seeing a lot
22 of clots in those segments.
23 When we did the root cause analysis of
24 that, it was definitely related to a mixing
25 problem, and that was even with the use of the

1 shakers. So, we addressed it by inserting a
2 process step where we manually ensure that the
3 initial blood collected into the bag is thoroughly
4 mixed with the anticoagulant before we leave it to
5 the automated shakers.
6 We found it critical in one other aspect.
7 Maybe the early mixing is the critical point rather
8 than the continuous mixing - who knows.
9 DR. RUTA: Thank you.
10 DR. NELSON: The next speaker, Dr. Rebecca
11 Haley from the Red Cross.
12 **Rebecca Haley, M.D.**
13 DR. HALEY: Thank you, Dr. Nelson and Dr.
14 Ruta for asking me to share the American Red Cross'
15 experience with leukoreduction.
16 [Slide.]
17 In the American Red Cross, we have looked
18 at our failures. We very carefully keep records of
19 our failures nationwide. The question is what
20 causes the failures, and are they internal, which
21 means do these failures never leave the blood bank,
22 or are they indicators of external failure, which
23 may deliver an unsatisfactory product to the
24 patients. I would like for you to think about that
25 as we go through these. And do these failures beg

1 for further action to protect the public?

2 [Slide.]

3 This is just a little glossary for tables

4 coming up. SD means that a filter is sterile

5 docked to a CPD red cell to be put into additive.

6 RC is the filter through an attached--we filter the

7 blood through an attached, in-line filter after the

8 plasma and platelets have been removed, and the

9 designation WB means that you filter the whole

10 blood before the components are made.

11 [Slide.]

12 So, if we are going to talk about

13 failures, what were our failure rates? We had

14 actually nine categories. I left out two of the

15 categories that were not very informative, so this

16 doesn't totally add up to 100 percent. There is

17 30,457 failures that we had reported out of our

18 3,619,169 leukoreduced red cell attempts, but clots

19 were by far the biggest category. That was 13,000.

20 Cold agglutinins, we had recognizable cold

21 agglutinins in about 1,000 user error, and that I

22 understand is a grab basket of things, was 3,000.

23 Unknown, the filter failed in some way, and usually

24 that meant it did not run, but there were not

25 visible clots was 11,000.

1 A very interesting category, we had 386
2 that were reported as sickle traits, and these did
3 not run. Now, the reason that we knew that they
4 were sickle traits was because we had looked,
5 trying to find blood for patients for red cell
6 exchanges of a sickle cell patient or an exchange
7 of a newborn, so for clinical reasons, we had found
8 that this was a sickledex-positive donor and had
9 attached that to their donor record, just as we do
10 special typing for antigens or special antibody
11 information. We don't routinely test for sickle
12 trait in our donors unless we have a special
13 patient request. So, that is why we have this
14 little bit of information.
15 [Slide.]
16 Out of our 3.6 million, 30,000 failed, a
17 rate of 0.8 percent. All failures listed were not
18 released to the public, so we can begin with the
19 fact that all failures were not released to the
20 public, and the thing that I failed to highlight
21 previously, we had 57 out of that 3.6 million that
22 had a white count that was too high to be released.
23 Now, we only counted 1 percent or 36,000
24 of these 3.6 million, so out of that 36,000, we had
25 57 that failed white counts.

1 So, that means that if this represented
2 100 failures, each one of these, it would have been
3 about a 0.1 percent white cell failure rate
4 supposing that you had all of those failures. FDA,
5 or course, requires no more than a 5 percent
6 failure rate.
7 [Slide.]
8 So, the type of filter, did that make any
9 difference? We are trying to figure out, okay, so
10 what does make the difference. Filter maker No. 1,
11 to filter CPD units, now, the CPD units are usually
12 either for pediatric transfusion where they don't
13 want the adenine in the preservative solution or
14 sometimes autologous units, so we had a filter
15 failure rate with manufacturer 1 of 1.1 percent.
16 Manufacturer 2 was 0.6 percent, but many
17 fewer filtrations. Manufacturer 1, we had 9,000
18 filters of the sterile docks out of 1.8 million.
19 This was obviously our largest category.
20 Manufacturer 2 was 0.3 percent, in the same sterile
21 docked group. Manufacturer 1, in-line, which means
22 they come with the filter attached, we had a 2.3
23 percent failure, failed to run, either designated
24 as clots or failed to filter out of 724,000, and
25 manufacturer 1 in- line, whole blood, was 0.9

1 percent failure while manufacturer 2 was 0.6
2 percent.
3 So, manufacturer 1 had the highest filter
4 rate, and one category of filters was worse than
5 the others. That was the in-line filters. My
6 technical people tell me that these are collected,
7 and they are collected usually, so that you can
8 make platelets out of these units. If you bring
9 the units back to the center, spin them down, take
10 the platelet-rich plasma off, and immediately
11 filter the red cells, they do better than if you
12 bring them back to the center and leave them in the
13 refrigerator over night, which we often do, because
14 that had a higher did not filter rate if they were
15 allowed to get cold.
16 So, the same filter manufacturer accounted
17 for the 386 sickle failures that we had, so they
18 accounted for the majority of those, as well. Now,
19 I did not proportion out how many of the sickle,
20 well, we don't know how many of the sickle donors,
21 what proportion were filtered through manufacturer
22 1 versus manufacturer 2.
23 Residual WBC failures were rare, and they
24 were evenly distributed among the manufacturers,
25 among the parts of the country, and every other

1 thing that we could find, because we had these
2 split out into sections of the country.
3 [Slide.]
4 So, our investigation of our filter
5 failures told us that our failures out of
6 manufacturer 1, 2 million, we had 27,000 failures,
7 about a 1 percent rate. Manufacturer 2 overall,
8 this is the number I was looking for before,
9 712,000 failures were 2,887, for a 0.4 percent
10 rate.
11 [Slide.]
12 So, our filter failure investigation tells
13 us that there must be many potential causes, and
14 that all causes we think should be investigated.
15 One cause for all failures is really not plausible,
16 and that sickle trait testing alone does not solve
17 the leukoreduction problems in a constructive
18 manner.
19 We have a possible, just figuring on the
20 back of an envelope, because we don't know what our
21 African-American population is, we do know that we
22 have more African-Americans in the Southeast than
23 we do, for instance, in the North Central part of
24 the country, we don't have a significant difference
25 in failure rate between the Southeast and the North

1 Central part of the country.

2 If we have about 15,000 sickle trait

3 donors, if we estimate we have 5 percent

4 African-American donors, and 1 in 10 of those is

5 sickle trait, we would have a maximum of 15,000

6 sickle trait donors to be filtered, our failure

7 rate or our failure rate was more than double that.

8 So, that is the first thing. Even taking

9 all the sickle traits, if they all failed, it would

10 not account for our failure rate, and the second

11 thing is that we only had 57 white cell failure

12 rates out of 3.6 million.

13 So, these may represent failures that

14 might get out to the public, but I think that that

15 number is really very small.

16 Can I answer any questions?

17 DR. NELSON: Toby.

18 DR. SIMON: If I am interpreting this

19 correctly, I am assuming that these 0.8 percent

20 units that did not filter, that something went

21 wrong, that ultimately those units could not be

22 used.

23 DR. HALEY: Right, they never left the

24 blood center.

25 DR. SIMON: So, this is making a

1 significant impact on the blood shortage in the
2 United States.
3 DR. HALEY: That, it is.
4 DR. SIMON: As a whole. That is almost 1
5 percent if we found up of our units we are losing
6 because of leukoreduction issues. So, there is a
7 lot of urgency, I guess, to solving the variety of
8 problems.
9 DR. HALEY: That is correct.
10 DR. SIMON: But you are suggesting that
11 the sickle cell is a small component of that at
12 most.
13 DR. HALEY: That is correct.
14 Unfortunately, we do not use the rocker mixers, and
15 I have been in favor of that for some time. Maybe
16 we will reconsider it.
17 DR. SCHMIDT: In the large number of
18 failures you had, you said that I think it was
19 about 57, you knew the donors had sickle trait.
20 DR. NELSON: No, it was 300.
21 DR. HALEY: 360.
22 DR. SCHMIDT: But you didn't test all of
23 the donors, all the African-American donors for
24 sickle trait, so that is sort of an accidental
25 number.

1 DR. HALEY: That is an accidental, that is
2 exactly right.
3 DR. SCHMIDT: I don't know what percent
4 you test, but it could be very, very much higher.
5 DR. HALEY: That is correct.
6 DR. BIANCO: Becky, could you tell us a
7 little bit about the methods you use to count the
8 leukocytes?
9 DR. HALEY: We use the Nageotte chamber,
10 just as you do, Celso. I read your paper, too.
11 You have to dilute the blood with the diluting
12 fluid, you have to charge the chamber, and you have
13 to wait for them to settle, and then you manually
14 have to count, and I understand that seasickness
15 medicine is a big item in the lab. It is a very
16 difficult manual process.
17 DR. SIMON: One more quick question.
18 Those failures, I don't know, can we make an
19 assumption that most of them are just sort of over
20 the line, or are the units that just didn't filter
21 and have normal white counts?
22 DR. HALEY: I don't have white cell count
23 information for you, I am sorry.
24 DR. SIMON: You don't have any impression
25 on that, okay.

1 DR. STRONCEK: How many units do you
2 count? You said this at the end. How many units
3 again do you count for residual leukocytes, and how
4 many, what percent exceeded the minimum criteria?
5 DR. HALEY: One percent we count, 57 out
6 of 36,000 failed. I don't exactly have that
7 percentage, but it is a very small percent.
8 DR. NELSON: One out of 800.
9 DR. RUTA: Becky, on the slide where you
10 showed failures, if I understand it correctly, all
11 of those are blocked filters except for the 57
12 where you had the white cell residual counts.
13 DR. HALEY: And the 15 that didn't have
14 adequate red cell, we lost too many red cells.
15 DR. RUTA: So, it comes out to about 70
16 that didn't even recover enough of the red cells or
17 in which the white cells were too high.
18 DR. HALEY: That's correct. Those are the
19 only ones that got outside of the center, that
20 indicated units that may have gotten outside of the
21 center. Those didn't get out.
22 DR. RUTA: There are a couple concerns.
23 One is units that leave the center. The reports in
24 the abstracts, I guess not literature, but
25 abstracts, I guess half of the individuals with

1 sickle trait will filter, but not leukoreduce, so
2 the concern would be for units that might get out,
3 that might be labeled leukoreduced, but, in fact,
4 not be properly leukoreduced.
5 So, I would like you to comment on that,
6 and then again the question of whether small clots
7 could also prevent proper leukoreduction, so that
8 we might actually have a problem of units getting
9 out that are not leukoreduced because of small
10 clots. Again, if you or anyone has data on that.
11 DR. HALEY: First of all, the possibility
12 that we under-represented the number of units that
13 did not filter, but got out to the public, again,
14 we estimate in this 3.6 million units, that we
15 might have had about 15,000 sickle traits. I don't
16 think that by counting 36,000 units we could have
17 missed all 15,000. I mean I am not quite good
18 enough a mathematician to do that--oh, Mike Busch
19 is getting up, maybe he can tell me.
20 But I have to believe that with a 1
21 percent sampling, that that may have been a fairly
22 accurate record of how many are getting out, and I
23 really think that probably we are having filtration
24 failures in our leukoreduced units rather than
25 white cell failures, and on your question of could

1 small clots cause channeling, so that units can get
2 by without leukoreduction, I think that is
3 certainly possible.
4 I think that we saw in this rather large
5 sample is very little evidence, however.
6 DR. RUTA: Just to clarify a couple
7 points, and that is the 95 percent confidence
8 interval that was put in the FDA guidance is really
9 an assurance that the process is working as you are
10 doing it. It is not going to pick up a systemic
11 problem that may be at a lower level, so if there
12 are small clots that prevent leukoreduction, that,
13 it may not pick up, and if there are individuals
14 with sickle trait, who will filter but not
15 leukoreduce, that, it might not pick up also.
16 It doesn't mean we don't think that people
17 should not be doing things about clots, and let me
18 lead that to the next question, at what percent of
19 implementation of leukoreduction is the Red Cross
20 right now?
21 DR. HALEY: About 85 percent. We are
22 about 85 percent leukoreduction.
23 DR. RUTA: So, if I understood, so about
24 15 percent of the units would not be filtered right
25 now, so there are some number of clots that we

1 didn't know about a year ago, that are still going
2 out and I guess that number might be higher within
3 the other organizations that collect blood, who are
4 not at the 85 percent level of implementation.
5 DR. HALEY: That is correct, and we also
6 have had a big diminution of complaints about
7 sending clotted units out to the field. You know,
8 you are saying we are losing some units, but the
9 difference is we are now losing them in the blood
10 center instead of at the bedside hanging up for
11 transfusion, and, you know, you can think which of
12 those is better.
13 DR. RUTA: I think in some ways we
14 recognize the argument that if a unit doesn't leave
15 the center, then, it may not cause a safety problem
16 for someone outside the center, they may not get a
17 unit that they think is leukoreduced, that is not.
18 On the other hand, you know, it does go to
19 supply, and if there are ways to deal with clots,
20 then, it might be reasonable to try and deal with
21 those.
22 If I could ask you, you had about 12,000
23 failures that were undefined, have you done
24 anything to try and define what else is causing
25 filtration failures?

1 DR. HALEY: We have not. We fill our
2 further paperwork, and we go over the process to
3 make sure that we are docking the filter properly,
4 and that sort of stuff, so we have done simply
5 process investigations, we haven't done scientific
6 investigations of the units.
7 DR. NELSON: Briefly, Mike.
8 DR. BUSCH: There is a study called the
9 VAT study, which was recently published in JAMA,
10 and it was a randomized study of leukoreduced and
11 non-leukoreduced patients, HIV-infected patients.
12 In that study, we performed routine
13 quality control of about 3,000 leukoreduced units,
14 and these were pre-storage, leukoreduced at 11
15 different clinical sites, blood centers, and the
16 samples for quality control were actually from the
17 transfusion service at the time the units were
18 issued, and then they were QC'd using a very
19 sensitive quantitative PCR method.
20 Something in the range of 1 percent of
21 those units failed to meet the 5×10^6 cutoff, and
22 it was about 3 percent on the 1×10^6, and most of
23 these were just above the cutoff. So, when you are
24 talking about over-leukocyte limits out in the
25 field, these are just barely above the cutoff.

1 At one brief period of the study, there
2 were actually like 10 units from one hospital that
3 were just off-scale, and they basically were
4 completely unfiltered, and that turned out to trace
5 to a problem in the labeling and the definition of
6 the units.
7 But I think when you talk about failed
8 units that are out in the field, released, that do
9 get through the filter, that when they are failed,
10 they are barely over that cutoff, and they are
11 defined as failed, but you need to remember that
12 the definition of that cutoff is based on extremely
13 limited data. The value of 1×10^6 versus 5×10^6,
14 the clinical consequences is very poorly defined.
15 DR. NELSON: I would like to move on.
16 Thank you. I am sorry. Go ahead.
17 DR. KOERPER: Could I ask you one quick
18 question? If I have done my math correctly,
19 because you mentioned a 5 percent rate of sickle
20 cell trait donors?
21 DR. HALEY: Approximately. That is an
22 estimate. No, 5 percent African-American, 10
23 percent trait of the 5 percent.
24 DR. KOERPER: Thank you.
25 DR. HOLLINGER: I have got one question.

1 Of the number of failed units, were any of these
2 repeat donors or what happened to people--you have
3 a lot of repeat donors, so what happened when they
4 came back to donate again, were they likely to also
5 have filter problems, or do you know the
6 percentage?
7 DR. HALEY: Blaine, we don't keep a
8 component record by donor, so we don't know which
9 ones came in and failed the next. We don't know
10 their quality control results. Quality control
11 results do not go back to the person, they simply
12 stay with the unit record.
13 DR. HOLLINGER: And there is no way you
14 can use these units again, you kept the units, did
15 not use them, but is there a reason that you can't
16 use these units for something, components or
17 otherwise?
18 DR. HALEY: Half of them is one bag, and
19 half is in the other. We did use the platelets and
20 the plasma. I mean we didn't throw out all the
21 components, we certainly used the ones that were
22 usable, and they are very usable.
23 DR. HOLLINGER: But you don't know if
24 there is a filtration problem from a person except
25 for the sickle cell, that there is a problem

1 related to their coming back again?

2 DR. HALEY: We do not know. We suspect

3 there is, but we don't know.

4 DR. NELSON: Next is Cheri Jennings from

5 Gulf Coast Blood Center.

6 **Cheri Jennings**

7 MS. JENNINGS: Good afternoon. Thank you

8 for the opportunity to be here and discuss our

9 experiences in Houston.

10 [Slide.]

11 In January of 2000, we implemented 100

12 percent leukoreduction of red blood cells, which in

13 2000 amounted to approximately 183,000 units. We

14 immediately saw that a lot of units failed to

15 filter completely, and some were filtering more

16 slowly than expected.

17 When the new FDA guidelines came out with

18 the proposed 1×10^6, we also noticed that we had a

19 number of units that would fail to meet the new

20 guidelines.

21 [Slide.]

22 We started looking and we decided that the

23 cause of our filter problems was donors that sickle

24 trait positive, and then we also were interested in

25 finding out if the preparation of platelets and

1 source leukocytes from a unit would help us meet
2 the proposed 1 x 10^6 standard.
3 [Slide.]
4 We do use the dock-on Baxter Sepa Cell
5 filter. We filter at 4 degrees. We tested all
6 donors who indicated they were African-American for
7 sickle cell trait using the sickledex test.
8 [Slide.]
9 Every unit that we found to be sickle
10 trait positive, we did a residual WBC testing on,
11 whether it was a filter failure or not, and we
12 screened with out Baker hematology instrument, and
13 if it appeared to be low, we then did testing with
14 the IMAGN.
15 [Slide.]
16 For four months, we did look at units that
17 did not meet the 1 x 10^6 standards to evaluate
18 whether platelets and source leukocytes were
19 prepared.
20 [Slide.]
21 As you can see here, we were kind of
22 surprised to see that sickle trait positive donors
23 were not the predominant cause of our filter
24 failures. Then, when we tested them, essentially
25 all of them were unacceptable with the 5 x 10^6

1 standard.

2 [Slide.]

3 These are the results of the problem

4 filters. Of the ones that are sickle trait

5 negative, most of them do have acceptable WBC

6 counts. Of these here, that were sickle trait

7 positive, they did meet the 5 x 10^6, but none of

8 them would meet the 1 x 10^6 proposed standard.

9 [Slide.]

10 This in the information on the units

11 whether we prepared platelets and source

12 leukocytes. We had the group, 74, that did not

13 meet the new proposed standard, and this is our

14 control group.

15 In the control group, of the 53 that we

16 made a platelet, we also made a source leukocyte,

17 and really, it appears that it might help to make a

18 platelet or source leukocyte, but what was

19 interesting is the ones that failed had a larger

20 average residual volume, and so there seemed to be

21 something going on with the volume of the red cell

22 compared to our control group.

23 [Slide.]

24 So, what we concluded was of sickle trait

25 positive units, 71 percent filtered within the

1 acceptable or our expected time frame of two hours.
2 We have now gone down to a one-hour time period for
3 what we consider as an acceptable time frame.
4 Again, of the ones that are positive for
5 sickle trait, 82 percent failed the current
6 guidelines for WBCs.
7 When you look at the ones that did not
8 filter or filtered in greater than two hours, 34
9 percent of those were sickle trait positive.
10 [Slide.]
11 Seventy-three percent of the sickle trait
12 negative units, that did not filter within two
13 hours, did have acceptable WBCs. Again, it appears
14 that the preparation of a platelet or source
15 leukocyte may help us reach the new proposed
16 standard.
17 [Slide.]
18 So, from all of this, what we are doing at
19 Gulf Coast is if we have a unit that does filter
20 and we discover that it is sickle trait positive,
21 we will do white cell testing on that. We are also
22 flagging all donors that we identify as sickle
23 trait positive, and we will not filter them in the
24 future.
25 We have found 9 donors who do not fit into

1 a category that repeatedly failed to leukoreduce,
2 and they all have more than four donations each,
3 and we are going to try to get those people in and
4 perform hemoglobin electrophoresis studies on them
5 to see if possibly hemoglobin C or something else
6 is the cause of the failure.
7 DR. NELSON: Thank you very much.
8 DR. SCHMIDT: I am going to ask something
9 which I think might have bearing on our vote. As I
10 remember what you said, you did the test on all
11 donors who indicated they were African-American.
12 Now, this is a very touchy area obviously.
13 Did you ask this of all donors or was this
14 asked in writing, did you only ask certain donors,
15 or how was it handled on a practical level, and
16 what was the response of the donors to this
17 question?
18 MS. JENNINGS: We have always had a
19 question on our questionnaire about race. It is
20 optional whether they answer that question or not.
21 So, if they indicated on the donor questionnaire
22 that they were African-American, then, that is
23 where we got the ones that we tested.
24 What I am thinking we are going to do now,
25 it is a very touchy area, is I think we will move

1 to testing all new donors, because we know there
2 are some people that are African-American that
3 aren't indicating it.
4 We also have a very diverse population in
5 Houston, so we find there are people that are of
6 mixed race and do not consider themselves to be
7 African-American.
8 DR. NELSON: Toby.
9 DR. SIMON: Just sort of following up
10 quickly with Dr. Schmidt, I assume the idea of
11 asking that was for patients with sickle cell for
12 these transfusion programs, to find them?
13 MS. JENNINGS: A lot of it was just
14 demographic information, but we do freeze, we have
15 a very large frozen inventory, and so we have
16 always tested people we are getting ready to freeze
17 for sickle cell.
18 DR. SIMON: The second question I was
19 going to ask is if you are now going to identify
20 those units and not filter them, but you are in 100
21 percent leukoreduction program, what are you going
22 to do with the units that aren't filtered?
23 MS. JENNINGS: We have been sending those
24 outside of the region.
25 [Laughter.]

1 DR. SIMON: So, if the country goes to 100
2 percent leukoreduction--
3 MS. JENNINGS: Then, we are kind of stuck.
4 DR. NELSON: Are you in Florida or
5 Arizona, Toby?
6 DR. SIMON: New Mexico.
7 DR. NELSON: Okay. It's close by.
8 MS. JENNINGS: We are going to try to
9 entice these people to donate platelets or plasma,
10 but from what I have heard, it isn't very
11 successful in trying to get them to do that.
12 DR. MITCHELL: You said that you are going
13 to move to test all of your blood units for sickle
14 cell, or are you only going to test
15 African-American, blood from African-Americans for
16 sickle cell trait?
17 MS. JENNINGS: No, I think what we will
18 end up doing is testing all new donors. That takes
19 away the stigma of doing genetic testing only on a
20 certain population, and it will also, again, some
21 people of mixed race would not check that box is
22 what we are thinking, and since it is an optional
23 question, the only way to make sure that we test
24 everyone at risk is just to test new donors, and
25 any one of those that we find that is positive, we

1 will do the white cell testing on.

2 DR. MITCHELL: Do you ever find trait in
3 people who are not African-American?

4 MS. JENNINGS: We have only been testing
5 those that indicate they are African-American. I
6 am sure there are other groups, you know, Northern
7 Europe, there are other areas where sickle trait is
8 prevalent.

9 DR. MITCHELL: Mediterranean region, yes,
10 there are a lot of places.

11 MS. JENNINGS: I am sure we are going to
12 find that.

13 DR. MITCHELL: I guess the other issue was
14 about usage of blood for fractionation. I know
15 that some of the other places use blood for blood
16 components.

17 MS. JENNINGS: There is enough call for
18 the non-leukoreduced red cells. We have been
19 sending those to other places, but we do have an
20 agreement with some fractionaters, and we could
21 start sending it there.

22 DR. KOERPER: I just want to point out
23 that there are a number of Hispanics who are sickle
24 trait positive, and we are following Hispanic
25 patients with sickle cell disease, so you are going

1 to miss people if you limit to someone who checks
2 African-American.
3 DR. SCHMIDT: You are going to be in a
4 very sticky area if you are not using or you are
5 making special usages of the blood collected.
6 When Dr. Ruta told us this morning, you
7 had to tell everybody who was deferred why they
8 were deferred, so you are going to have some people
9 who you did sickle cell testing on, and if you have
10 to notify them of that, that is the first time in
11 their life they knew that, this is going to be a
12 real morass. I don't know if you do have to let
13 them know.
14 DR. RUTA: Can I jump in a minute since I
15 was mentioned here? Actually, the reg now applies
16 to suitability criteria that are currently in the
17 regulations right now. So, it is not for other
18 conditions outside of the regulation.
19 So, if blood banks are deferring for
20 conditions outside of the regulations right now,
21 the notification regulation does not apply to them,
22 so if they are deferring for any other reasons that
23 are not currently in 640.3, or the testing reg,
24 then, the notification reg does not apply right
25 now.

1 DR. NELSON: You wouldn't necessarily
2 defer.
3 MS. JENNINGS: They are not deferred. We
4 are flagging them, just like we would flag them as
5 do not freeze.
6 DR. NELSON: You just wouldn't filter.
7 MS. JENNINGS: Right. They are being
8 flagged as do not filter or freeze. We have also
9 been in contact with the local Sickle Cell Anemia
10 Society, who is working with us on how to counsel
11 individuals when and if we decide to inform them of
12 this, but when this has come up before, everybody
13 has already known that they were sickle trait
14 positive. I think they are doing extensive testing
15 of infants for sickle trait.
16 DR. STRONCEK: Even though the deferral
17 issue isn't in the regulations, ethically, I
18 think--well, right now you can do what you are
19 doing, but down the line when we go to universal
20 leukocyte reduction, when these people come in, if
21 we know they have sickle cell trait, we do have to
22 not collect their red cells, because we collect
23 their red cells and then throw them out, that is
24 not ethical.
25 So, then it gets into a deferral thing.