1 control. We can have no growth coming back
2 out of that lot.
3 There are some papers that have
4 been written by the Michigan Tissue Bank on
5 the dosage that's required for chlostridium
6 and other bacterial forms.
7 DR. PETTEWAY: It's mostly
8 bacterial?
9 MS. WILSON: Exactly right,
10 bacterial.
11 DR. BOLTON: Any other questions?
12 Yes.
13 DR. SOLOMON: Thank you very much,
14 Diane. I don't know if you will know the
15 answer or someone in the audience. Could
16 you comment on of all the tissue banks that
17 are out there that we know of how many are
18 accredited by AATB and what percent are not
19 accredited but still follow the AATB
20 standards?
21 MS. WILSON: Ellen Heck actually
22 might be a little better at that as chairman

1 of the accreditation committee. I believe
2 there are approximately 60 accredited banks.
3 How many other banks there, Bob Rigney, do
4 you have an answer?
5 MR. RIGNEY: Ruth could probably
6 give us the number that had registered with
7 FDA at this point under the new registration
8 requirements. We have 74 banks that are
9 currently credited by the AATB involved in
10 either retrieval, storage, processing and
11 distribution, one or more of those
12 functions.
13 How many other banks are out
14 there, it depends on how you define what a
15 tissue bank is. Our members who are
16 accredited by us retrieve and process and
17 distribute the majority of the tissue in the
18 United States. Does that answer the
19 question?
20 DR. DOPPELT: That brought up some
21 more questions.
22 DR. BOLTON: Go ahead.

1 DR. DOPPELT: I was just going to
2 say, as Bob Rigney said, probably 90, 95
3 percent of the tissue distributed in the
4 United States comes from AATB-accredited
5 banks. If there are 73 or 74 accredited
6 banks there is a large discrepancy between
7 that number and the number that the FDA has
8 as registered organizations that in one way
9 or another deal with tissue.
10 But if you ask the question how
11 many banks are there functioning in a
12 similar fashion to the accredited banks,
13 that is, they are still distributing tissue,
14 that are not accredited, just as a guess I
15 would probably say there are maybe another
16 ten or twelve that are functioning in that
17 capacity. The discrepancies are other
18 organizations that are doing cells and so
19 forth.
20 DR. BOLTON: Dr. McCulloch.
21 DR. McCULLOUGH: This is a
22 question about the irradiation and

1 sterilization. Are there any issues about
2 whether all of the parts of a particular
3 piece of tissue are adequately irradiated?
4 I know in irradiating blood, for instance,
5 there are different doses of radiation that
6 actually apply to different parts of the
7 blood bag and you have to be sure that all
8 the dose you want actually gets to all the
9 blood. It seems like this might be more of
10 a problem for large pieces of bone or things
11 like that.
12 MS. WILSON: Right, AATB standards
13 state that the minimum dose is 1.5 megarads
14 and that process should be validated as to
15 the dosimetry of the boxes. The irradiation
16 company that you work with you should have
17 worked with to make sure that your boxes are
18 equally penetrated and that your bone is
19 penetrated.
20 DR. BOLTON: Any other questions?
21 Good, we'll move on.
22 Our next speaker is Ellen Heck

1 from the Transplant Services, University of
2 Texas and she will talk about the Eye Bank
3 Association of America. I also understand
4 that it is her birthday today so happy
5 birthday, Ellen.
6 MS. HECK: Who gave you that
7 information?
8 DR. BOLTON: We have our sources.
9 MS. HECK: Thank you. There are
10 many similarities in eye banking and tissue
11 banking but also some differences but like
12 with tissue banking we are concerned about
13 screening, sterility, and asepsis to arrive
14 at what we hope is a safe yet effective
15 product.
16 We really believe that the
17 screening process begins with public and
18 professional education because we are using
19 a second person historian. Unlike the blood
20 industry where you can interview the donor
21 themselves we are getting our information
22 secondhand, if you will, so we do want to

1 have an educated historian from whom we are
2 going to be gathering the screening
3 information as well as an expert resource in
4 our professional and medical staff to know
5 the sorts of questions and answers that we
6 are looking for when we begin to do our
7 screening process.
8 The consent process can never get
9 to that point because in our initial
10 screening we will talk with the hospital
11 about things such as the admitting
12 diagnosis, the temperatures, the fluid
13 volumes that were administered so we can do
14 hemodilution calculations, the white blood
15 cell counts, any high risk behaviors or
16 infections that might be present in a
17 potential donor before we even proceed to
18 take a consent.
19 Then, of course, the consent may
20 be declined. After that, however, if it is
21 accepted we have some 45 in-depth questions
22 that we will be asking the historian about

1 the person's medical history and their
2 social history. Now, these comply with the
3 EBAA guidelines that help us to reduce
4 transmission of disease.
5 These include things that are
6 applicable to the CJD. When we talk about
7 death with neurological disease of
8 unestablished etiology certainly we don't
9 know to what extent we are going to get the
10 correct answers based on the stage that an
11 individual might be as we have heard
12 repeatedly this morning but nevertheless we
13 feel that these questions have helped us
14 with both recipient safety and with
15 technician safety as we ask these questions.
16 The standard goes on to
17 specifically reference dementia. Unless it
18 can be attributed to cardiovascular disease,
19 brain tumor or head trauma donors with
20 metabolic-induced dementia may be acceptable
21 under certain consultations and the medical
22 director may decide to use those.

1 Our concern is that we are
2 excluding a number of cases that we don't
3 truly believe are infective such as
4 Alzheimer's Disease but we believe in doing
5 so, because we are doing this in the field
6 without the ability to do long-term studies
7 and without the ability to know when someone
8 says well, we think that grandmother may
9 have had Alzheimer's we don't know what
10 that's based upon in terms of a diagnosis
11 and what extent that was so we just go ahead
12 and rule those patients out of the pool
13 initially.
14 These are the things we are asking
15 about, loss of memory, inappropriate
16 responses, confusion, gait changes, human-
17 derived pituitary hormone, or dura mater.
18 We can prescribe the questions that are
19 going to be asked in the field but we also
20 need to remember that there is a certain
21 skill involved in eliciting this information
22 so we do a lot of training trying to get our

1 people to a point where they can ask these
2 questions and know what subsequent questions
3 to ask to get the information that they are
4 truly seeking in order to screen this.
5 Again, we don't know whether they
6 are valid until you get to late in the
7 progression of the disease with CJD but if
8 we do it can prevent an inappropriate
9 retrieval. Once again, we are not confident
10 in the diagnosis of Alzheimer's so we do
11 rule those out in the field.
12 In addition to screening we do a
13 physical assessment of the donor which has
14 been very helpful to us in diagnosing other
15 diseases, particularly some of the high-risk
16 behaviors, but we don't know that it has any
17 value for us in the CJD situation.
18 Following that, probably the most
19 relied-upon screening tool that we have is
20 serologic testing. Now, that testing may
21 occur before procurement but most frequently
22 is going to occur after procurement. Those

1 are the tests that Diane mentioned earlier
2 that are prescribed by the FDA or prescribed
3 by the EBAA and that may remove donors.
4 Unfortunately, at this moment we have no
5 such readily applicable screening test for
6 CJD. I wish we did. But those rule out a
7 number of other donors.
8 Finally, we get into the
9 processing and we may have rejections occur
10 here. We have more medical history review.
11 We are looking at whether anyone else
12 participated in collecting donor tissue and
13 getting information from them, trying to
14 construct a complete donor pool. So we have
15 again a lot of different opportunities to
16 reject a donor as we go through this
17 process.
18 Finally, by the time we get to
19 final labeling and release we get to this
20 point and hopefully we never get to the
21 second point right down here but we have all
22 been familiar with it because of FDA

1 regulations and certainly sometimes it does
2 happen.
3 But if we have done everything
4 right here we have had seven opportunities
5 to reject this tissue from transplant before
6 we ever get to actual release. There is the
7 initial review with the hospital personnel
8 where we go over the first five or six
9 questions. Then there is the family-
10 obtained history where I told you that we go
11 over at least 45 questions. There is the
12 medical chart review. There is the testing
13 that we do. There is the procurement review
14 which includes really a review of all of the
15 information that we have, the processing
16 review, which again is brought in-house and
17 allows several people who didn't go over it
18 in the initial stage to go over it again,
19 and then finally the final release of the
20 tissue review. So we have gone through a
21 number of steps.
22 The second step that we believe is

1 necessary to provide safety is sterility or
2 sterilization. Sterility is an issue that
3 has been a great deal of concern to us.
4 About six or eight months ago Paul Brown and
5 Nick Hogan talked to the Eye Bank
6 Association meeting and we talked a great
7 deal about sterilization of instruments.
8 So we want to reiterate something
9 that Diane said about the other tissues.
10 This is a single set of instruments per
11 donor. We do it a little bit differently in
12 eye banking because we do what we call an
13 inside and outside set of instruments. Here
14 are the outside instruments that come in
15 contact with the conjunctiva and the outer
16 portion of the eye and the inside
17 instruments and I want to particularly call
18 your attention, if I may, to these inside
19 instruments to these tiny little scissors
20 right here. These are very sensitive
21 instruments which require a great deal of
22 sharpness and proficiency to do the proper

1 type of surgical technique. Now, this
2 isolation of inside and outside instruments
3 has been very helpful to us in controlling
4 bacterial contamination. I don't know that
5 it helps us with CJD.
6 Many eye banks after Dr. Brown's
7 talk went to additional sterilization,
8 decontamination stage with their instruments
9 and what this really amounts to is that we
10 rinse the instruments immediately following
11 the procurement and we bring the instruments
12 back in a moist environment, keep them moist
13 all the time, and then we sterilize them at
14 135 degrees for 30 minutes. This is the
15 first decontamination step with the
16 instruments.
17 After that we go through a
18 cleaning process where we scrub the
19 instruments, we check them for function, and
20 we place them in Cidex for 30 minutes.
21 Following the Cidex 30-minute deactivation
22 we rinse them and put them into a milk bath

1 and we rinse them with 70-percent isopropyl
2 alcohol. We dry them, we lubricate them,
3 and then we sterilize them a second time.
4 That is before they would then be ready to
5 use on an additional donor.
6 These instruments are already
7 showing some corrosion and deterioration in
8 function based on this high sterility time
9 but we felt it was worth the extra effort
10 and the extra sterility exposure based on
11 what Dr. Brown had told us. He felt we were
12 reducing our risk significantly with this
13 step and it doesn't as yet cause us great
14 additional cost.
15 We are certainly looking into
16 disposable instruments but at the moment
17 don't have the kind of scissors that I told
18 you in the beginning were so important to us
19 because the surgical removal of the tissue
20 is a very important step in whether the
21 tissue is functional. If you put too much
22 stress and strain on the tissue in removing

1 it because you don't have the appropriate
2 instrumentation then you may indeed damage
3 the corneal endothelial cells that you are
4 actually trying to transplant.
5 The third step is asepsis and we
6 do aseptic procurements. Just like in the
7 tissue bank we establish sterile fields, we
8 use personal protective apparel, we use
9 sterile instrument kits which we just talked
10 about, and we try to do this in an aseptic
11 manner, OR technique, et cetera although we
12 do not need an OR for the retrieval of
13 corneas.
14 We can set up this environment in
15 a very limited space because obviously we
16 are removing a very limited tissue that is
17 fairly surface in its nature. This has been
18 very effective in controlling contamination
19 of bacteria and fungus and in limiting
20 environmental exposure and technician
21 exposure.
22 In the processing of corneal

1 tissue, which really does not under go a
2 great deal of processing, but if we have
3 removed a whole globe and come back to the
4 laboratory to excise a cornea we do that
5 with the same procedures as we would do the
6 removal except, of course, here now we have
7 a hood cleaning, which is a step we would
8 not have if we were doing the procurement in
9 situ. We do the hood cleaning, the personal
10 protective apparel, the sterile field, the
11 sterile instruments and the aseptic
12 technique.
13 We generally clean our hoods with
14 a bleach solution and an alcohol solution.
15 Hoods are required to be cleaned before and
16 after each use and are usually cleaned at
17 regular intervals in addition to that. The
18 majority of our hoods undergo culturing
19 processes on monthly intervals to validate
20 that the cleaning processes are indeed
21 working.
22 We do establish a sterile field.

1 Notice we have a sterile instrument kit
2 that's used for each procedure. When we
3 come back and do the decontamination steps
4 of these instruments they are kept in one
5 single container so that there is no
6 batching or mixing of instruments during the
7 sterilization process as well.
8 This just shows you what happens,
9 our personal protective apparel. Here you
10 see a corneal scleral rim being removed.
11 The things that are important to us here are
12 that we have a nice even rim, that we don't
13 put undue tension on this as we separate it,
14 causing loss of cells on the endothelium or
15 damage to the tissue.
16 Well, our final step in processing
17 to assure safety is labeling. All of our
18 tissue is labeled "Single Patient
19 Application Only." I think it is important
20 to consider the number of applications this
21 might represent from a single donor because
22 it is dramatically different from some of

1 the things that you have heard today.
2 Single patient application from a
3 corneal donor, assuming that we had both
4 eyes and were acceptable for donation, you
5 would have two corneal grafts. If it was a
6 whole eye donation you would have two
7 scleral grafts which could result in either
8 two grafts or up to eight grafts so that you
9 have a maximum potential here from one
10 corneal donor or approximately ten
11 applications.
12 Finally, the graft is labeled "Not
13 Sterile" and again for single patient use
14 only. It is sealed with a tamper-proof seal
15 and an individual number and labeling
16 process so that the graft can be tracked.
17 Finally, when FDA speaks eye banks
18 are used to jumping and we certainly are
19 willing to do anything that makes our grafts
20 more safe. But I think it's important also
21 for you to realize that we could not
22 complete our mission if we were not

1 concerned for safety. We have always been
2 eager to do what it took to be sure that the
3 recipients received a safe graft.
4 Finally, our mission affects
5 thousands of individuals each year, both
6 those who receive the transplants and those
7 who give the transplants, and we are
8 conscious of this and are concerned for
9 both. Now, these two children, this little
10 girl is the recipient of a corneal graft.
11 This young lady's sister died in an
12 automobile accident and was the donor of a
13 corneal graft. So when you are dealing with
14 recipients and donor families like this you
15 can't but take your mission seriously and I
16 assure you that our commitment to safety is
17 very serious. Thank you.
18 DR. BOLTON: Thank you, Ellen.
19 Are there questions from the committee or
20 comments? Ermias?
21 DR. BELAY: One of the donor
22 screening parameters that you describe is

1 getting historical information from the
2 families, historical information on whether
3 that donor received human growth hormone?
4 MS. HECK: Yes.
5 DR. BELAY: I always have
6 difficulty understanding how this historical
7 information is collected. If you take
8 cornea as an example, the donor is dead at
9 the time of the donation, one, and, two, the
10 next of kin may not be available to provide
11 the appropriate data.
12 In addition a good chunk of the
13 corneas, my understanding is, are collected
14 under what is known as legislative consent,
15 which would mean the family is not even
16 available to provide that kind of
17 information.
18 Now, do you have any information
19 on what proportion of, let's say, the
20 corneal donors we actually collect that type
21 of information on?
22 MS. HECK: Although at one time

1 that was a very high percentage of the donor
2 population, and I think back in the '70s it
3 may have accounted for as much as 50 percent
4 of the donor population, it is probably down
5 now to something closer to 15 or 20 percent.
6 The experience that I gave you is
7 our experience and we collect none of our
8 tissue without family consent and family
9 medical-social history interview. I think
10 you are seeing that becoming the more common
11 trend where back in the '70s, perhaps, the
12 medical examiner or legislative consent was
13 more prevalent but it certainly has
14 diminished.
15 In terms of getting that
16 information from the family, if I may, we
17 get it from the best historian available at
18 the time. That may be a family member. It
19 may be a close friend. It may be both a
20 family member and a close friend. We look
21 at multiple sources to try to do that. We
22 also may incorporate an interview with the

1 primary care physician if there is a primary
2 care physician. This is done by either
3 nursing personnel or trained transplant
4 personnel.
5 DR. BOLTON: When you say we
6 collect none without family consent is that
7 "we" the Eye Bank Association of America or
8 is that your local facility?
9 MS. HECK: That's our local
10 facility. The Eye Bank Association of
11 America does at this moment permit
12 legislative consent tissue but, again, I
13 think the majority of members have moved
14 away from that.
15 DR. BELAY: This has a direct
16 bearing on the questions that we are
17 considering because most of the additional
18 criteria in the FDA guidance relies heavily
19 on historical information provided by family
20 members.
21 MS. HECK: Yes.
22 DR. BOLTON: Other questions?

1 I'll just make a comment on that and that is
2 that again when you look at those potential
3 donors that are removed via donor exclusion
4 criteria through historical information it
5 still will only get those that are clinical
6 and a very small percentage of those that
7 would be pre-clinical like those that are
8 removed for variant CJD risk or other
9 iatrogenic CJD. But those that are
10 incubating disease will still not be picked
11 up by that so we go back to our
12 neuropathologists to save us in that case.
13 Yes, Dr. McCullough.
14 DR. McCULLOUGH: Can I pursue the
15 legislative consent donors? You say roughly
16 15 to 20 percent of all donations would be
17 so that essentially means that 15 to 20
18 percent of corneal donations would be
19 obtained without a medical history?
20 MS. HECK: Certainly without a
21 family interview. I won't say completely
22 without a medical history because the

1 medical examiner will be getting information
2 and providing information to you which is
3 helpful in constructing your donor profile.
4 But you will be absent that family interview
5 piece. That's correct.
6 DR. McCULLOUGH: That would always
7 occur just at the medical examiner's venue
8 and everything else does involve a family
9 history?
10 MS. HECK: The laws vary somewhat
11 state to state. It is called the Medical
12 Examiner and Coroner Law. If it's a coroner
13 that releases the tissue then you would not
14 have that advantage of a medical examiner.
15 I think it is worth pointing out,
16 however, that the majority of these cases,
17 certainly not all of them but the majority
18 of these cases, fall into that category
19 under age 50 that you talked about which are
20 less likely to be manifesting the disease or
21 have symptoms that we could track.
22 I know after you said the instance

1 I might get it after 50 I thought maybe I
2 should just go home and shoot myself because
3 I have, as you heard today, passed 50 but
4 then you said if I could hang out until 72
5 maybe I'd be all right so I'm thinking that
6 one over.
7 But those ME cases are usually
8 traffic accidents, violent deaths,
9 unexpected deaths of some sort, and do fall
10 primarily towards the lower end.
11 DR. McCULLOUGH: What percentage
12 of those would have experienced head trauma?
13 MS. HECK: A lot of them
14 experience head trauma. That is an
15 interesting question if I may step from EBAA
16 to AATB because our bank does both bone,
17 skin, and ocular tissue. In ocular tissue
18 sometimes the head trauma will preclude you
19 from getting the cornea simply because
20 there's enough edema that you can't get
21 them.
22 But that certainly doesn't

1 preclude me from getting bone and skin
2 because it doesn't affect that. So head
3 trauma, although reduced by helmet laws and
4 other things, it's still a major source of
5 donor tissue in this country.
6 DR. DeARMOND: What level of head
7 trauma? Everybody bumps their head. What
8 do you consider serious?
9 MS. HECK: Well, I'm thinking
10 primarily of motorcycle and MVAs, motor
11 vehicle accidents. That's the head trauma
12 that we see mostly that's caused the
13 fatality and there's a significant amount of
14 that.
15 DR. BOLTON: David?
16 MR. ASHER: I'd like to clarify
17 something about the medical standards. As I
18 understand it, the screening questions if
19 they detect a history of loss of memory,
20 inappropriate response, confusion, or gait
21 changes, those would be deferral factors but
22 a medical director has the option of

1 reinstating if in the medical director's
2 opinion those things are due to cerebral
3 vascular disease or other known causes?
4 MS. HECK: That's correct.
5 MR. ASHER: The reason I'm
6 concerned is that's an exact description of
7 our last dura associated case.
8 MS. HECK: I'm sorry. I didn't
9 hear the end of your statement.
10 MR. ASHER: That is an exact
11 description of what happened with our last
12 dura mater associated case. It had all
13 those things and the person doing the
14 medical review attributed them to cerebral
15 vascular disease, which the donor also had.
16 MS. HECK: I think that over the
17 last few months, several months and maybe
18 the last couple of years, you find that if
19 you get a composite of those things they are
20 much more likely to be rejected even if you
21 think you know what they are from.
22 I know certainly we have moved

1 that way and I believe that the majority of
2 eye banks have, too. It's when you may have
3 one of those but not the composite of the
4 three that the medical director might be
5 likely to say I know what this is from.
6 MR. ASHER: Since these cases are
7 almost certainly not autopsied on what basis
8 would the medical director make that
9 decision to reclassify such a donor?
10 MS. HECK: Those would primarily
11 be made on findings in the medical chart,
12 previous diagnosis by consultants, things
13 that we would review by calling primary care
14 physicians or getting information from
15 attending physicians in the hospital if
16 there's some specific documentation present
17 in the chart.
18 DR. BOLTON: Other questions?
19 Okay, very good, Ellen. Happy birthday
20 again.
21 We will move on. I think what
22 we're going to do is we are going to take

1 the next two presentations. We are running
2 about 45 minutes behind schedule. What I'd
3 like to do is take the next two, which have
4 to do with equipment and instrument
5 sterilization and what have you and process
6 validation.
7 Then we'll break for lunch before
8 the industry presentations on process
9 validation so we can get all of those in
10 without everybody feeling the pangs of
11 hunger and we'll come back after lunch and
12 have those.
13 So our next speaker is Dr. Robert
14 Rohwer from the VA Medical Center at the
15 University of Maryland, Baltimore. Bob will
16 talk on "Equipment and Instruments: TSE
17 Agent Disinfection in Routine and
18 Exceptional Situations." Bob.
19 DR. ROHWER: I was disappointed
20 that nobody asked Ms. Heck what the milk
21 bath was for. I'm curious and I'll have to
22 ask her myself.

1 I had the liberty of reengineering
2 this title to suit myself so this is what we
3 are going to discuss, disinfection and
4 sterilization of TSE-contaminated surgical
5 instruments. On the other hand I know of no
6 systematic study of this issue and as a
7 consequence we're going to have to beat
8 around the edges of this subject a little
9 bit.
10 What I'm going to do is review
11 some of the principles of inactivation of
12 these agents which I think are important and
13 we will look at their application to the
14 inactivation by sodium hydroxide and heat,
15 which are the only two really effective
16 inactivants that we know of. I'll mention
17 bleach and then I'll go over the WHO
18 recommendations for disinfection and also
19 tell you how we go about this job in our
20 laboratory and give you some anecdotal
21 evidence from the laboratory that suggests
22 that it does in fact work.

1 However, I'll finish by telling
2 you about an incident that occurred in
3 Windsor, Canada, in which they attempted to
4 apply these methods on a very large scale to
5 disastrous effect so they are still things
6 we have to do and learn about this process.
7 The main point I'm going to make
8 in the beginning of this talk is that the
9 susceptibility to an activation of TSE
10 agents if they are looked at in a kinetic
11 fashion are not that different from what you
12 would expect from normal viruses, the more
13 resistant, from the virus families or
14 spores. On the other hand even though this
15 is true, TSE agents are still very difficult
16 to disinfect. The reason for that, I'll try
17 to convince you, is not really due to the
18 failure of the inactivants themselves but
19 rather due to inadequate exposure of the
20 agents to these inactivants.
21 What I'm going to tell you is
22 based on some old work of myself and these

1 references and I highly recommend this more
2 recent document from the WHO laying out many
3 of the issues involved with disinfection and
4 infection control for these agents. This
5 document was put together as a consensus
6 document. It involved myself, David Taylor,
7 Paul Brown, a bunch of people, and I think
8 it really does represent the best current
9 thinking on this.
10 Now, I want to go over just the
11 inactivation process itself so that we are
12 all on the same wavelength when I discuss
13 the actual data for the TSE agents. I am
14 going to be discussing curves like this in
15 which the TSE agent is exposed to some
16 process, heat or chemical, for a period of
17 time and we have the surviving fraction over
18 here.
19 What's going on in an activation
20 curve like this is that if we started with
21 this many units of infectivity everything is
22 happening very fast and very early in the

1 inactivation process. By the time we are
2 here on this curve we have only got 10
3 percent of the population left and by the
4 time we are here we only have 1 percent of
5 the population left.
6 Another way of looking at it is if
7 you look on this axis down here 90 percent
8 of the effect occurs very quickly, 10
9 percent in the next tiny little time
10 interval here, 1 percent in this time
11 interval here, .1 percent of the effect
12 here, .01 percent here, et cetera.
13 This initial rate of inactivation
14 describes the way 99.99 percent of the
15 infectivity is behaving in this curve;
16 however, in this curve right here we are
17 describing how only one part per thousand is
18 behaving. It is a bimodal distribution of
19 effect.
20 The properties intrinsic to the
21 agent are reflected in the initial rate of
22 inactivation. This is basic chemistry. The

1 vast majority is being inactivated and the
2 interpretation is much less complex than for
3 the residual fraction, which is a complex
4 function of environmental parameters much
5 more complex but depends on the context of
6 the agent, the milieu in which it finds
7 itself to some extent.
8 Here's an example of this type of
9 inactivation using bleach at the
10 concentration in which it is recommended to
11 be used on the bottle. This is not the
12 highly effective concentration which is
13 recommended from David Taylor's work which
14 is 5 percent, ten times this concentration,
15 or undiluted bleach. Nevertheless we see
16 that we have a very rapid destruction of
17 infectivity on contact with bleach and then
18 a residual survival from that point forward.
19 We get the same kind of thing when
20 we put conventional viruses into the same
21 brain homogenate milieu showing you that
22 there is a protective effect of the

1 bioburden of the brain homogenate itself.
2 These same viruses in highly purified form
3 are killed to undetectable levels almost
4 instantly.
5 The inactivant of choice in our
6 hands has been sodium hydroxide. This is
7 some work that Paul Brown and I did many
8 years ago comparing Creutzfeldt-Jakob
9 disease and 263K scrapie which can be
10 challenged at higher titers to these various
11 concentrations of sodium hydroxide.
12 The main thing I want to point out
13 is that at one normal we had inactivation to
14 undetectable levels for both CJD and scrapie
15 after 60 minutes of exposure but almost as
16 good an effect from 10th normal; however it
17 falls off dramatically when you go below
18 this concentration. Somewhere between here
19 and here we are losing efficacy. Also, even
20 after 15 minutes of exposure we have done
21 most of the work.
22 On the other hand this is not a

1 consistent finding and the reason for the
2 inconsistencies probably has to do with the
3 details of the experimental approach. So
4 where it has been highlighted in yellow
5 these are all experiments from the
6 literature in which complete, and by
7 "complete" I mean inactivation to no
8 survivors given the size of the challenge
9 that was actually assayed, these experiments
10 represent that experience but in the same
11 chart we also have a number of instances in
12 which very high levels of inactivation were
13 reached but some residual infectivity was
14 nevertheless recovered.
15 A similar picture pertains for
16 heat except the initial activation is even
17 much more dramatic. This was a kinetic
18 experiment looking at what used to be the
19 standard autoclave temperature of 121
20 degrees centigrade. This was set up in an
21 oil bath so samples could be taken very
22 rapidly.

1 Once they came to temperature and
2 then cooled and then assayed what we see
3 here is that by the time the sample got to
4 temperature, and the ramp time here was
5 about 30 seconds, we had 99.999 percent of
6 the population destroyed. The surviving
7 fraction is at the level of parts per
8 million but it does survive and it drags out
9 here for another 15 minutes or so.
10 Similar data have been developed
11 by David Taylor using a somewhat different
12 system in which brain macerates rather than
13 homogenates were statically exposed to these
14 temperature regimes here for these times and
15 temperatures, giving the result here.
16 This is somewhat shocking. At
17 first glance this looks like a shocking
18 result in the sense that survivors, and by
19 "survivors" I mean infectivity survived this
20 treatment to the tune of four infections out
21 of 13 animals inoculated under these
22 conditions, et cetera, but if you look at

1 this data and plot it the same way that I
2 plotted my previous curve these points would
3 all fall down on this part of the curve.
4 The reason for that is that these
5 are only partially effective in killing the
6 infectivity. We are at limiting dilution
7 and when you put it back on a chart like
8 this it would be in the same range. So
9 these data are entirely consistent with each
10 other.
11 Well, of course, what we would
12 like to know is what gives rise to this
13 residual surviving population. This is
14 where our public health problem actually is,
15 in this material that we can't get rid of
16 completely. A clue is these dry heat
17 inactivation experiments that were conducted
18 by Paul Brown about ten years ago.
19 Here again we have even higher
20 temperatures than I showed you just a moment
21 ago for ten 60-minute exposures, 160 degrees
22 centigrade, and only getting two to three

1 logs of inactivation where nine logs is
2 possible. What this is telling us is that
3 dry heat inactivation is much, much less
4 effective than wet heat inactivation for the
5 destruction of these agents. Well, this is
6 not a big surprise because there are a
7 number of sporulating bacteria that can
8 survive to about these levels under these
9 same kinds of conditions.
10 I think it gives us a clue as to
11 what may be going on with these surviving
12 populations. Now, 132 degrees is a very
13 significantly higher temperature than 121
14 degrees for a steam sterilization where an
15 activation takes place in just seconds,
16 really. On the other hand 132 degrees is
17 only incrementally more effective than 121
18 degrees under dry heat sterilization
19 conditions where an activation could take
20 days under these particular conditions.
21 And the thing that we have to
22 remember is that the surviving population is

1 very small. This is a very small fraction
2 of the starting infectivity. This is parts
3 per million of what we began with.
4 So I'm going to offer you this
5 model which is that in this case there is a
6 horizon line here. This is fluid down here.
7 What I'm trying to show you here is that in
8 a tube or a bottle or in the circumstances
9 under which these experiments were done in
10 the case of homogenate there is an
11 opportunity for the material to boil as it
12 comes to temperature and throw the
13 infectivity on the glass where it might dry.
14 In the case of the macerates the
15 macerate is forced into the tube and leaves
16 a streak behind it which can dry as the tube
17 heats up on the way to inactivating
18 temperatures. And it's important to
19 remember that in brain we have about 50
20 percent fat, which can form actually a layer
21 over this which could be quite impenetrable
22 to steam and water, especially if you think

1 that this is happening only at the level of
2 parts per million.
3 This leads to the next slide.
4 What this data show is that the infectivity
5 is not intrinsically resistant to steam
6 sterilization. The problem is with delivery
7 of the inactivant and it is for that reason
8 that we make the following recommendations.
9 It's very important to prevent drying.
10 We're very careful in the laboratory when we
11 are done with an instrument to immerse it in
12 water or sodium hydroxide, typically sodium
13 hydroxide in our case, and to do that prior
14 and during steam sterilization to make sure
15 everything is wet and nothing can dry on the
16 instrument.
17 It's very effective to combine two
18 or more methods. This is has been
19 demonstrated in a number of publications
20 now, either pre-treating with sodium
21 hydroxide followed by heat or, of course, an
22 even more stringent situation is to put the

1 instrument in sodium hydroxide and then
2 sterilize it.
3 These factors also contribute, we
4 feel, to an effective sterilization.
5 Surfactants, homogenization, good dispersion
6 of the material, agitation if it's possible
7 to eliminate sanctuaries in the vessel
8 itself. And in our experience refinement,
9 and this is strictly anecdotal, seems to
10 reduce the potential for protective
11 associations and as the material becomes
12 more and more refined it becomes more
13 susceptible to inactivation.
14 Well, all of this is reflected in
15 this WHO document which I recommended to you
16 earlier. Basically the recommendations
17 there are that instruments should be kept
18 moist until cleaned and decontaminated and
19 they should be cleaned as soon as possible.
20 Avoid mixing these kinds of things. I'm
21 going to leave this for you to discover on
22 your own because it is available on the Web.

1 Then there is this hierarchy of
2 recommendations for inactivation which were
3 recommended. For disposable instruments,
4 materials, and these types of things, they
5 should be incinerated if at all possible.
6 And the recommendation was even made for
7 instruments exposed to high infectivity
8 tissues, for example, known surgery to a CJD
9 brain, be destroyed in this way. I'm not
10 myself in agreement with this but it
11 certainly is the most absolute way to go
12 about it.
13 In terms of the hierarchy of heat
14 and chemical combinations the most stringent
15 is to immerse in sodium hydroxide, heat in
16 an autoclave for 121 degrees for 30 minutes,
17 clean rinse in water, and subject to routine
18 sterilization. If the instrument won't take
19 this then use sodium hydroxide or sodium
20 hypochlorite for at least an hour and then
21 transfer to water and do the same thing.
22 Third on the list is to immerse in

1 sodium hydroxide or sodium hypochlorite for
2 an hour, remove and rinse in water, and then
3 transfer to an open pan, heat and gravity
4 displacement or porous load autoclave. And
5 then finally immerse and boil in sodium
6 hydroxide. This has been shown to be
7 effective by David Taylor. Hypochlorite can
8 also be used in this way though there are a
9 lot more problems associated with
10 hypochlorite in our opinion than sodium
11 hydroxide with corrosion, et cetera.
12 In our laboratory we use the
13 following regime. We start with a sterile
14 instrument. We use it on infected material.
15 We keep it wet. If it's convenient or
16 possible to clean it or we are cleaning it
17 anyway before we put it in the wet
18 environment and we do that with a ChemWipe
19 (?) or whatever, we keep it wet. We make
20 sure it's kept immersed before it's put in
21 sodium hydroxide overnight.
22 It then goes to an autoclave where

1 it is cooked for 132 degrees for an hour
2 under sodium hydroxide or for some
3 instruments in water if they can't take this
4 process. Then from this position, and I'm
5 trying to indicate the loss of infectivity
6 here by the diminution of the red in this
7 scheme, we take it to our cleaning bath
8 where it gets sonicated again in a detergent
9 cleaner that is about one-tenth normal
10 sodium hydroxide at 60 degrees and it's
11 probably inactivating in and of its own
12 right. Once it comes out of the cleaner it
13 is packaged and then goes through a standard
14 autoclave sterilization before it goes into
15 our sterile pool and is reused.
16 We have some evidence that this
17 works because we have been doing these
18 experiments in the laboratory for the last
19 several years looking at very low levels of
20 infectivity associated with blood-borne TSE
21 infectivity. In particular I'm going to
22 show you this experiment just because I

1 think you will be interested in it as well.
2 This is new data where we inoculated a large
3 cohort of animals, hamsters in this case, at
4 a low concentration of infectivity and at
5 three-week intervals we pooled the blood
6 from 20 animals and then we inoculated 100
7 recipients with each pool of blood.
8 We have the data on the next
9 slide. This is the incubation time of these
10 animals, of the animals got sick. These
11 were the times in which we created the pools
12 over here on the side. So what we have here
13 are two pools containing 100 animals each
14 with no infections at all in them. There's
15 really no distinction between the
16 instruments that were used here and used
17 here. Here, just as we had expect, we
18 started seeing cases as the disease
19 progresses. And in fact on the next slide
20 we get the status plotted and we see these
21 two points without cases here and about
22 mid-clinical disease we start seeing effect

1 and an increase in effect as the disease
2 progresses.
3 One more example of this is we
4 have done plasma fractionations on
5 endogenously infected hamster blood where we
6 fractionated all the fractions given here
7 out of the plasma by the cone fractionation
8 and again reinoculated 100 animals or more
9 in this case with each one of these
10 fractions. And the data here, all the
11 little dots are animals that contracted
12 scrapie in the course of this experiment
13 according to their incubation time here and
14 the actual fraction that was inoculated
15 here. But it is important that as we got
16 farther and farther into the fractionation
17 we see fewer and fewer cases and we never
18 did see cases in the case of fraction 2 and
19 fraction 5.
20 Well, that slide didn't make it in
21 here. Anyway, I was just going to point out
22 that the actual schema for fraction 2 and

1 fraction 5 are the end products of two
2 branches of the cone fractionation scheme
3 and it would make sense that the removal
4 would be highest for those two fractions
5 but, again, it shows you that we can
6 actually do these experiments without cross-
7 contamination into these types of materials.
8 This method that we use contrasts
9 very strongly with the method that is
10 typically used in a hospital setting where
11 this part of the scheme is missing. In fact
12 what happens is you start with a sterile
13 instrument, you use it for surgery or
14 whatever, and then it goes to a cleaner.
15 This is done in a specialized washer using
16 heat and detergents. From there it goes to
17 a package and is sterilized in an autoclave
18 under conventional conditions and then goes
19 back into circulation.
20 So this is a far less stringent
21 procedure; nevertheless, it is likely to be
22 highly effective, I think, in terms of

1 obtaining sterile instruments for reuse.
2 The one thing that bothers me about it is
3 this step right here where the cleaning step
4 occurs before a disinfection step, which is
5 what we have tried to achieve in the
6 laboratory over here.
7 From my perspective this cleaning
8 apparatus overwhelms all other contamination
9 issues. It creates a secondary
10 decontamination problem. If contaminated
11 instruments are actually introduced into
12 this machine you have a problem of the
13 contamination of the washer and
14 contamination of the waste stream from that
15 washer.
16 This, again, has not been done in
17 any systematic way but talking with the
18 people who are responsible for this type of
19 process they are adamant that this has to be
20 done, it can't be done any other way, and
21 these machines are adequate to the task. On
22 the other hand I don't believe that they

1 should be presumed to be adequate and this
2 should be investigated carefully.
3 I'm going to finish with an
4 example in which a CJD case was identified
5 after neurosurgery. This happened in the
6 Hotel Dieu Grace in Windsor, Ontario, last
7 year about this time. What happened is
8 there was a neurosurgery on a patient. I'm
9 not sure what sex it was. They were
10 subsequently diagnosed with dementia on
11 subsequent observation. A 14-3-3 CSF
12 sample was taken and it turned out to be
13 positive.
14 This patient did eventually have
15 Creutzfeldt-Jakob disease, as you will see.
16 That, at least, is a relief. But on this
17 discovery right here the hospital staff
18 decided that they had to pull all of their
19 instruments and sterilize them.
20 Now, they had already been through
21 this wash cycle and sterilization cycle and
22 the instruments that were used in the

1 neurosurgery had not been tracked and so
2 they had been mixed with the general pool
3 and so the hospital felt that they had to
4 sterilize everything. They sterilized the
5 entire pool at once overnight. They didn't
6 do any test samples and they used
7 autoclaving and one normal sodium hydroxide
8 as recommended in the WHO guideline for
9 this.
10 The next morning they had a lot of
11 electrolysis corrosion and destruction of
12 these instruments. There was a lot of
13 complaining about fumes. I'm not sure where
14 that came from but there was some chemistry
15 that we don't experience going on in this
16 process. In the end they estimate they
17 destroyed about $10 million worth of
18 instruments.
19 The complete details from this
20 incident haven't been released yet so it's
21 hard to evaluate exactly what happened.
22 There were obviously chemical

1 incompatibilities in these mixtures that
2 were autoclaved and I would have to point
3 out that the WHO guidelines are based on
4 laboratory experience and they were our best
5 good faith recommendation for how to deal
6 with these agents in that type of setting.
7 Clearly we need to develop and
8 validate procedures that will work for a
9 hospital setting as well and sort out the
10 issues. This incident could be of great
11 value to us in terms of sorting out the
12 factors that led to this wholesale
13 destruction.
14 My concluding remark would be that
15 the instrument washer was not considered a
16 source of vulnerability even during the
17 debriefing on this incident which I was
18 invited to attend, getting very cold stares
19 from lots of people.
20 Sterilization of the instruments
21 is pointless without sterilization of the
22 washer from my point of view and if the

1 washer itself is sterilizing then re-
2 sterilization of the instruments was
3 unnecessary.
4 I think there is one final slide.
5 I want to make the following point, that in
6 terms of dealing with this episode in
7 particular I think we can't lose track of
8 this feature, that this was a case of
9 Creutzfeldt-Jakob disease which they
10 identified. They might not have. He might
11 have had the surgery. He might have died in
12 the surgery, whatever, in which case the
13 instruments would have been processed the
14 way they always would have been and we would
15 never have known the difference.
16 We can really only identify a
17 minor proportion of the potential exposure
18 to this disease and that is these cases that
19 we defer. We have no way of identifying
20 incubating sporadic cases, at least not yet.
21 For that reason I feel it's
22 pointless to implement measures that attempt

1 to reduce the risk from known cases to below
2 the irreducible risk from unidentifiable
3 cases. It's important to find out what that
4 irreducible risk actually is by modeling and
5 risk assessment but we also have to maintain
6 some realism in terms of what we are
7 attempting to do.
8 The only caveat here is that this
9 is true unless we apply a uniform higher
10 standard to the whole process and not just
11 apply it to point instances of
12 contamination.
13 DR. BOLTON: Thank you, Bob. I
14 think in that last slide you made the same
15 point that I had made earlier about not
16 being able to identify these incubating
17 sporadic cases. There are questions. We
18 need to hurry along so I'll try to make
19 these brief.
20 Steve?
21 DR. DeARMOND: How do you test for
22 residual infectivity on surgical

1 instruments? Is there any way to do that in
2 a practical way?
3 DR. ROHWER: I think we need a
4 paradigm because that's going to be tricky.
5 We don't usually do neurosurgery on hamsters
6 and mice, at least not large-scale
7 neurosurgeries, which you would want to do
8 in a case like this.
9 A paradigm has been advanced by
10 the Weissmann group. They are using these
11 stainless steel sutures which they then
12 expose to infectivity and then implant in
13 the brains of animals. We have looked at
14 that a bit and I think it has quite a bit of
15 promise, actually, as a way to go.
16 They are rods, not canulas, which
17 means that you can control exactly what
18 happens to the entire surface that's
19 exposed. The animals do tolerate it quite
20 well. So it is a way to go, I think.
21 I know that there is some funding
22 for this in Europe that comes out of the EC

1 program, TSE Program, but I haven't heard
2 any updates on it recently as to whether
3 there is new data on that.
4 DR. BOLTON: Sue?
5 DR. PRIOLA: You hypothesize that
6 this small fraction of agent which is
7 protected in your inactivation studies might
8 be due to protection by the high-fat content
9 of the brain. It perhaps forms a varnish
10 over the agent.
11 Have you done inactivation
12 kinetics with infectivity from other tissues
13 such as spleen or lymph node and do you see
14 a similar kinetics?
15 DR. ROHWER: We haven't. We have
16 not. We are looking for high levels of
17 infectivity when we do these experiments
18 because we are showing such high levels of
19 inactivation. But there are lots of things
20 like that that could be done. I mean, you
21 could delipidate (?) the brain and do that
22 experiment as well or you could look at

1 spleen. That's a good suggestion. I think
2 there is a huge parameter space associated
3 with inactivation which has not been
4 explored and should be.
5 DR. BOLTON: Briefly.
6 DR. DeARMOND: I was just going to
7 say that have you tried sonication with
8 lipid solvents, chloroform, methanol,
9 acetone, something that wouldn't really
10 corrode the instruments, and then go through
11 the process?
12 DR. ROHWER: We have done
13 sonication with sodium hydroxide but that
14 doesn't count. It's very effective.
15 DR. BOLTON: Dr. Solomon.
16 DR. SOLOMON: This is a very naïve
17 question and we might want to save it for
18 the discussion but if you were a
19 professional organization and wanted to
20 develop some standards now what would you
21 recommend?
22 DR. ROHWER: My feeling is that

1 making those recommendations in the vacuum
2 of not having, obviously, a full
3 appreciation of the compatibility issues
4 with the recommendations that were made in
5 the WHO guidelines would be difficult. But
6 in terms of looking at compatibility those
7 experiments are much easier to do than
8 infectivity experiments. It's a matter of
9 putting various instruments in various
10 combinations and various alloy combinations
11 and that kind of thing in a pot and trying
12 it and seeing whether you get corrosion.
13 That kind of thing, I think,
14 should be done to see if we can get a handle
15 on just what the issues actually are. We
16 know that when we use our cheap -- not all
17 of them -- we have a survival of the fittest
18 program in the laboratory for surgical
19 instruments. But we only buy cheap
20 Pakistani stainless and some of it is very,
21 very resistant to sodium hydroxide in heat
22 and some of it isn't.

1 DR. BOLTON: David, the last
2 question before our next presentation.
3 DR. DeARMOND: You will be pleased
4 to know that investigators in the FDA Center
5 for Devices are conducting exactly the kinds
6 of experiments with instruments that you've
7 mentioned.
8 DR. ROHWER: Very good.
9 DR. BOLTON: We will anxiously
10 await those results.
11 Our final presentation before
12 lunch will be "Process Validation for
13 Conventional Agents" presented by
14 Dr. Mahmood Farshid. I hope, Mahmood, I
15 didn't butcher your name too badly.
16 DR. FARSHID: "Mahmood" was very
17 close. It is like saying "my mood." My
18 mood, your mood, good mood, bad mood. I
19 hope everybody is in a good mood.
20 My presentation will be a brief
21 overview of viral validation studies and
22 also the approach that we take in evaluating

1 such studies. The product which currently
2 requires viral validation studies falls
3 loosely into three different categories:
4 Monoclonal antibodies and recombinant
5 products produced in cell culture. These
6 are highly characterized products and
7 extensively tested and they have an
8 excellent viral safety record. Blood and
9 blood products and other human blood
10 products, probably tissue and soft tissue
11 and bones also fall in these categories.
12 But my main focus would be in the plasma-
13 derived product where I draw most of my
14 experience.
15 Also, animal-derived product which
16 these are, for example, lymphocyte produced
17 by rabbits or antivenin produced in horses.
18 So it would depend on what kind of a
19 starting material you are using the approach
20 to the viral validation study will be
21 somehow different.
22 There are some complimentary

1 approaches in basically reducing the risk of
2 viral infection, so viral inactivation
3 essentially is only one component of this
4 multi-faceted approach which includes donor
5 screening, donor history assessment, and
6 written and oral questionnaire.
7 Donor testing, which in the case
8 of whole blood includes testing for
9 antibodies to HIV-1 and 2 and p24 antigen
10 and serological tests for HCV and HB_SA_G and
11 anti-HB_C and anti-HTLV-1 and 2, and
12 syphilis. For plasma, for instance, HTLV
13 and Anti-HB_C is not included in the test.
14 Pharmaco-vigilance and finally in case of
15 the pooled and plasma-derived product the
16 last line of defense will be viral
17 inactivation and removal and basically
18 validating the manufacturing process for
19 removal of this virus.
20 So basically the aim of viral
21 validation is to provide evidence that their
22 production process will effectively

1 inactivate or remove viruses which could
2 potentially be transmitted by this product.
3 So here we are talking about a relevant
4 pathogen which may be present in a starting
5 material.
6 Also to provide indirect evidence
7 that the production process has the
8 capability to inactivate or remove novel or
9 yet undetermined viruses. Basically here we
10 use an array of viruses to cover also those
11 who are undetermined or emerging viruses
12 because not all viruses are screened for.
13 For example, HGV and TTV and SLV and other
14 viruses also will be covered by the
15 inactivation or removal process.
16 This is basically a common virus
17 clearance method. This list is by no means
18 complete and basically is drawn, as I
19 mentioned, from my experience with plasma-
20 derived products. There are other
21 methodologies which are being used. It can
22 be divided. The viral clearance basically

1 includes viral inactivation and removal
2 which inactivation could be a chemical
3 inactivation like, for example, using a
4 solvent detergent, which is very well known
5 or physical inactivation like heat which we
6 just heard about. The removal includes also
7 chromatography or precipitation or using
8 nanofiltration.
9 In evaluating the viral validation
10 studies there are different components in
11 the study that we need to evaluate. One
12 will be the scale down process step. The
13 viral validation studies not done in actual
14 manufacturing settings and for very obvious
15 reasons because it is not desirable from a
16 standpoint of G&P and also is not practical
17 because you need a huge amount of virus to
18 do that. Therefore the laboratory model or
19 the scaled down model of the manufacturing
20 process is being basically designed and it
21 will be used for the validation studies.
22 The other will be spiking, to do

1 that deliberately, the one step which needs
2 to be validated will be spiked with high
3 titer virus.
4 Finally, the reduction of that
5 virus, either inactivation or removal, will
6 be determined in subsequent steps. If the
7 number of steps being validated then the
8 reduction of clearance from these steps will
9 be summed up and that will be basically
10 total log reduction value for that
11 particular process.
12 In evaluating the studies we look
13 at the choice of viruses, what kind of
14 viruses may have been used and if it is
15 appropriate. And the design of the
16 validation study, which is essentially
17 validity of the scaled-down process, this is
18 very important in order to determine that
19 what is obtained is relevant to the actual
20 manufacturing process.
21 The study should provide evidence
22 that the scaled-down model actually is

1 relevant and basically mimic the actual
2 manufacturing process. The kinetics of
3 inactivation also needs to be shown as we
4 saw when Dr. Rohwer showed some of the
5 kinetics of some heating activation.
6 And if it is removal we need to
7 know the whereabouts the virus was removed.
8 Basically the mass balance needs to be shown
9 and determined. Also, the robustness of the
10 process as a whole also needs to be
11 determined and that maybe by introducing
12 some deliberate changes in the process to
13 see if the process as a whole is robust.
14 Also, the limits of sensitivity of the assay
15 used for infectivity also needs to be
16 determined and finally the log reduction of
17 how much log reduction is achieved in the
18 whole process.
19 In terms of viral selection the
20 viruses that can potentially be transmitted
21 by product, basically we refer to them as a
22 relevant virus. These are pathogenic

1 viruses. So the first choice will be to get
2 the virus which is relevant. Of course,
3 this is not always possible because some of
4 the relevant virus do not grow in tissue
5 culture such as Hepatitis B and Hepatitis C.
6 In that case we use a specific
7 model viruses which basically are viruses
8 which physically and chemically are similar
9 to the relevant viruses as close as
10 possible. In addition to that non-specific
11 model virus also will be used. These are
12 simply to show basically the overall
13 capacity of the step inactivating viruses
14 and for this purpose usually viruses which
15 are highly resistant and small ---- viruses
16 will be included in the panel of the
17 viruses.
18 Therefore the selection of viruses
19 basically is dependent on the nature of the
20 starting material, if it is cell-derived or
21 human-derived or animal-derived and for the
22 reason that I stated because we need to be

1 close to the relevant viruses in this
2 product. Also we need to consider the
3 practicality of using these viruses, for
4 example, availability of suitable culture
5 system and availability of high titer stock
6 which is necessary to do this kind of
7 experiment. Also the availability of
8 reliable methods for quantitation of this
9 virus.
10 These are a panel of viruses that
11 are used for doing viral validation studies
12 in a plasma and plasma-derived product and
13 probably can be applied to, I would say, all
14 human-derived product if one wants to do
15 their viral validation study.
16 HIV-1 is being used as a model
17 virus for HIV-1 and 2 and also HTLV and this
18 is required for any kind of studies which
19 are done and any type of viral validation
20 studies.
21 For Hepatitis B basically it is
22 not modeled directly. There are some model

1 viruses like ---- Hepatitis B or ----
2 Hepatitis B which could be used as model
3 viruses; however, in revalidation studies we
4 do not require any of these viruses to be
5 used and the capacity of a system to clear
6 Hepatitis B basically would be extrapolated
7 from looking at the panel as a whole.
8 For Hepatitis C there are a number
9 of specific model viruses. Some of them are
10 used more often. The bovine viral diarrhea
11 virus is one which in terms of its size and
12 genomic structure is very close to Hepatitis
13 C and being used in the validation studies.
14 CMV also which is a ---- virus is
15 larger than HCV, is more resistant in terms
16 of inactivation, and is probably a better
17 choice if one wants to do the inactivation
18 studies. We encouraged the manufacturer
19 that they can use both of them because BVDV
20 because of its size would be better in
21 removal and validation of removal studies
22 and CMV is because of high resistance

1 probably would be better to be used in
2 inactivations.
3 HAV, there is a laboratory strain
4 of HAV which can basically be used as a
5 relevant model for Hepatitis A and this is
6 also because of small non-envelope viruses
7 which can also qualify as basically being
8 used to show the rigor of the overall
9 capacity of the step in inactivating
10 viruses.
11 Because of the presence of a
12 number of herpes viruses inclusion of one of
13 them in the validation studies is desirable,
14 for example, like PRV, and that is also
15 qualified as having one DNA virus in the
16 panel which basically covers maybe for
17 Hepatitis B as well, although it is a larger
18 virus.
19 For B19 PPV can be used for human
20 parvovirus B19. This is a small highly
21 resistant ---- virus and it basically is a
22 good virus to show overall capacity of the

1 inactivation or removal step and also it can
2 be basically used as a surrogate or as a
3 model for B19.
4 This is a panel of the viruses
5 which are used for the cell line driver.
6 Basically the panel which we use, as I
7 mentioned, is dependent on what kind of
8 starting material basically is used in the
9 manufacturing. Here the required virus is
10 the retrovirus because the presence of this
11 virus is endogenous in mice and hamster cell
12 line. Also the PRV may cause latent
13 infection in some of the cell line and that
14 need to be there.
15 And ---- virus is present in a
16 number of different cell cultures and that
17 is included in a panel and MVM, which is
18 also parvovirus, is a highly resistant virus
19 and again it will show basically to
20 determine the overall capacity of the system
21 in clearing the viruses.
22 So if we look at the selection of

1 viruses it is intended to include the DNA
2 and RNA viruses, both single and double
3 stranded, lipid and non-lipid viruses should
4 be included and in terms of their sizes it
5 should include large, intermediate, and
6 small size.
7 In term of their resistance it
8 would be from highly resistant to
9 inactivation to very easily inactivated. By
10 doing so basically we cover the viruses
11 which are undetermined or emerging viruses
12 and that will basically increase the
13 assurance of the overall capacity of the
14 system to remove the viruses in general.
15 The other component for looking at
16 the viral validation, as I mentioned, is the
17 scaled down purification process which
18 usually is one-tenth to one-hundred of the
19 full scale. In this, as I mentioned, some
20 data should be provided to indicate the
21 relevancy of the small downscale to the
22 actual manufacturing process. That is the

1 only way that we can determine that the
2 result that obtains is really relevant to
3 the actual manufacturing process.
4 For example, the buffers, the pH,
5 protein concentration, and the product
6 should be the same as full-scale
7 manufacturing. The test material which I'll
8 use for this kind of testing should come
9 from actual manufacturing process. The
10 intermediate material should come from the
11 actual manufacturing process and put it
12 through their scale down.
13 All the critical operation
14 parameters should be kept as that has full
15 scale, for example, bed height, flow rate,
16 and so on and the absolute values, which I
17 mentioned, temperature, pH, should also be
18 kept as that of the actual manufacture.
19 Also, make sure that in term of
20 product specification that the product is
21 identical to the production scale. So
22 basically the scaled-down models should be

1 substantially equivalent to the actual
2 manufacturing process if you want to
3 basically extrapolate from the result
4 obtained in a scaled- down model to that of
5 actual manufacture.
6 So the overall criteria for
7 effective virus should produce significant
8 viral kill and should be reproducible and
9 controllable at the process scale and
10 modelable at the laboratory scale because
11 some processes are difficult to model;
12 therefore, it would be difficult to
13 determine the actual capacity of that step
14 in clearing the virus. So modelability of
15 that method, basically this step will be
16 important.
17 And it should have minimum impact
18 on the product yield and activity. It
19 basically should not affect the product. It
20 is intended to kill the viruses and should
21 not kill or remove the product itself. It
22 should not generate new antigens or leave

1 any toxic residue. It should not be
2 mutagenic or carcinogenic.
3 The manufacturing process for
4 blood-derived products should contain at
5 least two effective steps for removal and
6 activation of viruses and "effective" refers
7 to one which basically produces significant
8 viral removal or inactivation. At least one
9 step should be effective against
10 non-envelope viruses.
11 At least one stage in the
12 production process must inactivate rather
13 than remove viruses. So total reliance or
14 removal may not be sufficient. The removal
15 process is very difficult to basically model
16 in a lab and inactivation are more robust;
17 therefore, if total reliance on removal we
18 ask that one inactivation step also be
19 included.
20 In evaluating the result if one
21 single step having a large effect gives more
22 assurance of viral safety than several steps

1 having the same overall effect. You may get
2 studies which show the same level of overall
3 viral reduction. One of them may be
4 obtained by two single steps and one may be
5 obtained by five or six different steps.
6 The one which is obtained by two different
7 steps will definitely provide more
8 assurance, that is, more effective in
9 basically clearing of virus.
10 Even under the best of
11 circumstances there are limitations. One
12 needs to realize that viral validation study
13 just provides an estimate and assurance of
14 how the system will work and they are not
15 absolute. The limitations include that
16 laboratory strain may behave differently
17 than native viruses because most of the
18 viruses, even the relevant ones, are
19 laboratory adopted viruses and they may
20 behave differently as the one that they are
21 present and why.
22 The source of plasma or

1 immunoglobulin may have neutralizing
2 antibodies so that may affect the overall
3 viral kill and overestimate the viral so the
4 presence of neutralizing antibodies is a
5 variable which needs to be considered when
6 doing inactivation or removal. For example,
7 in case of Hepatitis A that anti-A is
8 usually present and in many cases is the
9 result of over-estimation of the capacity of
10 that particular step in killing the viruses
11 where the killing may be as a result of the
12 presence of a neutralizing antibody and not
13 because of the effectiveness of that
14 particular step. And there may exist in any
15 virus population a fraction that is
16 resistant to inactivation. I think this has
17 been mentioned this morning.
18 A scaled-down process may be
19 different from full scale. Sometimes it is
20 difficult to basically model the actual
21 manufacturing process and there will be some
22 differences and it is difficult to determine

1 how those differences are going to affect
2 the overall clearance. So that also needs
3 to be considered, that what we see is done
4 in the lab and is not an actual
5 manufacturing process. That will be another
6 limitation.
7 The total virus reduction may be
8 overestimated because of repeated and
9 similar process steps. The different steps
10 that will be validated should be orthogonal.
11 They should work by independent mechanisms
12 in order to be acceptable to add the total
13 viral reduction from this different step,
14 basically to sum them up.
15 The ability of a step to remove
16 viruses after repeated use may vary. This
17 is probably true for chromatography, which
18 the residents sometimes use repeatedly, so
19 what you get in the beginning in the course
20 of the validation may not be after a number
21 of years.
22 That concludes my presentation.

1 Thank you.
2 DR. BOLTON: Thank you. Briefly,
3 questions or comments from the committee?
4 DR. EPSTEIN: I just wanted to
5 make two quick comments. Thank you,
6 Mahmood.
7 The first is that we also care
8 about the absence of log removal and we try
9 to develop a standard that is applicable to
10 what we think is the pathogen burden in the
11 product. So there's the idea of overkill
12 relative to some upper limit of potential
13 contamination.
14 Then you touched on this
15 indirectly but when you have a series of
16 processes it's true that we look at the
17 summation of logs clearance. But it's also
18 true that we more or less routinely will ask
19 for some thru-put experimentation to show
20 that at least for the critical steps it's
21 valid to sum the logs reduction.
22 DR. BOLTON: That was a good

1 point, Jay. I would have made that except
2 I'm too hungry to think about it so an
3 excellent point.
4 So here's what we will do. I want
5 to cut lunch short from an hour to 45
6 minutes. We'll meet back here at 2:00.
7 (Whereupon, at 1:17 p.m., a
8 luncheon recess was taken.)
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