These represent data accumulated over 20 to 30 years of inoculating tissues from human patients with Creutzfeldt-Jakob Disease into primates, which are far and away the most sensitive and susceptible bioassay animal for human tissues. Most of this data come from inoculation of squirrel monkeys. Some of it comes from inoculation of chimpanzees. They both have rates of transmission when inoculated with brain tissue of something like 95 percent so they are an extremely sensitive host.

What you see is that brain and eye contain infectivity more often, the brain, as I say, 95 percent, the eye a lower percentage but still very high. You will see the numbers in a bit.

Dura mater and pituitary gland in fact were not inoculated. I have included them in the slide simply because we know they are infectious in humans from the fact that they are a transmitted disease in

humans. Spinal cord, spinal fluid.

But then a variety of peripheral organs are also infectious in a small proportion of patients that were tested, lung, liver, kidney, spleen, lymph nodes. Our experience in primates indicates that human blood is not infectious but I think it is arguable in view of the established fact that blood is infectious in a number of experimental models, including rodents, sheep, and monkeys.

The muscles which were tested that had no infectivity were heart, skeletal muscle, adipose tissue, testis and prostate, and none of the excretions or secretions yielded infectious material.

Now, as I say, that is a pretty generalized slide but when I show you the numbers on which this data are based you will see that this is perhaps a more optimistic judgment than might be made.

Brain was inoculated into 259

patients. This data, by the way, you may say well, why don't you give us some titers? We would all be interested to know what the titer of spleen is in a human being compared to brain.

This alone represents experiments that probably consumed 1,000 squirrel monkeys and it is just not done, especially today. So what data there is is likely to be the only data that there ever will be in primates. Whether humanized transgenic mice turn out to be as good a bioassay as primates remains to be seen. On the other hand humanized transgenic mice if they over-express may actually be unrealistic. You may get, for example, a transmission and an over-expressing humanized mouse that has no basis in reality at all. So beware of transgenic bioassay animals until it is established that they represent realistic results.

You can see that most of the other

tissues with the exception of liver, kidney, and spleen were not inoculated into or they did not come from very many patients. These denominators represent the donors, that is, the number of humans that were tested, not the number of animals that came down.

So lung, for example, four humans were tested, two of them were positive, but that involved somewhere between 8 and 12 monkeys. We did not achieve transmission from any of the other tissues shown, marrow, whole blood, leukocyte, serum, the muscles that were shown on the previous one, but, you see, adipose tissue was only inoculated from one patient, gingiva and intestine from a single patient.

So you cannot really be on the basis of this data in any way confident that infectivity would not be present in peripheral tissues were 10, 15, or, better yet, 100 patients used for bioassay experiments.

As I say, the data is very meager with respect to peripheral tissues. Such as it is it's primarily negative but I wouldn't necessarily go home thinking that we have shown that there is no infectivity, for example, in peripheral nerve. Inoculated five patients, that's not bad. No transmissions. What we can say from this for sure is that if infectivity does exist in peripheral tissues it exists at very low levels. Otherwise we would have a transmission every time we inoculated a tissue.

That really is all we know from primate experiments about infectivity in human tissues. I did want to show you one other slide, however, which is a very interesting slide because it illustrates the fact that these are all patients with Creutzfeldt-Jakob disease on the left. The species inoculated, spider monkeys, squirrel monkeys, chimpanzees, all very susceptible

hosts, all transmitted in short order when the brain was inoculated by the intra-cerebral route. But the incubation periods following peripheral routes of infection were typically longer, sometimes much longer, and sometimes apparently long enough so that a transmission never occurred and these were animals that were observed for up to ten to fifteen years.

Point: When you inoculate material directly into the brain you will have transmissions far more often than when you give the same material by a peripheral route of infection.

So even when you have tissue that is infectious it is not a guaranteed transmissible tissue and most products that are used by humans are not inoculated directly into the brain.

Now we go to iatrogenic disease.

DR. BELAY: May I ask a question?

DR. BOLTON: Paul, would you like

to entertain some questions on this part first?

DR. BROWN: This is so quick I think it will --

DR. BOLTON: You want to move on? We'll hold them then.

DR. BELAY: So what are the consequences of infectivity in human tissues? Well, here they are. Growth hormone has accounted for almost 160 cases of CJD worldwide, dura mater has accounted for what is probably now about 90 cases of CJD worldwide, and there are, as you know, very rare and now getting increasingly old cases of cross-contamination of instruments at corneas in which transmission has occurred.

When you break this down country by country it looks like this. So Japan is the overwhelming champion of dura mater graft transmissions. France is a comparable champion of growth hormone transmissions.

And the US and the United Kingdom together have had about 60-odd transmissions between us.

The other sources of iatrogenic disease, cross-contamination, surgical instruments, corneal transplants, gonadotropin in Australia, are really trivial compared with these three major sources and these three major sources will not have escaped your attention that they are all from tissues developmentally or in close proximity to the brain.

You may want to ask why Japan has such an overwhelming number of dura mater graft transmissions and why France should have had such a large number of growth hormone transmissions. We don't and never will know the answer with confidence to either of those questions but Japan probably did not import a greater proportion of contaminated dura mater than any other country. What it did do was import enormous

numbers of dura mater grafts relative to other countries.

If you use the estimated denominator for risk of iatrogenic CJD from Japan you get a risk that is something like 0.03. So compared with growth hormone, which, for example, in France is now running along at the rate of about one in 18 recipients, you can see that even with dura mater, pooled as it was, inadequately sterilized as it wasn't, it has not been relative to the number of dura mater grafts used a very florid source of iatrogenic disease.

So I think the answer to Japan probably is the fact that the Japanese used 20,000 dura mater grafts a year in its heyday and that represents a multiple. We don't know what multiple, but a multiple, probably 10 to 20 times more frequently used in Japan than elsewhere.

With respect to growth hormone,

that is a very interesting story, I think, and not to bore you with the reasons for the conclusion it is likely that France ran into trouble from a combination of difficulties.

My reading of the French experience is that their primary problem was the fact that in France autopsies of the brain typically were only done on patients with neurologic disease; that is, unlike the US where a complete autopsy is specified and you do the brain and you do a complete autopsy in someone who has died of a myocardial infarction in France this was not typical.

In France when you had a neurologic disease you often had the brain examined. If you didn't die from a neurologic disease you didn't even if an autopsy was done. It didn't include the brain.

The consequence of that is that fully one-third of brains and brain

autopsies came from hospitals with large neurologic services, which is a huge bias because the pituitaries were only able to be removed, obviously, from patients who had had autopsies of the brain. So one-third of the autopsies in France came from hospitals that were primarily neurology hospitals. That's bad. It was obviously a very wonderful source for pituitaries, the only source that was available, but in retrospect a bad idea.

A second element of the French story is that every one of the patients in France so far has shared a treatment period that is relatively constricted. Unlike the US and the United Kingdom where there has been a random incidence of CJD starting from about 1967 all the way up through 1977 in France all of the cases of CJD shared a window of treatment between the beginning of 1984 and the middle of 1985, a period of 18 months.

So it appears that this large ever-expanding number of autopsies that were done and pituitaries that were collected as growth hormone use became more prevalent combined with a rather bad, risky source combined with what is probably either both bad luck in terms of having a few patients with CJD in the pot, so to speak, and probable massive disregard of cross-contamination during that period at some point.

In France, as elsewhere, the pools of pituitaries varied from several hundred to 10- to 20,000 and in the period at issue here in the mid and early 1980s batch pools typically ran to at least 10,000. So there was an opportunity for a greater dispersion of contamination.

The final issue is that the method of processing, and I haven't got the details on this, the final chromatographic step for the purification of growth hormone gave you

the opportunity to take either a large number of fractions or a smaller number of fractions and although I've never seen hard data to support the contention Dr. Parlow, who in this country made the growth hormone after 1977 and whose hormone evidently has remained and was then free from infectivity because no US patient who has only received the Parlow treatment has come down with CJD.

So that the Parlow method of chromatography and the cuts he took, which he says were very narrow, may have made a big difference in terms of the amount of infectivity that might have gotten through. So this is a combination of factors. Again, I don't think we will ever work them out with precision, although the French Inspector General and the Director of INCERN once again want to rehash the whole thing and go over it and find out if they can a little more precisely what the cause was because they have a dozen lawsuits on the

table in France.

Finally, here are four anecdotes. They represent cases of Creutzfeldt-Jakob disease in recipients of 57, 63, 54, and 55 years. The fourth column, the organs donated were liver, bone, pericardium, and kidney.

The diagnosis of the donor is known in three instances, 42 cerebral aneurysm, 46 myocardial infarct. The other ages aren't known but one gentleman died of an infarct and the final patient nothing is known about.

So in no case was the donor visibly suffering symptoms that could possibly been Creutzfeldt-Jakob disease. Autopsies were not done on them so we have no idea whether they had incipient Creutzfeldt-Jakob disease.

The age of the recipients is very satisfactory for sporadic Creutzfeldt-Jakob disease and the organs that were donated, to

the best of our knowledge, two of the organs, bone and pericardium, are unlikely to have contained infectivity even if the donor had CJD. The liver and the kidney, as you have seen, could have been infectious.

My reading of this is that all four cases were probably sporadic Creutzfeldt-Jakob disease but we just don't know because we don't have enough data on the donors. But I thought you would like to see it, anyway, to know that there are a few cases, a handful, in which iatrogenic transmission from peripheral tissues might have occurred. And that will conclude it.

DR. BOLTON: Thank you, Paul.

Questions?

DR. BELAY: I just wanted a clarification about the infectivity data for the various organ tissues that you provided in the previous tables, I believe three or four slides back.

DR. BROWN: Shall we put that on?

DR. BELAY: The question is were all the data derived from primate models or was it a combination?

DR. BROWN: No primate models. Well, I don't know what you mean. The tissue that was inoculated was all human; that is to say, these were tissues from humans with CJD completely.

The recipients were either squirrel monkeys or chimpanzees. That is the bioassay animal.

DR. BELAY: All the recipient animals were primates?

DR. BROWN: The bioassay animals were invariably squirrel monkeys or chimpanzees. While I think of it let me point out something. If I'd known this was going to be an issue, it has nothing to do with we had to I've just said, but there was this whole question raised in previous discussions about if you did a biopsy would you stand to miss the diagnosis because it

wouldn't have been an area that was either neuropathologically or immunologically involved.

We do have information from our review of cases in France long ago from about 1965 through 1975, when it was habitual in France as a routine measure for anybody coming in who was suspected of CJD to have a biopsy and who in many cases went on to have an autopsy. Somewhere between 50 and 100 of those cases had both biopsies and autopsies done.

Our analysis indicated that no matter where the biopsy was done in France, at least, in the way that they did it, and they were usually frontal temporal biopsies or parietal temporal biopsies, the rate of positivity relative to the autopsy was 95 percent. So you may miss some but you aren't going to miss many.

I think this concern about doing a biopsy and missing is a legitimate concern

but you have to understand that if you get a piece of frontal temporal cortex you have an overwhelming likelihood of finding tissue that is pathological.

DR. BOLTON: A follow-up?

DR. BELAY: I think Dr. Brown would agree with me that it might be appropriate to add that the dura mater graft associated with CJD cases worldwide, well, actually, a vast majority of them were associated with a single brand of dura mater or Lyodura, which was produced by a single manufacturer, which I believe is very, very significant because a vast majority of the worldwide dura mater-associated CJD cases actually received one brand of dura.

At that most of those dura, which is Lyodura, those grafts were produced before 1987. I think that is worth mentioning.

DR. BROWN: Yes, I think it is worth mentioning too, thanks. Lyodura is

bad news. I don't know why Brown and ---- are still in business. I would have thought they would have been sued out of existence a long time ago but they are.

In 1987 they introduced urea exposure to their proceedings. Before that they hadn't and to the best of my knowledge there are only two cases of dura mater transmissions using dura mater that was provided by other sources. One was in Italy in which a university produced dura mater and one was in this country in which a probable, almost certain, dura mater transmission occurred from a different brand.

DR. BOLTON: Dr. Gambetti?

DR. GAMBETTI: Another point of information concerning the infectivity of the cerebral spinal fluid which is important because nowadays CSF is shipped all around many countries.

DR. BROWN: Most of it comes to

you, Pierluigi.

DR. GAMBETTI: You had in one slide two classes, I believe, concerning infectivity.

DR. BROWN: Let's go on because the numbers are better.

DR. GAMBETTI: Then you have a number 3 out of 26.

DR. BROWN: Yes, that's the number, something like that. Let's go back one more.

DR. GAMBETTI: If I remember correctly in that paper among these 26 cases there were also Kuru cases?

DR. BROWN: That's correct.

DR. GAMBETTI: Kuru cases. Do you know. Is there a way to know whether --

DR. BROWN: Well, I can tell you that some of them, one or two. No, I don't. I can't tell you whether one, two, or three were from CJD or Kuru. My guess is that probably all three were from CJD.

I have to go back on my word. I don't think very much cerebral spinal fluid was ever collected in New Guinea. I think the likelihood is that these are probably CJD.

I can go back and later today tell you exactly if you would like or you can give me a call. In the slide as a whole Kuru is represented but it is not disproportionate; that is, some of them are Kuru. Most of them are CJD for the whole slide.

DR. GAMBETTI: So you would consider CSF infectious?

DR. BROWN: I would. I do. It is. I mean, I don't think anybody can argue that CSF would not be infectious. The nice thing about this number and CSF is the Kretchmar's technique, the so-called SIFT, screening for intensely fluorescent objects to determine the presence of pathological PRP, his numbers match these numbers. You

get the same rough percentage of positives as this. That's a point that was not picked up by many readers in the paper, but it made me quite sanguine about the reliability of that particular test.

DR. BOLTON: Are there other questions of comments?

DR. DeARMOND: Nobody can duplicate the squirrel monkey experiments that I know of, again, and the transgenic mice present a possibility. What would have to be done to convince you that one of the transgenic mouse models, the MHU_2M or a pure humanized or whatever would be valid? Because the monkey is a gold standard.

DR. BROWN: Ideally it seems to me the most convincing proof would be to take archived specimens of these same tissues and put them in the transgenic mice but I don't know whether that would ever be possible. If it were God knows when we go to our archives, which have suffered 40 years of

various problems and misfilings and label losses, whether we could retrieve enough tissue to make that possible I don't know.

Alternatively, less ideal, I suppose we could certainly find a handful of these tissues to titer, for example, in parallel. John Collins (?) and I have already done an experiment which has never been published and probably never will be in which the same three human tissue specimens from three different patients with CJD he titered in his transgenic mouse assay and we titered in squirrel monkeys. From that it appeared that the transgenic mouse assay was as good or better in terms of the end point of the infectivity level as were the primates.

So what news there is in that regard is good news. I am just worried in transgenics whether, as I pointed out, they might not risk being too sensitive.

DR. DeARMOND: There can be

spontaneous disease and you worry about things like that. Another question perhaps related to a future topic this morning is what sort of a decrease in infectivity titer, say with decontamination, do you think is safe? One hundred percent loss would be ideal but it seems unlikely today and still preserving an expensive instrument.

From your studies with the monkeys and other studies do you have a sense of if you have ten to the ninth (10^9) in brain and you were able to decontaminate it what level below would you have to go to to feel pretty comfortable that it wouldn't transmit?

DR. BROWN: Some people in the audience are going to think I have seeded these questions from you because that's a question that I love to answer. The answer is obviously it depends on the likely burden of infectivity in whatever it is you wish to decontaminate.

If it is a surgical instrument used on the brain from a CJD patient I think you would want to decontaminate the works. I don't think you would probably accept anything short of full sterility.

If it is a plasma product and you could guarantee a one log drop in infectivity 100 percent of the time it would be enough because there is no way in the world a plasma product is ever going to contain as much as one log of infectivity per mil. It just won't happen. If it had happened we would see hundreds of patients already who had gotten CJD from plasma products.

So it has to be balanced against what you expect from tissue. As a rule I would think viewing these experiments and data from other laboratories if it's a question of non-central nervous system tissue or tissues which are developmentally associated with the nervous system like the

eye or in proximity like the dura mater if you are outside the central nervous system and its associated tissues the amount of decontamination that you ought to require shouldn't be massive.

Let's just carry it through one more step. It looks very much as though in patients with variant CJD there may be regularly infectivity in the spleen and maybe the tonsil and the rest of the lympho-reticular system. We don't know about the peripheral organs yet, and incidentally we do have titer information on human brain from sporadic CJD and on average it is a little lower than five logs per gram. It is not like a hamster. Even in the most infectious tissue in human beings titered in squirrel monkeys and in chimpanzees the average end point dilution was about ten to the five (10^5). It was a little less than ten to the five (10^5).

I would suppose based on an

analogy with other diseases like scrapie, which has been very well studied, that the spleen titer is not going to be likely to be much more than ten to the three (10^3) if that. It might very well be down to ten to the two (10^2) or ten to the one (10^1)

So you would still want, I think, if you were a general surgeon operating, taking a spleen out of a patient that you knew had variant CJD, you would want full sterility. I don't think the same is true for sporadic CJD in general surgery. It seems to me that you could undoubtedly get away with less sterility and be happy.

The other thing is you don't always have to go to autoclaving at 134 and two normal sodium hydroxides. There is a tendency for people to think that well, either this works or it doesn't, "works" meaning full sterility or nothing, but the fact is that the tenth normal sodium hydroxide is a damn good decontaminant. It

is not as good as normal but it's damn good.

Diluted bleach is good, urea is good, and there are a couple of other chemicals that have been looked at by you, your group, I think, and a couple of others. They work and they work to the tune of three or four logs as opposed to seven or eight logs. These things ought to be considered for decontamination where they might be more appropriate than the really harsh methods.

DR. BOLTON: Dr. Epstein?

DR. EPSTEIN: In the earlier discussion today it was suggested that screening might be done on the ocular tissue directly rather than trying to access the brain. In you studies I think you had four out of five patients had a positive ocular tissue.

Could you just comment on how the eye tissue was sampled? In other words were these whole eyes, were these retinas? What did you actually culture?

DR. BROWN: They aren't specified, Jay, because they typically were a pool, lens, cornea, retina, and so we have no data indicating which of the components of the eye produced the transmission, the infectivity that transmitted.

Based on studies that Nick has done and Collins the presumption is, and I think it's a plausible presumption, that most of the infectivity in the eye itself is in the retina and progressively less in the tissues forward of the retina in a very orderly way so that by the time you reach the conjunctiva and the cornea you're not talking about much infectivity.

Of course, the problem is by definition one infectious unit transmits disease so you don't need much infectivity, particularly in a graft that is going to sit there for the duration instead of a plasma product which is going to be metabolized quickly.

DR. BOLTON: Dr. Doppelt?

DR. DOPPELT: On this list of tissues that can be infective do you have any data on bone or other peripheral soft tissues like ligaments and tendons?

DR. BROWN: I think bone was inoculated from just one patient. It was negative for what that's worth. Bone, however, is a tissue that has been looked at. I don't think we need to bring the slide up again.

DR. BOLTON: It's on our handout.

DR. BROWN: Well, I think both were at some point inoculated. I think it's the second slide or the one after that that we might as well leave on, bone marrow from two patients and I don't see bone at all. So we don't have any data on bone. Two bone marrows were negative.

Bone, however, has been inoculated in several studies on experimental and natural scrapie and on experimental BSE and

it has been negative. These are good studies. This is cattle to cattle, taking bone and inoculating a gemisch (?) of bone into the brains of other cattle. So by analogy with other TSEs bone apart from marrow is not likely to be infectious.

DR. BOLTON: Any further questions? Thank you, Paul. Thank you for coming in even when you are under the weather. We always appreciate your input.

I think what we will do is we will take our next speaker, Dr. Aliza Eshkol, and then we will take the break, okay? We are a little bit behind schedule, but really because we took Paul out of order.

Dr. Eshkol will speak on the experience of a commercial manufacturer of a pituitary-derived hormone. This is really the Serono experience.

Dr. Eshkol.

DR. ESHKOL: Thank you for inviting me to share with you some of our

own experience on pituitary-derived growth hormone, which is not available any more for distribution in any case, but one can still learn from the history of it and of the various validation studies which were done in the past.

Pituitary growth hormone was started to be prepared from pituitary glands in the early '50s. Initially the pituitaries were collected and they were put into cold acetone and kept there until they were processed and the process was quite a harsh process of extraction, of generally ---- things and subsequently ---- by either a strong acid or a strong alkaline procedure, mainly the Wilhelmy procedure. These products were only semi-purified and they contained a lot of aggregates and dimers which are frequently antigenic.

From the '70s on extraction and purification were done on frozen glands by significantly milder methodologies and they

included some size exclusion chromatographies in order to isolate them on a ---- and exclude the dimers and the aggregates.

Now, regarding the safety, as you all know better than me, probably, that it depends on several issues. It depends on the raw material. Obviously pituitary glands can become contaminated.

It depends on the process, on the capacity of the process to eliminate the agent, if present, on the decontamination procedures, on avoiding cross-contaminations on the back sides and eventually, if available, final quality control.

Now, the first issue is the source. The pituitary glands are collected post-mortem. The majority of the donors come from the older population. Donors of nervous system disease or mental illness were excluded but that obviously has its limitations and mostly no brain histology

was done. So the conclusion is there is absolutely no certainty that the donors are free of CJD, actually the opposite. Everybody is sure that they are very included between the respondents who harbor the CJD or who had the disease.

In March of 1985 the NIH learned that a 22-year old man died of CJD. He received growth hormone from 1966 to 1976, namely growth hormone produced by the harsh old methodologies and in spite of that it was transmitting the disease. In April '85 two additional deaths were reported.

Now, factually it was known in principle to some investigators and particularly the concern was is the transition to the milder methodologies better able to exclude such agents during the process of the purification and a validation, a clearance study, was initiated in the UK in '79. It was published in August '85 by Taylor in Lancet and actually

they showed that the process which was used and the process is called the Lumley-Jones (?) process was capable of eliminating about five logs of infectivity.

The process includes an aqueous phase protein extraction, a selective isoelectric precipitation of the growth hormone at pH 4.9 subsequent solubilization, concentration, chromatography. On various types of columns we use sephacryl in order to isolate finally the monomeric growth hormone.

In '85 after the occurrence of the first CJD cases it was recommended to introduce two additional steps, mainly a millipo filtration with a cap of 100,000 daltons or a nanofiltration with a filter with a cutoff of 20 nanometers or six more of urea treatment. These two were introduced in 1985 only.

In view of the introduction of these two steps we first initiated a

clearance validation of those two steps selectively. We analyzed two filters, a 100,000 cutoff filter and a 25-nanometer filtration step, each of them followed with urea treatment subsequently.

The control of the strain which was used for the challenge had a 9-log infectivity. After the filtration there was about a 2-log reduction in infectivity with both filters. It seemed, however, that the urea was more effective in further reducing the infectivity following the molecular weight filtration rather than the ----nonomole filtration by reducing by more than five logs of infectivity or about 4.4 logs the infectivity.

In spite of the fact that it was clear that growth hormone from pituitary glands is going to be discontinued and the recombinant growth hormone was available at that stage we decided to continue our investigation in order to know even in

retrospect what was the risk, actual, with the growth hormone which we produced so we proceeded to do more extensive validation studies looking at the whole process.

In that study we first of all checked whether there is any kind of background contamination in the equipment which was used. So the AGH purification procedure without a challenge was done up to the Sephacryl chromatography step. The monomeric growth hormone was concentrated and the whole product in its entirety was inoculated into 66 weaning hamsters and none developed scrapie, indicating that they did not have any kind of a background contamination.

Then the process was done in three separate steps. One, starting material was from the same batch for all the three events. This is the original purification methodology and that is the material which was prepared up to '85 and there was a

reduction of about four logs of infectivity which is quite similar to the one that was initially described by the Taylor in the Lancet '85 paper.

Now, this material when subsequently subjected to an additional filtration and the six mole of urea there was a further reduction and a total elimination of nine logs of infectivity so quite effective.

Subsequently all the equipment which was used during this clearance study was decontaminated by either steam autoclaving or by one normal NaOH. Then the same equipment was used again for the production of a scaled-down process of growth hormone and aliquots of the monomeric after the Sephacryl stage of growth hormone were inoculated into 12 hamsters and the residual material was again ultrafiltered and urea treated and the entire product again was inoculated into 135 hamsters.

None showed clinical signs of disease, no histological lesions of the brain, indicating that the decontamination process apparently was effective.

The next issue is cross-contamination, obviously, so what we did is we divided the whole process into four risk categories. Each step was performed in a separate isolated area from the other.

The flow of the process goes from gland handling, first extraction, crude extract, semi-purified protein to purified protein whereas the traffic of people and of equipment can go only in the reverse direction so in order to avoid any kind of potential cross-contamination from one risk category to the other one.

The effect of batch sizes: Based on the assumption that there might be one out of 10,000 CJD-bearing donors in donors of the pituitary glands, if the batch size would have been 5,000 it would mean that

about one out of two batches could be contaminated and that would translate into 25,000 vials which could potentially be contaminated.

We have opted actually from the very beginning of using small batches, between 5- to 600 glands per batch, which means that about one-tenth of that would be potentially contaminated and, as you heard earlier from Paul Brown, some people actually use even much higher batch sizes of 10,000 glands or even more.

Based on all these issues, based on the batch size, based on the clearance levels, and based on the incidence of potential inclusion of contaminated glands the calculated worst case and also best case, which is not on this slide, of contamination of growth hormone produced with and without the added ultra-filtration and urea treatment steps and the bottom line is that the worst case, the old process, it

is one in 50,000 vials which would have been contaminated and which means that about one in 64 patients or children could have been exposed to such a contaminated vial.

With the new purification process with the added filtration and urea treatment the risk obviously is significantly lower in view of the additional five-log clearance. The best case would have been about 1,000 times lower.

The problem is that we cannot know whether in effect we did have ever any case or we will have ever any case. Up until now we have no reported case of CJD with the product which we have distributed but we know that the incubation periods can be extremely long. Here we have up to 37 years, 38 years reported recently, and the incubation with the gonadotropins in Australia, the four cases between 13 and 16 years, a little bit shorter, but with the growth hormone the incubation periods in

some cases are extremely long.

So therefore we cannot exclude today that we did not have ultimately probably some contamination, but if it was it was very low and our worst case calculation is definitely wrong because if it would be true then we would have had already a case so we are somewhere between the worst and the best calculation in reality.

What it shows is that growth hormone, which is a single-chain protein and non-glycosylated, can be purified with a large safety margin provided that the procedures do include steps which can eliminate and inactivate CJD or any other TSE agent and the process is carried out under strict conditions and includes effective decontamination procedures.

As Paul Brown alluded earlier, in France they had actually exactly a similar process, almost 90 cases of CJD up to the

years of '87 where only this kind of procedure was used, but my suspicion told me that there were very severe problems of cross-contamination and I even had a hunch at which step.

Now, the question is can total elimination of potential infectivity be guaranteed? Well, the total elimination is already a loaded term. What does it mean? We have no CJD case reported but in view of the long incubation periods we are still watching out whether there is going to be something. What is sure, that if there was any residual infectivity in some batches it must have been extremely low.

Total absence of risk would need assurance of the safety of the whole material which is particularly important in the case of older age group donors and in view of the fact that this is produced by pooling of the pituitary glands but unfortunately the currently available test

metals do not have the sensitivity which would make this feasible.

Thank you very much.

DR. BOLTON: Thank you, Dr. Eshkol. Are there questions or comments from the committee? Steve?

DR. DeARMOND: When I was a medical student we took endocrinology. I remember one of the endocrinologists said to us there was a great benefit from using hormones extracted from the pituitary because they were never purely the hormone; there were other factors associated with it which were beneficial. But this was a long time ago before synthetics were available so I don't know how they could have tested that hypothesis.

Is there any place today in medical practice for hormones or products derived from human pituitaries or do we rely entirely on synthetics?

DR. ESHKOL: Today we rely totally

on the recombinant growth hormone but actually even before that already with the modified purification methodology which was employed using the chromatographic methodologies actually all the other pituitary hormones they're already excluded, which remained the TSH, LH, and FSH.

What people were thinking was that a little bit of FSH and a little bit of LH might be useful for these children but actually in practice they have not been exposed to it already from the '70s even with the extractive growth hormone, which did not contain these hormones. So what did not change was the transition to the recombinant anything in that sense.

DR. BOLTON: Dr. Brown?

DR. BROWN: Another data point on that, an experimental study was done once in our lab to see if we could transmit disease from archived aliquots of various batches in growth hormone. The results fit very nicely

into the concept that there was only a scattered transmissible dose here and there in hormone batches that were given to humans.

The data were that out of 70-odd different lots of growth hormone, which represented about half the lots of growth hormone distributed in this country, only one lot transmitted disease and in that lot only one of three monkeys that were inoculated. So that would give you a little idea of how little infectivity there was.

DR. ESHKOL: That is obviously true for what has been done in the USA and what raises the big question is the experience in France. Why did they have so many cases? So is it really, as you have pointed out, because they had a higher number of pituitary glands which came from CJD patients? I think it is a combination. That is definitely one of the contributing factors but I think that also the

procedures, particularly cross-contamination and decontamination, which were probably not appropriately respected, could have played a bigger role in that. But it shows that pituitary derived growth hormone does have the potential of transmitting in large numbers of patients the disease.

DR. BAILAR: I have been a little puzzled here as a newcomer to this field by the concern about older donors when we seem to know so little about when potential donors might acquire the disease and what their titers are during all the years it is lurking around unrecognized.

Could you comment on that? Does anybody know about these things?

DR. ESHKOL: I am not an expert on that and I think that Paul Brown could probably answer that much better if he is ready to do that.

DR. BROWN: Yes, there is no information whatsoever apart from a single

observation in a patient with variant CJD by which I mean there simply is no information to know whether during the incubation period of disease in human beings there is infectivity and if so where. That's in humans.

The one exception that I know about is the demonstration of pathological PRP. I think it was in the appendix of a patient who was operated on about 13 months before the first symptoms appeared. So that is probably the single data point that I know of in human beings.

In experimental models there is no question that infectivity typically occurs in a rising curve throughout at least the mid to late incubation period of the disease. So the gist or the thrust of your question is is there a risk of transmitting disease from pre-clinical CJD patients. The answer is almost certainly yes, there is but we can't put a number on it.

DR. DeARMOND: Our experience, of course, with the mouse and hamster models is that clinical signs begin in the last 10 or 20 percent of the incubation period. So over a four-month period, say, in a mouse it is the very last stage. But we can follow the protein and infectivity going up to that stage.

In fact the brain is very filled with abnormal protein and shows lots of vacuolation when they finally get sick. But as you go back further you see bits of that already occurring. So, extrapolating to a human, if there is a similar event, say the disease begins spontaneously in one location in the brain and from the start of symptoms to death it is about four to six months, that could mean that it has been percolating in the brain for two to three years, maybe two years, before it manifests itself with clinical signs if we can extrapolate from hamster and mouse to human.

DR. BOLTON: Ermias?

DR. BELAY: I just wanted to make one point. As you know, the human growth hormone associated with CJD, the outbreak of human growth hormone associated with CJD is an ongoing outbreak. It is not over yet. We still continue to have cases pop up almost every year.

So I think it's too early to say that the patients who received a purified form of human growth hormone would not develop CJD in the future because these patients received a human growth hormone after 1977 and close to 1985 and it's possible that the purification would actually reduce infectivity and possibly increase the incubation period.

So we need to observe this outbreak for some period of time in the future before we say that the purification would probably have prevented the CJD in the human growth hormone recipients.

DR. ESHKOL: I full agree with you and this is why I had actually on my last slide that we can not exclude that we are not going still to see some cases which might have very long incubation periods because of very low residual infectivity in the product. This is not excluded.

DR. BOLTON: Additional comments and questions?

Okay, what I would like to do now is to shorten our break slightly to ten minutes. So I have 10:36. Let's meet back here at 10:46.

(Recess)

DR. BOLTON: Our next presentation is by Dr. Tom Lynch from Clearant. He will be telling us about limiting batch size, the effects of batch size on risk of contamination with infectious agents, and I believe this is work that Dr. Lynch actually did while he was at CBER. Tom.

DR. LYNCH: Good morning. Yes,

the slides are coming up. I can certainly talk to the donor standards but I think Ms. Wilson would be upset if I did. If I could have the right slides I can begin.

The remarks I am going to make this morning are in fact based on a study we did some years ago at CBER in the context of human plasma-derived proteins, including a product referred to as pooled plasma solvent/detergent treated.

Our experience with hepatitis and HIV has taught us that the dissemination of an infectious agent into a population contribute can be far more efficient to the use of administration of pooled therapeutic products than through the use of products derived from single donors. We performed an analysis in the context of these two product categories, pooled plasma-derived proteins and pooled plasma, in order to get a quantitative measure of this risk.

We were specifically asking two

questions. One was whether limiting the size of manufacturing pools from which plasma derivatives were made would enhance the safety of these products. This is question isn't really relevant to today's proceedings but the second question may be, which was should any single donor product be converted to a pooled product for any reason.

Our approach at the time was a far less sophisticated mathematical model than the one presented by Dr. Taffs this morning. We simply related the prevalence of an infectious agent in a donor population the size of the pool to the risk of the recipients of that product made from those pools.

Prevalence would include for an agent for which no screening or testing is performed the natural prevalence in that population whereas for an agent such as HIV for which testing is performed prevalence

would represent the residual risk of a false negative test. The size of the pool was measured in donors rather than donations simply because the number of donors would reflect more accurately this underlying prevalence risk.

Finally, the risk to the recipients was something we struggled in defining. We finally settled on a very conservative measure. We defined risk as the probability of exposing a recipient of a given product to a product made from a pool that included a donation, a unit, and material from an infected donor. Now, this definition of risk was actually fairly flexible, another virtue it had. It also was a measure of the proportion of the total product made derived from these "contaminated pools" and the risk of contaminating a given manufacturing pool was also equivalent to this definition of risk.

What we did not attempt to assess

was the risk that such a product would pose to a recipient of that product of actual infection. The relationship between exposure and infection was recognized as a very complex relationship driven by a number of factors shown on this slide. But certainly the risk of infection is no greater than the risk of exposure. It could be somewhat less depending on these factors so the use of exposure as a measure of risk was taken to be a conservative or worst case estimate of risk.

This is an example of data from that analysis. It shows on a three-dimensional plot the risk at any given combination of parameters. Pool size is shown here. You will notice it goes up to very large numbers relevant to plasma derivatives and it encompasses a range of prevalences within the donor population from one in a million up to one in 600,000. What this slide illustrates is that the risk of

contamination or exposing the recipient of the product to the infectious agent increases as pool size goes up and as the prevalence of the agent in the population goes up.

A number of things needs to be changed in order to make an analysis like this relevant to your deliberations. Pool size, for example, is one. A pool size of 600,000 is simply not relevant, I think, to the tissue industry.

Another issue that is of little or no importance is the risk that a chronic user of a therapeutic product faces a cumulative task. Transplants of allografts, for example, tend to be single episodes in a patient's life so I have made no attempt to calculate the cumulative risk associated with repeated exposure.

There were a couple of other assumptions that we made that are relevant to the model I'll show you today. First of

all, we assumed that there was no dilution effect. This idea that somehow by taking a contaminated unit and diluting it with a far greater number of uncontaminated units you could reduce the titers of infectivity below the level of infectivity, we assumed that that would not be effective and we also discounted any effect of antibodies that may be present in the pool that might potentially neutralize an agent.

The reason for that is that obviously not all infectious agents are neutralized by antibodies effectively and, secondly, for an emerging agent, a new agent, the antibody titers in the donor population may not be sufficient to protect the recipients of a product.

So this is a scaled down tissue-based version of the model I just showed you. Again, the pool size in donors is shown along this horizontal axis. The risk is shown on the vertical axis. Note

that it goes up to one percent here because the pool size is smaller and therefore the amplification of the primary risk associated with the prevalence of an infectious agent is also less.

But the same relationship exists on this slide. The risk increases as the prevalence increases and as the pool size increases as well. So clearly increasing pool size is associated with an increased risk, although here that risk according to these parameters would be below one percent.

What I did want to elaborate on a little bit is this large green area. I don't want to give the impression that this is a flat or neutral area or that there is no influence of the parameters there so I've replotted some of this data in the next slide.

This is simply converting the vertical axis to a log and you can show that even at very low levels of risk the

influence of pool size and prevalence are the same.

This slide illustrates that the unitary risk, the pool size of one, this is the unitary risk reflected by the prevalence of the infectious agent and you amplify that risk almost as intuition would predict in proportion to the increased size of the manufacturing pool.

Now, the actual numbers on which these graphs are based I think were provided to the committee in tabular form. There are lots of numbers there, so it is a little bit more difficult to grasp than the graphical presentation but they are available for your use.

Also, the model that I have just presented is a simple binomial model. The rationale for using it was elaborated in a publication of the original analysis and a copy of that publication has also been provided to you. In the interest of

historical accuracy, your past chairman, Dr. Brown, performed a similar analysis, specifically in the context of TSE agents.

This is just a replot of the same data, next slide in linear form. So the conclusions from analyses such as these are as follow: For the conversion of a single donor product to a pool product the theoretical risk of an exposure to a recipient of this product increases nearly in proportion to the size of the pool from which those products are made. This is especially true for small or moderately sized pools and especially true for agents with relatively low prevalence.

Now, we recognized originally at the time that this risk is one factor and it may be mitigated or offset or outweighed by other factors. This is where judgment comes in and where this committee is charged to operate. For example, the incorporation of a broadly effective pathogen and activation

step might justify pooling where the inactivation requires the pooling process and there are numerous cases of therapeutic products that cannot be made from single donor source materials. They must start from pooled material in order for the manufacturing process to be carried out.

The classic example of that are the plasma derivatives themselves. Here the risk-benefit, however, can be evaluated quantitatively only when you know what the risk associated with the given infectious agent is.

I think I'll conclude there and take any questions that you may have.

DR. BOLTON: Thank you, Dr. Lynch. Questions? Steve?

DR. DeARMOND: It seems it gets down to, as I see it and I'm not in business or in industry and we don't worry about money in academics because we don't have any, but the cost of doing all of this has

to be weighed, I guess, to some degree.

Ultimately it seems to me the best test is whether the material is infective and to date we don't have an idea test but bioassay is by far the most sensitive but that would be expensive to do bioassays. You have to take the animals and it takes a while for them to get sick. Especially if you have low titer it could be two years or a year and a half and your time lines are probably not in that length. You make a batch of plasma and you would expect to have it on the market, I guess, after processing, within the order of months. What sort of time lines do you deal with?

DR. LYNCH: You are right about the time to market for most allograft tissues. My familiarity is primarily with musculoskeletal tissues and that certainly fits with the sequence that you just mentioned. But the products can be on the market for quite some time. Current dating

puts a fairly substantial shelf life on some of these products.

With regard to testing you are absolutely right. The impact of testing is going to be on what I refer to as unitary risk of prevalence. You can start with a relatively high prevalence of an infectious agent in a donor population and drive the effective prevalence down substantially by introducing effective testing.

To the best of my knowledge there is no perfect test so that risk has never been driven to zero but it can be driven to very small numbers. So in using these three-dimensional plots what you would be doing by introducing a test is driving that Y-axis back toward the lower numbers and you can assess the impact of risk from there.

I should point out that one other distinguishing feature relevant to your question and relevant to the application of these numbers that distinguishes the tissue

industry from the plasma derivatives industry is that a fairly large number of products can be derived from a single donor so that unitary risk when you view it from the number of patients that are exposed to material derived from an individual donor can be amplified by the number of products made. But to the extent that this practice is uniform across the tissue industry the proportions of risk would not be altered but the absolute values might be increased.

DR. DeARMOND: One of the questions I was asking Paul Brown was how low does the amount of, say, prion protein have to be before it's no longer a problem? Obviously, we don't know the answer but there is with each product perhaps a different threshold for creating disease in a recipient.

DR. LYNCH: And I made no attempt to distinguish between the risk levels associated with the material such as dura

mater where tests show it can have a substantial risk associated with it to a product like a bone dowel where absent contamination with blood or marrow we think the risk would be substantially lower than that. I haven't tried to distinguish between those types of products because one would really need to know what proportion of each would derive from an individual donor.

Perhaps it's best to think of these numbers in terms of the discrete products and again we are talking about exposure so the levels of infectivity don't enter in but ultimately that's what you are trying to get to, what is the true public health risk in terms of transmitting these diseases by these various practices.

DR. DeARMOND: Could I ask one more?

DR. BOLTON: Sure.

DR. DeARMOND: This is has to do more with the people who are donors. There

are in some cases with plasma derivatives from what I read in the material that is sent to us some are repeated donors of blood for plasma derivatives. What proportion are repeat donors?

DR. LYNCH: Well, as I understand it a cadaveric donation is a once in a lifetime event.

DR. DeARMOND: Yes, that you can only do once. My family doesn't get any money for it.

DR. LYNCH: I assume that the idea of a repeat donor, which is quite prevalent in the source plasma sector and somewhat less common in the recovered plasma sector, is of little significance in the tissue and organ donor population but I stand ready to be corrected on that.

DR. BOLTON: Additional questions? Yes. Please introduce yourself.

DR. SOLOMON: Ruth Solomon, FDA. That's is correct but you have to remember

we will be overseeing reproductive cells in tissues and the semen donor can donate almost as often as the source plasma donor.

DR. LYNCH: Excellent point, yes.

DR. BOLTON: Other questions? I would like to bring up one issue and get comments especially from our epidemiologists and that is the difference between the prevalence of disease which we talk about a lot with sporadic CJD, which is somewhere between one-half and one per million population per year, and the prevalence of infection, which is going to depend, as we discussed a little bit earlier, on the incubation time and if that is a 20-year incubation time in sporadic CJD then the prevalence of infection may be 20 times higher. Instead of one in 1,000,000 it may be one in 50,000. So comments?

DR. BELAY: I think another way of looking at it would be what's a lifetime risk of acquiring CJD for a person. That

would be obviously higher than one per million. One per million would be for each year. Accumulated over time it could be right about one in 10,000 is the best estimate that I have of potential lifetime risk of CJD.

DR. BOLTON: John?

DR. BAILAR: I agree. There is a potentially big difference between the lifetime incidence of infection and the lifetime incidence of disease. It need not be all related to the length of the incubation period.

Some people may carry the infection asymptomatically forever, never come down with the disease if they live to 150. I have been puzzled by the numbers I have read in this package and some I have heard this morning by what does seem to be a discrepancy between the size of the lifetime risk and the size of the risk in the donated products. The latter seems to be generally

bigger so there may be a lot of this stuff lurking around out there that we just don't know about.

DR. BOLTON: Other comments? Steve, did you want to say something?

DR. DeARMOND: I did but I didn't know how to do it. First of all, I presume you were talking about sporadic CJD and the idea that it may be caused by an infection. There was an implication that carrying infection is not the way I think of sporadic CJD, that it is like Alzheimer's, a change in the protein configuration that leads to the disease that occurs spontaneously for some reason.

It seems like once that happens, although we have no evidence that there can be CJD that percolates along without producing disease although a person can have an auto accident and die before it manifests itself, but it seems once it takes hold it goes to completion. You could argue that we

haven't proven that so I am having some trouble with the one per million but that is really based on the number of people who die. Is there really something much more prevalent in the population? We have no evidence that there is something more prevalent that could transmit the disease to somebody else.

DR. BOLTON: Yes, let me frame my question or hypothesis a little better. First of all, I think it was stated earlier that we have no way of knowing what the incubation time, and I use that term in the sense of from the point of either infection of spontaneous conversion of the initial PRP molecule from PRP-C to PRP-SC, what that time is to the onset of clinical disease in sporadic CJD.

It could be six months, which would be a good extrapolation from infection by iatrogenic transmission in the cases of direct intracranial inoculation, or it could

be 20 years. So it's probably somewhere between that, six months and maybe 20 or 40 or 50 years, but it's in there somewhere.

Now, during that time once that event has begun those individuals are going to have some abnormal PRP in their brains and our experience with transmission of sporadic CJD would indicate that they are infectious and therefore they are infected in that sense and whether they initially got the disease through a rare infection or whether it was spontaneous conversion of PRP is really immaterial at that point.

So then the question of the prevalence of infected individuals becomes important because that number is going to be much higher. If the incubation time of the average sporadic CJD case is 20 years the prevalence of infected individuals is at least 20 times higher.

As John said, if there are people who have the ongoing process in place but

will never come down with clinical disease then the number could be much, much higher. Of course, we just don't have a way at this time to assess those numbers.

So I just wanted to bring that out and get some discussion on that because clearly when we start talking about issues of donor screening, tissue screening, and what have you, the prevalence of infection or disease, whatever it is, is very important as we begin to deliberate on how to best regulate that or how best to increase the safety factor.

DR. BELAY: I think it's going to be very, very difficult to come up with a specific number for the kind of data that you are looking for. That is why I say it is probably better to look at a lifetime risk of acquiring CJD wherever it is coming from, sporadic, iatrogenic, or otherwise, but I would think that the number that you are looking for would probably be anywhere

between one per 10,000 to one per 1,000,000 but it will be very difficult to come up with a specific number.

DR. BOLTON: And as we look at different age demographics how does that change? I mean, my inclination would be to think that the older you get the closer to the one in 10,000 that number becomes but that may not be correct.

DR. BELAY: I agree with you. The older you are your chance of potentially incubating CJD would definitely be higher or at least sporadic CJD.

DR. DeARMOND: In terms of just looking at the deaths of CJD certainly over the age of 50 it's almost up to four to five per million compared to the overall population because they are all concentrated essentially over the age of 50. Ninety, ninety-five percent occur over the age of 50 and drop off after the age of 72.

DR. BOLTON: Yes, that was always

my recollection, that there is a drop with age over 70 or so. How dramatic is that drop? I don't know if this will ever come into play with respect to tissue donations because at that point the tissues that could be donated might not be optimal.

But is that something that we should be considering as well? It doesn't seem like it because, as you say, they prefer younger tissue.

DR. BELAY: But I think you brought up a very good point because if you look at the data from CJD it tends to rise in the 5th decade and then the 6th decade and all of a sudden tampers down when you reach the 8th decade.

So the incidence or the death rate from CJD in persons in their 80s is almost similar to persons in their 50s so there is some difference as people age. Particularly after 78 or 79 the incidence of CJD drops down.

DR. BOLTON: So we will have to keep that in the back of our minds here as we discuss this further.

DR. DeARMOND: Do Pierluigi and my lab and Nick Hogan's lab have to start a study to try to find PRP scrapie of infectivity in every autopsy brain that we do?

DR. BOLTON: Sure, I think that sounds great. You can do that for free, right?

We will move on now. We are going to pick up part of the section that Paul Brown has already presented. This is Topic #1-3, "Single Donor Aseptic Recovery and Processing of Human Tissue," and our next presenter will be Diane Wilson, R.N., from Tissue Services and she will be talking again on the issue of cross-contamination of bone and soft tissue with higher risk tissues during the recover of the tissue.

Diane.

MS. WILSON: It's a pleasure to be here. What my main topic today is talking about, what the AATB standards are and how they apply to donor processing and donor procurement in a single donor procurement.

Some of my slides are graphic and I apologize for that but I didn't really know what level of understanding that the advisory committee had about donor procurement and processing so I wanted you to get a general overview of the entire process.

I wanted to start real quickly on donor screening just to give you an idea of how we go through the process. The first thing that happens is a call from the hospital, the evaluation of the hospital records, then a consent from the next of kin and a total medical-social history. This does include all high-risk activities and neurological function of the potential donor, past history, past surgeries,

hospitalizations, anything that we can paint into a picture to make a total puzzle to make sure that our donor tissue will be safe.

Serological and infectious disease screening, the first five tests listed there are mandatory by AATB: Hepatitis-B core, HIV by DNA, DNA by PCR, and HIV Antigen are optional tests. Most AATB-accredited banks are doing those but they are optional.

The physical exam is a mandatory screening test and they are done on all categoric donors. Then you will find tissue banks with a little bit of variety. We use a physician letter to the family physician to find out anything else about the donor that we may not have picked up in the hospital records. And because we are part of a blood center we will look at the blood donor registry for past deferrals but those are also optional. You will find tissue banks will vary a little bit on the amount

that they do above and beyond the standards. Then we have a medical director review.

Informed consent is extremely important to us and AATB puts a lot of emphasis on this. We have to make sure that no coercion has been applied, that all of the information that we give the next of kin is accurate and not misleading, that everybody is done in understandable terms, that infectious disease consent has been obtained in order to test them for hepatitis and HIV and the other testing, and that we have access to those medical records because we will be getting a copy of an autopsy if it has been performed and we need a total copy of the medical records from the hospital or at least be able to summarize those records.

The new things that have come up in the last year, AATB has added to the standard saying that we should, not a "shall" at this time, discuss with the

families whether they agree to profit versus non-profit use of the tissue, cosmetic use, use of tissue outside the United States, and in order to use tissue for research we need to have consent for that. And then always give the families the right to ask questions because this is the most devastating time of their life and at the time you are going to be talking about consent you want to make sure that they have a full understanding of what you are talking about, whether tissue to them means eye tissue, bone tissue, or a piece of bone marrow.

Donor identification, the first thing that you'll do when you get to the hospital is they must identify the donor and it will be assigned a unique ID number for the tracing of all the tissues and this number will then follow that donor through the entire process.

Prior to retrieval at least one of the staff members shall identify the

potential donor's ID against the consent for tissue donation knowing that they are retrieving the proper tissues and they have the accurate donor. Donor identity will also be documented in each and every chart as to how you identified, whether it was a toe tag, a wristband, or whatever source of identification was used.

We do have time constraints written into AATB. Up in the top corner of the slides I have written the standard for AATB in case anyone wants to see more detail on these standards. If a donor has been refrigerated or placed in a refrigerated cooler prior to 12 hours we have 24 hours to begin the retrieval of that tissue. If they have not been refrigerated within 12 hours then we must begin tissue procurement within 15.

The environment must be documented. We must do all donors in an aseptic fashion. We are going to use the

standard surgical prep, the same that would be used on any other surgical procedure in a hospital, sterile packs, sterile instrumentation, and the technique must be trained upon, documented, and according to standards.

The general site where the tissue was removed has to be documented. This means was it removed in an operating room or a morgue or a procedure room or a facility room that the tissue bank may own. All working surfaces before and after retrieval will be scrubbed using bactericidal and antimicrobial agents for cross-contamination.

The preparation of the donor, we'll start with cleansing, preparing and draping the skin as well as soon as the technician will do a gowning and gloving as any surgeon or scrub tech would do on any other surgical procedure and this shall be accomplished with the same diligence as used

routinely for all operative procedures and this is the standard. Whether or not you are going to terminally sterilize the tissue this is the standard. The aseptic technique that will be followed and the one we train on is the American Association of Operating Room Nurses or the AORN standards.

The surgical staff shall perform the scrub of their hands and their forearms prior to retrieval of any tissues. They will wear head coverings, eye shields, mask, and scrubs at the time that they do these procedures. This is whether they are in a funeral home or in a morgue or any other place and sterile gowns and gloves shall be donned after they scrub and all tissue recovered, again, will be used under aseptic technique.

Here are just a few slides of scrubbing the forearms and gowning in a proper procedure. The gloving technique is usually closed glove because the first

person to glove is alone. We don't have the luxury of a scrub person to help us to begin a proper surgical technique.

In prepping the donor they will shave first any areas where an incision site will be. We also will stay away from any abraded areas or open wounds. Here we begin our surgical dissection, usually start at the hip, go down to the ankle, go through the fascia lata, which is one of the grafts that now are used in place of dura mater since we don't have any dura.

Here they are removing the femur. The same surgical technique is used for every part of this process. They are disarticulating the knee at the knee joint. This is the femur and the tibia with the deflected patella.

Going down to the tibia to the ankle, just making a clean dissection. The rest of the muscle and all will be left with the donor for reconstruction purposes. And

removing of the tibia down at the ankle and here is the Achilles tendon coming around the backside of the calcaneus there.

The last graft that is usually removed from the donor will be the ileum. They will go back up to the hip and remove the ileum. In case of contamination this would be your number one place to get contamination would be by nicking the bowel at this time.

Pre-processing cultures are mandated by AATB. There has been a lot of information out recently about some recent infections and preprocessing cultures must be done on all tissues retrieved. Every individually recovered and packaged tissue shall have a pre-processing culture and this is prior to the treatment of any antibiotics or sterilizing of that tissue.

Even if the tissue is going to be sterilized you must have a culture taken to give you an idea of what bio-burden the

donor may have had at the time. It can be used in the evaluation by the medical directors to see if possibly there was some systemic infection. Those results will be maintained in the medical chart and the M.D. will review those prior to release.

Immediately after removing the tissue from the donor they will be wrapped individually in aseptic containers. These will be labeled with the donor ID and the type of tissue. This is just to keep the tracking of the tissue number throughout the process.

Here she is doing a swab culture on a tibia. They will swab the entire graft top to bottom or any cut surfaces prior to putting it into the package. After each tissue is removed it is individually wrapped prior to the removal of the next tissue. So if you have a contamination problem your problem will probably be picked up as you go through the case so each graft is wrapped

individually as it comes out.

Here she is wrapping it in one single barrier and then she is going to wrap it this way. After this point then it will be labeled with the identification.

The transportation of the tissue from the procurement site to the freezer, it will be packaged in a manner to permit environmental conditions to be maintained during that transport time. The containers do not require monitoring during the transport time, according to AATB, as long as the containers have been validated. If they have not been validated containers then they would need to put a temp tail in there or something to show that they had been kept at refrigerated or frozen temperatures. The maximum time that AATB allows on wet ice or refrigerated temperatures prior to freezing or processing is 72 hours.

The transport receptacles must contain the following labels and this is

even if you are coming from a coroner's office back to your own facility. Any time it is carried in a car or shipped on an airplane it must say "Human Tissue Enclosed," the name and address of a retrieval agency and processing center if different than the retrieval agency, and it must state, "Quarantine, Not Suitable for Transplant in its Current Form."

Reconstruction of the donor is mandated on all tissue donors no matter whether they will be cremated or not. We do follow whatever funeral home guidelines they have. Usually we use dowels, rods, PVC piping, and our closure technique will either be usually a baseball stitch or a running stitch. Here is some muscle being sewn back up on the thigh around the internal brace and then a closure to the donor's skin after that time.

Most tissue banks will tell you that this is their signature as to their

care of the donor; therefore, much care is taken during this process in cleaning the donor up.

Single donor tissue processing, processing methods, all tissue shall be processed by methods known to be validated to prevent contamination and cross-contamination. Some tissue banks will use AORN draping such as you were use in the operating room. If they are using any process other than draping of their back tables they must have validated their surfaces and processes to show that there is no cross-contamination on their work surfaces and before and after processing they will be scrubbed again and wiped down with bactericidal and antimicrobial agents.

And this is just a typical clean room on a draped surface. You will see other banks that use stainless steel counters and process directly on those. Those should have been validated prior to

use if that is what they are using.

A typical central supply area where instruments are wrapped after each donor whether being processed or procured and then sterilized with the use of steam or gas sterilizers. Reagents and supplies according to AATB standards used in processing and preservation shall be of appropriate grade for the intended use and sterile if indicated. On the receipt of all supplies we do record all reagents and supplies, including the type of agent, manufacturer, lot number, date of receipt, expiration date, and inspection of that reagent. This is also in case there is a recall on any type of agent that we may have used on a donor; therefore, we will know what tissue to quarantine.

Tissue ID numbers, in processing each tissue shall be assigned that unique ID number which still will follow it through the process. All tissue units shall be

assigned the same ID number as if they are identical in a lot. So once the donor has been given a number that becomes your lot.

In pooling AATB does address this issue and it is not allowed. Tissue from multiple donors shall not be pooled during retrieval processing, preservation, or storage at any time. Cross-contamination, written procedures shall be prepared, validated, and followed for prevention of infectious disease contamination or cross-contamination by tissue during the processing. So it is very important to us that when a donor tissue is brought into a room no other donor tissue is there and no two tissues ever touch at any time during that process.

Here is a processing lab. What you see on that right hand is a metal glove that is actually on top of his other gloves. This is just for safety. Frequently you will have a lot of sharps in your hands so

this is a safety glove.

This is a typical cutting with the use of a sterile band saw. All of this is autoclavable. The entire band saw is taken. That is an ileum he has in his hand and that is the acetabulum of the ileum he is cutting there, just as a reference.

Small saws may be used. These, of course, too, have to be able to be autoclaved and sterile at all times. These are little cancellous blocks that you may see used in a cervical fusion or upper neck.

Some people will centrifuge tissue. It depends on what their validated process is or their process is for tissue banking but this is a centrifuge cup. It will be centrifuged to bring out all of the lipids and marrow. You will see a lot more of that in the other speakers that are talking about processing.

Each graft is individually packaged and sealed in the room that it was

processed in or in the packaging room to make sure that, again, there is no cross-contamination.

Ultrasonic baths are used on some donors. This is again to bring out fat and lipids, you will see. It just depends on what type of methods the tissue banks are using.

These are patella ligaments. I just put a couple of pictures in here of different grafts that you might hear about over the years. When you talk about young donors you are talking about patella ligaments, tri-cortical blocks, and some of the weight-bearing grafts that are extremely important in tissue banking. You will hear tissue bankers say that young donors are of extreme importance because of the type of grafts you can get.

The older donors also are because the young donors are going to be used for more of these structural grafts and the

older donors for more of a ground type of material that may be used as a packing material on a hip surgery. So you will see the need for both types of donors.

Femoral heads and short distal femurs or femoral condyles, cortical struts used in hip surgeries, back surgeries, tri cortical blocks, it's an ileum with a tri cortical block. You will hear about three sides of the cortical there with a trabecular bone in the middle. These have to be from young donors. It is very important. These are the grafts, the teller ligaments, femoral grafts, and tri cortical blocks, that you will always see massive back orders for, surgeons waiting for these grafts in very high demand.

Fibular segments may be used in a back surgery. Crushed cancellous can come from a younger donor or an older donor. It is used a great deal in hip surgeries, knee surgeries, packing materials, defects in

bone, but this is not a weight-bearing structure; therefore, it can come from some of those older donors.

Final tissue culturing, once the tissue has been processed terminal sterilization by a radiation or ethylene oxide on those lots of tissue. You must do 10 percent destructive sterility testing to show that you have no contamination after the process or an equally validated procedure such as 100 percent swab testing.

If no terminal sterilization has been done and you are calling the tissue aseptically processed, you are not going to terminally sterilize, then you would do 100 percent swab culturing according to standards.

Tissue release, the final release after all the grafts are finished, you put the whole puzzle together and quality control will review all of the following areas again prior to release. That will be

the procurement and processing records, the medical director approval. AATB does require that a physician release the donor suitability, the serology review again, the culture review again, meaning the procurement cultures and the processing cultures, any water residuals from the freeze-dried tissue, irradiation results will all be put together prior to the release of that.

Then once your tissue has been released it is an AATB standard that the record shall include the name and address of the tissue bank, the type, quantity, and unique ID number of each tissue graft, the recipient name and hospital number or Social Security number of the recipient, the transplantation site, the date and the time, the ordering physician, the dispensing physician, and the tissue preparation person if applicable.

Most tissue banks will have some

type of a tissue log that they will give to the hospitals to help them in filling out these papers. It's not mandatory that they return all of these. We strongly encourage it and most tissue banks will have a way to go back and look at those that have not been returned because in case of a recall or any problems we certainly need to know where the tissue has been transplanted.

And that's it.

DR. BOLTON: Thank you, Diane. I particularly appreciate that last slide. I'm going on vacation next week to go fly-fishing, so I'm ready.

Questions? Ermias.

DR. BELAY: You say you don't allow cross-contamination of the products. I was curious about some of the instruments you showed us on the slides, particularly the saws, the different saws you use to cut different bone parts. Are those instruments sterilized in between donors after

processing between one donor from the next?

MS. WILSON: Yes, they are. According to AATB standards you must sterilize in between donors. The saws, a lot of them that have any type of a rubber cord cannot be steam-sterilized but they can be gas-sterilized.

You would then validate them in your gas sterilizer to show that you do not have any residual tissue left in them or growth on the grafts but even the large band saw can be steam-sterilized and the other things with the cords are gas-sterilized.

DR. BELAY: The other question I had was do you have any demand and supply problems with bone in the United States?

MS. WILSON: Yes, we do. The demand far exceeds the supply, especially of weight-bearing grafts, the patella ligaments, the femoral grafts, Achilles tendon, tri cortical blocks.

DR. BOLTON: With respect to the

band saw, aside from the sterilization you said that can be autoclaved. Is it scrubbed? Is the saw itself scrubbed in any way to remove tissue or particulates?

MS. WILSON: The saw is. The saw will have a large blade that goes around the wheels. It will not only be cleaned but blown out with an air hose. Then it will also be soaked with a disinfectant and then it will be sterilized after that period of time.

The blades on the saw usually are a one-time use only. They can be re-autoclaved but because of just cuts in handling the blades to the personnel we usually just discard those.

DR. BOLTON: So the blade itself is used for one patient?

MS. WILSON: The blade is for one-time use but the saw itself that has to be autoclaved, too, has to be cleaned and you have to get out the other residual bone

powder that it will create.

MS. KNOWLES: And I have a question. When you blow it out with the air hose is that connected up to a vacuum?

MS. WILSON: Yes. Most people will have that, yes.

DR. BOLTON: Yes, Dr. Wolfe?

DR. WOLFE: You mentioned that since you don't harvest dura mater that you have been using fascia lata. Certainly autologous fascia lata is one of the alternatives to dura mater. Is it your experience that there has been an increased amount of fascia lata request or demand as the use of dura mater has decreased? Could you just comment on that?

MS. WILSON: From our experience there was initially when we stopped doing dura mater.

DR. WOLFE: When did you stop doing dura mater?

MS. WILSON: 1996, our bank did

and most banks stopped very shortly thereafter. We had an increase for fascia for probably about a year. I believe most places now are using more synthetic or bovine.

DR. WOLFE: Stopped in '96 for what reasons or what was the context?

MS. WILSON: We had done dura probably since 1987 and when the new pre-market approval, 510(k)s were required on dura mater, we did a very small amount of dura mater and elected to not do dura.

DR. PETTEWAY: As far as the sterilization of the tissue, the ethylene oxide irradiation, what types of organisms have been validated for sterilization with these types of tissues, just to give us an idea of how effective they may be?

MS. WILSON: I probably can't answer for all banks. I know that we use bacillus pumilus as an impregnated strip that goes in every lot of irradiation as our