COMMISSION OF INQUIRY
ON HORMONE RECEPTOR TESTING

BEFORE THE HONOURABLE JUSTICE CAMERON - COMMISSIONER

September 15, 2008

Appearances:

Bernard Coffey, Q.C. . . . . Commission Co-counsel
Sandra Chaytor, Q.C. . . . . Commission Co-counsel

Rolf Pritchard/Jackie Brazil . . . . Her Majesty in Right of NL

Peter Browne/Jane Hennebury . . . . . Doctors Kara Laing et al

Daniel Simmons . . . . . Eastern Regional Integrated
. . . . . Health Authority

Ches Crosbie, Q.C./
Laura Brocklehurst. . . . . Members of the Breast Cancer
Testing Class Action
Mark Pike . . . . . NL Medical Association
Jennifer Newbury . . . . . Canadian Cancer Society (NL Division)
Blair Pritchett. . . . . Central, Western and Labrador-Grenfell
Regional Integrated Health Authorities

LIST OF EXHIBITS

TABLE OF CONTENTS

THE COMMISSIONER:
Q. Please be seated. Ms. Newbury.
DR. NEBOJSA (NASH) DENIC, EXAMINATION BY MS. JENNIFER NEWBURY
MS. NEWBURY:
Q. Good morning. Good morning, Dr. Denic. Jennifer Newbury for the Canadian Cancer Society, Newfoundland and Labrador Division. I have a few questions for you this morning, dealing first with retesting of the positive ER/PR patients. And I believe that you'd mentioned last week that you had retested some of the patients recently?
DR. DENIC:
A. That's correct.
MS. NEWBURY:
Q. Okay, and was that done in 2008?
DR. DENIC:
A. That's correct, 2008. The latter group of the patients wanted to be retested.
MS. NEWBURY:
Q. Okay, and these are patients that identified themselves as wanting to have a retest done, is that correct?
DR. DENIC:

Page 5

A. That's correct.
MS. NEWBURY:
Q. Okay, and about how many of these patients have been retested?
DR. DENIC:
A. I think the list that was given to me recently, it was around ten patients, and they come from the different years of original testing.
MS. NEWBURY:
Q. And is anyone at Eastern Health tracking the numbers and the results of those tests?
DR. DENIC:
A. That's correct. To best of my knowledge, actually, and I was being supplied basically by the risk and quality management department.
MS. NEWBURY:
Q. Okay, and they were from various years, is that correct?
DR. DENIC:
A. That's correct.
MS. NEWBURY:
Q. Okay, so these weren't random test results, as such, these were just patients that identified themselves?

Page 6

DR. DENIC:
A. That's correct.
MS. NEWBURY:
Q. And I assume that the patients would have been advised of the test results upon receipt?
DR. DENIC:
A. They were advised. Even one of the patients I called directly.
MS. NEWBURY:
Q. Okay, and would you consider this to be a sufficient sample size to draw conclusions about the ER positive group in its entirety?
DR. DENIC:
A. I think it's a good indicator, you know. Whether statistically is valid, it's difficult to say, but I think it's one of the good indicators that one should have in mind.
MS. NEWBURY:
Q. And do you know if anyone has done a statistical analysis or plans to do a statistical analysis of these results?
DR. DENIC:
A. You mean just these positive or -
MS. NEWBURY:
Q. Yes, just for the -

Page 7

DR. DENIC:
A. - overall what has been done?
MS. NEWBURY:
Q. - positive test results?
DR. DENIC:
A. That's very small--oh, you mean--excuse me, I don't understand. The new ones that we received, ten patients statistic -
MS. NEWBURY:
Q. Well, the new--so far there have been a number of what have been called retroconversions or false positives.
DR. DENIC:
A. That's correct.
MS. NEWBURY:
Q. And there have been some other ER positive test results that were confirmed to be positive?
DR. DENIC:
A. That's correct.
MS. NEWBURY:
Q. And now you have a new group of about ten. And I believe that you indicated that those results, there were no retroconversions, there were no false positives in that group?

Page 8

DR. DENIC:
A. That's correct.
MS. NEWBURY:
Q. So you would have a number of samples now. And I'm just wondering if there is a plan or any discussion about doing an actual statistical analysis there to see if you can draw any conclusions about all of the ER positive test results done to date?
DR. DENIC:
A. I don't know to any organized way, but I'll let you--what I did recently since Dr. Makretsov came on board and he is very much a research oriented and he has a statistical analysis program in his computer. So I did draw some of the numbers, you know, just these are ball park figures, and I put like 2700 tests as a denominator, then I put 380 tests that had a negative ER and that we have a problem with and then I put the number of false positives, which were four, but at that time I even put a little bit higher number because this was done looking into the thousands of patients, so I said, okay, let's give 15. So we give it a higher number, as

Page 9

such, so just to try to get any better statistics to all of this. And the remaining number are the positives, really. And based on the test that we did, the statistical analysis positive predictive value, which means what does it--how objective is the test to pick up the positives. That number is 99 percent. So in 99 percent based of this rough analysis, I'm not saying that this is--you know, I think it should be done in a different way, but this is my rough analysis of this. So in 99 percent we should be able to pick up positives.

MS. NEWBURY:

Q. Okay, based on the results that you've received to date?

DR. DENIC:

A. That's correct.

MS. NEWBURY:

Q. Okay, and that's assuming that the data that you relied upon is appropriate and that instead of using the four, what you consider to be false positives, you used a figure of 15?

DR. DENIC:

Page 10

A. Yes, I gave extra numbers just in case that we may encounter, but we may not.

MS. NEWBURY:

Q. Okay, and is this an analysis that you've kept a record of?

DR. DENIC:

A. Yes, I did.

MS. NEWBURY:

Q. Okay. I'm not sure, is that something that's been provided?

DR. DENIC:

A. No, that was done just by myself. I was interested--this was done in August. I just want to see what would be the--is there any statistical analysis can be done, but it hasn't been done, actually, and should be done more properly, I would say, by statistician, probably NLCHI or somewhere else.

MS. NEWBURY:

Q. Okay, and is this something that you've shared with any of your colleagues at Eastern Health, even your rough analysis and your thoughts that perhaps it could be done more formally?

DR. DENIC:

A. I don't think so I shared it. Maybe within

Page 11

the group of pathologists at St. Clare's or, I mean, Dr. Makretsov knows about it because he did that for me, really.

MS. NEWBURY:

Q. Okay. Nothing on a formal basis, anything shared -

DR. DENIC:

A. No, nothing on a formal basis.

MS. NEWBURY:

Q. Dr. Denic, it was Dr. Laing's evidence last week that it was her understanding that some of the retroconverters could be explained by over--by a pathologist overcalling a slide due to background staining. And she had indicated that the pathologists that were involved in looking at the retroconverters had taken out some of the original slides and reviewed them. And she thought the pathologists could speak better to that whole issue and, but her understanding was that they felt that the staining was more background staining than true staining of the cells and that lead to an overcalling of the slide. And I'm wondering if you were one of the pathologists involved in that review?

Page 12

DR. DENIC:

A. Yes, I was.

MS. NEWBURY:

Q. Okay, and Dr. Laing thought it was you and Dr. Cook and Dr. Carter. So is it the three of you that looked at that?

DR. DENIC:

A. I believe it was only me. I am not quite certain about Dr. Cook and Dr. Carter. They might have looked one or two cases, you know, because we looked at large number of cases. I cannot be stating this with absolute certainty, but I know I looked at these cases.

MS. NEWBURY:

Q. Okay, and do you know if anyone else, aside from Dr. Cook or Dr. Carter may have looked at these or did you do this on your own?

DR. DENIC:

A. That's what I am saying, I cannot be certain. I know that I looked at it.

MS. NEWBURY:

Q. You looked at it, okay. So you don't know if anyone else did for sure?

DR. DENIC:

A. No.

Page 13

MS. NEWBURY:
Q. And if overcalling a slide due to background staining occurred in some of those cases, can you recall how many of the four retroconversion cases fell into that category?
DR. DENIC:
A. Yeah, all four.
MS. NEWBURY:
Q. All four, okay. So if that occurred with those four particular slides, how can you be sure that it didn't occur with other slides, other ER/PR positive test slides?
DR. DENIC:
A. Well, you cannot be absolutely sure about anything, as such, that happened. You know, these are some of the pitfalls in testing and interpreting that it's recognized, you know. But the literature states, really, that this is not a common problem. This is not a problem, as you can see, with the false negatives, you know. So could we find one or two more, we may or we may not, you know, so I cannot state with any degree of certainty what are there.
MS. NEWBURY:

Page 14

Q. Okay, you've indicated that the literature says that this isn't a common problems. Now I believe Dr. Banerjee had indicated that while he saw no evidence that the Ventana system was overcalling the--creating false positive results, he had indicated that the system still requires optimization to involve non-specific staining. So you're aware that that's something, that's a necessary step to avoid non-specific staining?
DR. DENIC:
A. Any machine needs optimization.
MS. NEWBURY:
Q. Right.
DR. DENIC:
A. And validation. And that's been done in our lab.
MS. NEWBURY:
Q. Okay, and that's something that Trish Wegrynowski had also pointed out, that you need to optimize your test results. And she'd also indicated that you need to run negative controls and that would help to ensure that you're not getting any non-specific staining?
DR. DENIC:

Page 15

A. That's correct. And they have been run.
MS. NEWBURY:
Q. And in a decision whether or not to look at false, possibility of false positive test results or to do a review of some of the positive test results, did you take into account any possible flaws, I guess, or issues that were apparent regarding optimization of the systems that you had in place between '97 and 2005 and also the absence of negative controls?
DR. DENIC:
A. I didn't take because obviously I didn't have any kind of role and I didn't oversee immunohistochemistry, so I really don't know what was in place. And I think the people who should know any kind of knowledge, as such, would be Dr. Ejeckam, who overseen the immunohistochemistry, Mr. Barry Dyer and Mr. Gulliver and obviously the technologists who were working on the system. So I wouldn't have any kind of knowledge in that regard.
MS. NEWBURY:
Q. At the time, but do you think now that looking back at it, now that you've had the reports of

Page 16

Ms. Wegrynowski and Dr. Banerjee, can you say with confidence that there were no issues with non-specific staining due to failure to optimize the tests or that there were no issues because of the lack of negative controls, and have you taken that into account into any decisions?
DR. DENIC:
A. With certainty of course I cannot say about it. What I was thinking about my approach to the, whether we should look into this group was from the perspective that obviously scientific road, as you can read now, try to come to some kind of conclusion what would be the error rate that one test may acquire during performing. We are talking about, what, minus five percent these days about it. While we would all like to, for every test to be 100 percent certainty and specific, we know that this is not reality for any single test. Any instrument you buy in your house, that tells you a performance, plus, minus confidentiality rate, 95, so you hear of plus, minus five. So when I look all of this, as well, and so a five percent is something that

Page 17

even in Dr. Allan Gown's lab, he said concurrence rate was 95 percent, I think he stated that in Wall Street Journal, as well, which is a high concurrence. So if you look at these are four cases which were picked up from 1000 of patients, they needed to be taken off the Tamoxifen because they fell in a group of false positive, that's far below even a point five percent of the such. So that's one way to look at it. The other ways to look at it is these random patients that are coming up and said that we would like our test to be done, we did those ones where the number is not big. Obviously, thankfully or luckily, the results never changed. They were positive then, they stayed positive now. The other thing is that, as well, literature says that this group may exist but is not even close significant as a false negative. And the last thing that I see that if there are still patients out there that would like to be retested, and I think Eastern Health did say that, that we'll do it, we'll do it for the benefit of them getting again the results, confirming their results and for the benefit

Page 18

of knowing the decision that we made was right. While we did state it that we cannot go onto the mass retesting because you can only imagine, it's now we have another 1500 right away coming to the lab, I think the lab would go to a halt and nothing would have been performed and we still have hundreds and hundreds of new patients that has to be done. So this is a factor that I looked, and in my opinion is how we should treat this issue.

MS. NEWBURY:

Q. A couple of points on your explanation, Dr. Denic. I mean, first of all, you've indicated that four patients had to have a change of treatment because they were false positives and you said that there were 1000 patients in total, approximately. But wouldn't you have to look at, when you're looking at the error rate for the positive test results, wouldn't you have to look at the positive test results that converted as opposed to the whole group? And I understand, perhaps we can bring up Exhibit P-2662, please? I understand that the total number of positives was actually 16, so if you have four out of 16, that would be a

Page 19

much higher percentage. Perhaps you can explain that? This is a document, an e-mail that was sent to you. And I believe attached to that there are lists--now, this is from November, 2006, I believe. So it has total retested. And I know that these numbers have been updated -

DR. DENIC:

A. That's correct.

MS. NEWBURY:

Q. - since then. But just looking at this point in time. They were confirmed negative 341, patients panelled, 422. And if you go down towards the bottom of the page, it says "Confirmed positive, 12" and then a little further down, "retroconverters, four." So it would appear to me that the only positive test results that were tested would be the 12 plus the four, which is 16. And those are the ones, the four out of the 16 are what converted, so that would give an error rate of 25 percent.

DR. DENIC:

A. I think that's the very wrong way to look at it, Ms. Newbury. I mean, we are doing against

Page 20

the 12, four, completing disregarding that another how many, 1500 tests been done.

MS. NEWBURY:

Q. Um-hm.

DR. DENIC:

A. And these 12 patients, these patients' original results were considered to be positive by the treating clinician and treated appropriately. There was slight change in the patients' ER/PR status, but review by the panel confirmed that ER/PR status are still being positive. So they are being positive, they stayed positive, so they should be put in a group, really, of all of these remaining positive which we didn't test it. But I don't see that this is a good stat, that, you know, just to look for over 12, no, I don't think so, that isn't the right way to do it.

MS. NEWBURY:

Q. And I guess the point is is that it is a very, very small number. You have only 16 positive patients that were tested. So I guess the point is that that is a very small sample size, and wouldn't you have to do a much a larger sampling size to be able to draw any

Page 21

conclusions one way or the other about positive test results?

DR. DENIC:

A. So the question is how large sample should be done and how this can be performed without affecting helping the patients, as well, and not affecting the regular daily service, as such. That's why we came to that conclusion, that let's offer this to the patients.

MS. NEWBURY:

Q. Yes.

DR. DENIC:

A. Because even as you understand the benefit of Tamoxifen therapy in the patients who are negative, there's some benefit of Tamoxifen, as well, like preventing the breast cancer in the opposite breast.

MS. NEWBURY:

Q. Um-hm.

DR. DENIC:

A. So not that some of these patients didn't benefit and such. The other thing that's what you should look at is probably there's a ten percent of the chance, as the literature said, that even if they're negative, they might be

Page 22

responding to Tamoxifen therapy. So there's some benefit even of Tamoxifen to these four patients, while they were taken off for the various reasons, they could even have benefitted because they're alive. And so I think this is the way that to look at it. And the other thing is that I think I brought that on my last day last week is about how much we know about the tissue, as such. I think that some of the expert witness may have testified about the heterogeneity of the estrogen receptors into the tumour tissue. And what we are examining is the five micrometres of the one's tissue, while the tumour could be five to six centimetres big. And we are taking representative section because that's a part of the standards of practice. So you would take your three or four representative sections from the tumour and thinking that you have an appropriate sample.

MS. NEWBURY:

Q. Um-hm.

DR. DENIC:

A. So is there anybody going to say that in this particular patients why we said they were

Page 23

negative. Wouldn't have been a clone of the cells, one percent, in the billions of the billions of the cells. And I think people testified that even sometimes different clone metastasis and they found the different clones in the lymph nodes that can we absolutely guarantee, absolutely guarantee, that there is nothing positive even in this negative patient. So these are my thoughts about the process being done and this is my opinion.

MS. NEWBURY:

Q. Okay. I appreciate your opinion on that Dr. Denic. I guess a couple of other points arising from that. Was it your evidence last week that when selecting the sample for the ER/PR testing that you tried to select a portion of the tumour that's well differentiated, knowing that that would be the most likely part that would be ER positive?

DR. DENIC:

A. Yeah, you're trying to do that from the tissue already taken.

MS. NEWBURY:

Q. Sure.

DR. DENIC:

Page 24

A. Because when you look at the tumour on the gross bench as a gross specimen, you cannot distinguish what part is well differentiated, which part is moderately differentiated, which one is poorly differentiated, because the tumour looks the same. So only under the microscope, so if you already sampled those one and then when you look under the microscope, you'll try to see is there any areas that the tumour is well differentiated. Again, this is a random sample of three to four that we are obliged to take. Sometimes we take more, depends what we are looking at. If you are looking if the tumour is close to the margin, surgical margin, of course you take more tumour and the margin with it in order just to identify whether or not the tumour is crossing the surgical resection margin, because it has clinical implications. So obviously you cannot say that on a gross bench, but when you're taking the slides, yes, you can identify whether or not there are any better differentiated area.

MS. NEWBURY:

Q. And that's with a view to trying to find out

Page 25

if there's any benefit at all to the patient in taking hormonal therapy?

DR. DENIC:

A. That's correct.

MS. NEWBURY:

Q. Such as Tamoxifen. So you try to overcome the problem about the tumour not being heterogenous and try to actually target those areas that you anticipate might be ER positive?

DR. DENIC:

A. Exactly. To try to--we anticipate, not necessarily, because biological makeup of the cells could be completely different from the various portions of the tumour. And that's something that you cannot even do on your regular microscope. That's what molecular geneticists do.

MS. NEWBURY:

Q. Dr. Denic, if there were to be further testing, at what point would you feel that there is value in, you know, looking at a further, broader sampling of ER positive test results? I'm assuming, you know, there is some value, obviously, into doing the ER/PR

Page 26

testing, otherwise it wouldn't be done. So even though there are some impediments due to the, you know, the heterogenous nature of the tumour, that there is a value in doing it and it's done for a purpose and it's done to give options to the physician and the patient for treatment. At what point would you have a lack of comfort about the PR positive test results?

DR. DENIC:

A. I believe there's going to be still patients around that are going to be coming asking to be retested, you know, and I'm going to be monitoring that--those ones as well, you know. So we have to give a period of time that patients come forward. As I said, we've been offering and some of them did come forward. So I think to me that's going to be the best indicator, as such, whether if we start seeing changes in the results, then I definitely would be alarmed myself.

MS. NEWBURY:

Q. Would you think there would be a value into doing some of those retests on a random basis, recognizing that if patients are self-

Page 27

identifying, it might be patients who are still alive, as an example, or perhaps patients that haven't done as well as they had hoped, and maybe by virtue of the fact that they are self-identifying rather than being randomly selected out of a database, then it may not be a true representation of all of the ER positive patients?

DR. DENIC:

A. But random sampling is usually a true representation rather than the direct going with--I understand your question, how many of the cases that we should be doing and that we would be satisfied that this is done right. Obviously, in order to be satisfied that this is done right, you have to do all of them, you know, and I believe that for the various reasons as I said, and herein mentioned, the biggest one that going through all of this, we're going to need a lot of human resources. I'm going to lose the manager, I'm going to lose the program director again, and I'm going to probably lose myself and the oncologists as well has to be dedicated through all of this, and thousand patients took us two years and

Page 28

probably this going to take us another three or four years, and in the meantime, I think, we won't be able to sustain really the service, but what would be the number really. I think, if statistician comes with any kind of proper number that they say, then we can revisit that issue and look into it.

MS. NEWBURY:

Q. Would that be your only impediment in terms of doing a further larger sampling size? Is it only the financial and human resources?

DR. DENIC:

A. It's not financial as much as it cost--you know, I don't think so that anybody would put the dollar figure against the patient care. It is the human restraints as well.

MS. NEWBURY:

Q. Sure.

DR. DENIC:

A. And the other factors, as I stated, that influence decision whether we should go further on on additional 1500 patients for tests.

MS. NEWBURY:

Q. And you've made reference to literature and

Page 29

that your conclusion from the literature is that false positives are not likely to be the problem as compared to the false negatives?

DR. DENIC:

A. That's correct.

MS. NEWBURY:

Q. And I believe it was Dr. Mullen's evidence that he thought, as a concept, that it would be unlikely that you would get a false positive, but his qualifications on that is that if you had a properly validated antibody, he thought that a false positive would be unlikely, and Dr. Banerjee also had testified on the issue of false positive results, and his conclusion was that it shouldn't be--I believe he thought it wasn't likely because if a pathologist would see cytoplasmic staining, then that should be disregarded, and he would be surprised if a pathologist would interpret cytoplasmic staining as positive, but in light of the fact that the four retroconversions were, in fact, in that category, does that give you concerns that the pathologists did all, in fact, recognize that cytoplasmic staining should not be called a positive

Page 30

results?

DR. DENIC:

A. Well, this is the experience of what they have. I'm stating from the literature, which was not written by either of them in that regard, stating that you can find the issue--probably one of the issue would be because there is overstaining cytoplasmic versus nuclear, but that doesn't appear--when you look at the slide, it's not always straightforward as one would think it's black and white because even these days when they're reporting the, for example, HER2/neu, and if you just look at the statement of Dr. Gown's office, they're trained to subtract the background staining from the staining of everything else.

MS. NEWBURY:

Q. Uh-hm.

DR. DENIC:

A. So it's not always possible to clear up the background staining, which may--it depends on the date and the time that the pathologist might look at this and conclude, yes, this is positive, but eventually it's not. Could be

Page 31

tired, could be part of the day, as I said, you know, that's why we don't see a large number of these cases.

MS. NEWBURY:

Q. I think one of the physicians, I believe it was Dr. Mullen, had indicated that the problem with false positives is more likely with HER2/neu as opposed to ER, but even if factors such as a pathologist looking at a slide later in the day and being tired and not being able to distinguish cytoplasmic staining from nuclear staining, you know, those could very well have been factors between 1997 and 2005?

DR. DENIC:

A. It might have happened, and as I said, this is only four cases and if you just look what current opinion is about it, how much error rate one should allow this would have been plus/minus 5 percent because you have to give some kind of leverage in any test. So 4 percent really is below in this group that had been done. It's very low.

MS. NEWBURY:

Q. But where does the 4 percent come from? That's what I don't understand. Where did you get

Page 32

that figure?

DR. DENIC:

A. What 5 --

MS. NEWBURY:

Q. The 4 percent? Did you say 4 percent?

DR. DENIC:

A. No, no, four patients.

MS. NEWBURY:

Q. Four patients, but that's out of a total of how many positives?

DR. DENIC:

A. That we looked here, that we looked all of this cases that were picked up because there was the group of the patients, they fell in a group below 30, and I exactly don't know how many of them are below 30 cutoff. So they would have been considered positives today.

MS. NEWBURY:

Q. Uh-hm.

DR. DENIC:

A. So you can probably compare them against four as well, and you can compare them against those ones that we never retested.

MS. NEWBURY:

Q. But the ones that you haven't retested, you

Page 33

have no idea how many of those might also convert. So would it be appropriate to use that as a denominator in figuring out what your error rate is?

DR. DENIC:

A. While you say it's inappropriate, we have to recognize that some patients are treated right, and even in--we showed that even this error, some patients stayed negative as well. So while we didn't test them, you cannot just assume they were not done right either.

MS. NEWBURY:

Q. And I agree, you can't make an assumption one way or the other about that.

DR. DENIC:

A. That's correct.

MS. NEWBURY:

Q. Dr. Denic, another point on the literature and what is expected in terms of false positive, there were a number of PR results that converted to negative, and perhaps we can bring up Exhibit P-0720, please. I think the focus in the false positives has been the ER test results as opposed to the PR test results. This is--I'm not sure if you're

Page 34

familiar with this document. These are results that were provided to Mark Quinn following an access to information request, and these, I think, were compiled by quality and risk management. Just looking down at line 15, the original ER result was negative, and the PR result originally was 50 to 60, and then it converted to zero ER and 2 PR. So would that be considered--it hasn't been referred to in the group of retroconverters or false positives, but would that essentially be a false positive PR result?

DR. DENIC:

A. It's still positive as you can see. It's 2 percent, and if you recognize everything over 1 percent is positive, but --

MS. NEWBURY:

Q. Clinically is that your understanding?

DR. DENIC:

A. I don't know about this particular case. You have to look at case by case.

MS. NEWBURY:

Q. Sure.

DR. DENIC:

A. Because the clinicians were treating the cases

Page 35

even then. It depends on the case as such. Even below the cutoff, sometimes above the cutoff, sometimes a patient would be--well expressor of ER/PR, but wouldn't be treated because of the various other reasons for.

MS. NEWBURY:

Q. Uh-hm, but that is a significant reduction in the PR result?

DR. DENIC:

A. But I think since we have Dr. Dabbs here, I think he would be better to give the overview, but in my opinion, what I know about PR, PR is more volatile antibody and you're going to see a larger variability in the PR.

MS. NEWBURY:

Q. Uh-hm.

DR. DENIC:

A. While it has the clinical implications, as we know, based even on a CIQC proficiency testing, as you know, that's a new body that's been developed in Saskatchewan they're doing, you can see larger variability in the PR testing among the labs than with ER. So this is something that I wouldn't be surprised, and if you had Dr. Brendan Mullen's retests for

Page 36

these positives, you can even see that in his lab one of the tests changed from 5 to 30 on a PR. I think that must be an exhibit here. So it doesn't surprise me.

MS. NEWBURY:

Q. Okay.

DR. DENIC:

A. And still it could be a different level of section expression, and the nature of the beast is the PRE's.

MS. NEWBURY:

Q. And there are a number of these. I just wanted to show those to you briefly. Line 20 --in fact, there are two line 20s. The first line 20 was negative ER/75 PR, and that changed to 2 and zero ER/PR respectively, and on line 61--it's actually three lines 61. So the third line, there was a result of zero ER, 50/60 PR originally, and that went to zero and zero.

DR. DENIC:

A. Ms. Newbury, it doesn't surprise me.

MS. NEWBURY:

Q. It doesn't surprise you at all.

DR. DENIC:

Page 37

A. It doesn't surprise me.
MS. NEWBURY:
Q. And does it surprise you that not only did it vary, but went from a high significant PR result down to zero or very close to zero?
DR. DENIC:
A. As I stated, you can go even to the Mount Sinai's results and you can see the variability on the retests, the ER and the PR as well. So if I want to compare us with a gold standard lab, you're going to find that this kind of changes may occur in anybody's lab.
MS. NEWBURY:
Q. And it isn't so much the fact that there is variability because I can appreciate what has been said to date, but it's the fact, number one ,that there is a significant difference there; number two, it goes from a positive result down to a negative result, and does that cause you any concern about the fact that theoretically or according to the literature, false positives are not likely to occur?
DR. DENIC:
A. Because we are talking here about PR,

Page 38

obviously, as I said. PR is more volatile and not much even work up in the literature. We know there's a positivity, but even if you read the literature about the PR, they're going to tell you that it is not as well shown optimization, validation. We know there is significance of patients who are PR positive and ER positive appears to have better responded to therapy. So I cannot explain all of them because this kind of results you're going to find in labs.
MS. NEWBURY:
Q. Dr. Denic, you testified last week a little bit about the issue of tissue reprocessing, an are you aware--you were aware, I understand, that it was taking place at St. Clare's, and I'm wondering if you're aware of the step by step procedure that was used in the tissue reprocessing at St. Clare's?
DR. DENIC:
A. While I don't do tissue reprocessing, tissue reprocessing means trying to put tissue back through the same process as it went the first time, but before you do that, you have to take the paraffin off. So the paraffin has to be

Page 39

melted off and then you put the tissue through the same procedure. What reprocessing does is not re-fixation.
MS. NEWBURY:
Q. Uh-hm.
DR. DENIC:
A. Reprocessing is moving the tissue again through the solutions, such as alcohol, which takes the water out of the breast tissue because breast tissue is a fatty tissue and has high content of water. So reprocessing really harden the tissue in order to make more suitable for cutting.
MS. NEWBURY:
Q. Okay, that's the purpose for it, and I think you indicated last week as well that during the process of reprocessing, the sample would be exposed to alcohol?
DR. DENIC:
A. That is correct.
MS. NEWBURY:
Q. Part of taking the sample out of the paraffin. You've referred also to an article of Clive Taylor and Goldstein, I believe?
DR. DENIC:

Page 40

A. Yes, this is Dr. Dabbs actually ad hoc group.
MS. NEWBURY:
Q. I wonder if I could call up Exhibit P-1767, please. I just want to confirm that this is the article that you were referring to. If you want to scroll through that, you can.
DR. DENIC:
A. Okay. I think if you have the questions and answers in the back of this.
MS. NEWBURY:
Q. I believe there's a reference there on page 13.
THE COMMISSIONER:
Q. You're looking for page 13 on this exhibit?
MS. NEWBURY:
Q. Yes.
DR. DENIC:
A. Oh, I think we might have --
MS. NEWBURY:
Q. Did you want to go back a bit.
DR. DENIC:
A. I think this is--okay, I think this is Dr. Badve, and this is the part which is marked "Q", the middle column, first paragraph. He said, "Standard processing protocols entail

Page 41

the use of alcohol out of the tissues fixed in formalin. Alcohol is also fixative with a relatively rapid penetration. So if unfixed tissues are loaded onto a processor, they are more likely to undergo significant alcohol fixation which can lead to altered immunohistochemistry and false positive results".

MS. NEWBURY:

Q. Okay. So that was the reference you made last week?

DR. DENIC:

A. That's correct.

MS. NEWBURY:

Q. And so that was a concern, a potential concern that you raised regarding the reprocessing?

DR. DENIC:

A. My point is that we have 380 patients that were false negative, and if alcohol reprocessing does anything, it would be false positive results.

MS. NEWBURY:

Q. But you can't say --

DR. DENIC:

A. So that doesn't really answer 380.

Page 42

MS. NEWBURY:

Q. Right.

DR. DENIC:

A. Does it?

MS. NEWBURY:

Q. Now the tissue reprocessing wasn't taking place in all cases, though?

DR. DENIC:

A. No.

MS. NEWBURY:

Q. And there's no way to identify, as I understand it, which samples had been subject to tissue reprocessing?

DR. DENIC:

A. No.

MS. NEWBURY:

Q. Okay, and you don't know because there has been very limited testing of positive patients, you don't know if this, in fact, may have played a role in some of the positive test results?

DR. DENIC:

A. No.

MS. NEWBURY:

Q. Okay, and it was the evidence of Les Simms

Page 43

that there is evidence to support the fact that there was alcohol being exposed to the samples during the retesting. I think the evidence of Les Simms said that to get the wax out, you would soak it in xylene and rinse it in alcohol and put it back in formalin. So he's confirming your understanding that there was exposure to alcohol, the samples?

DR. DENIC:

A. The tissue was exposed to alcohol in the first place.

MS. NEWBURY:

Q. Right.

DR. DENIC:

A. With the processing as well.

MS. NEWBURY:

Q. Before it's fixed in formalin?

DR. DENIC:

A. No. You first fix the tissue in formalin, as I said, with this breast, except for the few that fell out of--of the working hours of the labs, and we know that some of tissue received in lab had less formalin in. The tissue eventually would be fixed 24 hours. So the fixation wouldn't be any more affected in

Page 44

terms of--the tissue comes fixed into the tissue processor. So the fixation is done in 24 hours. So then you move the tissue from one alcohol to another to take the water out because fixation is one process. Moving the water out of alcohol is a different process. So again if you reprocess the tissue, fixation is already done, but what he's talking here about is most likely--so if unfixed tissues are loaded onto the processor.

MS. NEWBURY:

Q. Uh-hm.

DR. DENIC:

A. So unfixed tissue, and there's testimony--actually the reports rather than testimony, because most of them I didn't listen. Fixation was taken as in account of as being responsible for the false negative results.

MS. NEWBURY:

Q. But the question, Dr. Denic, that arises from this, what if a tissue had not been properly fixed and then was later exposed to tissue reprocessing, and there is evidence--Dr. Banerjee had indicated in his first report that there appeared to be inadequate attention

Page 45

paid by grossing pathologists to the tissue thickness, quality, and adequacy of fixation, and that there was no standardized fixation protocol, and Trish Wegrynowski also commented in her report that prior to 2003, formalin fixative was prepared in house and distributed, but no documentation concerning the pH of this fixative was found. So there's some possible inadequacies here in fixation and my question is whether or not there's been any analysis or investigation of whether or not an inadequately fixed specimen, then subject to tissue reprocessing, could have led to false positive results?

DR. DENIC:

A. But what you quoted here in Dr. Banerjee's report he's talking about false negatives. He's not talking about false positives in the report as such. So from that perspective, Dr. Banerjee--Ms. Wegrynowski has said--they said just can be affected, they never stated how, and obviously the whole point was about false negative. None of them really, as I understand, and I read this so many times, I couldn't find that reference to the false

Page 46

positive results in neither of these reports, but Dr. Banerjee was talking about false negative, so if the tissue are unfixed, and the other reference that they made is Sakura, I couldn't find actually in the literature as such. This is the first-- this was written in 2007 as such, reference to the alcohol and--so they are conflicting statements really to me.

MS. NEWBURY:

Q. Dr. Banerjee, in writing his report, of course, he was involved in a review that resulted from an index patient that was ER negative, and the focus all along has been on the ER negative patients, and, you know, granted the context there would be based on that, however, Dr. Banerjee is pointing out clearly that there are problems or concerns that he had with fixation as did Ms. Wegrynowski, and my question is whether or not Eastern Health, or whether you or anyone else has considered the implications of there being inadequate fixation? It could affect the ER positive--or negative test results clearly, but has there been any consideration--we know that we've had tissue reprocessing going on,

Page 47

we know that could expose tissue samples to alcohol. We also know there are problems with fixation. Could those two combined factors for some of the cases lead to problems with false positives?

DR. DENIC:

A. I don't think so a significant problem, in my opinion, and I don't consider myself an expert in immunohistochemistry as such. I don't think so it's a significant problem, and again maybe more light is going to be shed by Dr. Dabbs because I'm still seeking the right answers too.

MS. NEWBURY:

Q. Sure.

DR. DENIC:

A. Obviously when Dr. Banerjee wrote all of this, he found that the fixation was inappropriate, and I agreed to the certain extent, and whether the fixation was the real culprit, the fixation or the processing as such, you would expect--then the tissue went to Mount Sinai, thousand tissue went to Mount Sinai, thousand tissue came back with the results from Mount Sinai.

Page 48

MS. NEWBURY:

Q. Uh-hm.

DR. DENIC:

A. So what happened--so there got to be some other factors involved in all of this, that Mount Sinai was using in 2005 that we were not using, and we know that the technology changed, we know that the protocol they were using is completely different from the protocol that we were using down there in '97, '98, '99, up to 2004 when we using the DAKO. So antigen retrieval obviously the one that somebody should look into. The other thing that I mentioned, I know that I still cannot explain cases turning from zero to hundred, and that's--since nobody has ever examined the piece of equipment, and there is the evidence in the other authorities, and even in UK that equipment could have played some of the role, I'm not saying the full role, but could have been playing some role in all of this. I think it's a complex process. I don't think so it's straightforward, and we seen even in Mount Sinai's results changing from one block. We send the one block, zero, zero, internal

Page 49

control positive, fixation adequate. Second block, ER 50, internal control positive, fixation adequate. So that makes all of this very complex to me still. That's why I'm saying I'm still seeking myself the answers about all of this.

MS. NEWBURY:

Q. But does the fact that there was tissue reprocessing going on, and the fact that there was fixation, does that not at least raise a question in your mind as to whether further testing, a larger sample size, if not all of the positive test results should be looked at, just to satisfy yourself that there is not a broader problem going on?

DR. DENIC:

A. Ms. Newbury, I need the evidence-based literature in all of this to reflect and to tell me exactly how was done, what is the evidence about this, and then we can--we can take some of the cases and we can experiment with that.

MS. NEWBURY:

Q. So you're looking for evidence-based literature to show that there is a problem?

Page 50

DR. DENIC:

A. Exactly.

MS. NEWBURY:

Q. How about looking for evidence-based literature to show that there isn't a problem? I'm wondering why you're assuming that there is no problem unless there's some evidence out there to show that there is, but perhaps there hasn't been a lot out there that had a problem with fixation at the same time that they had tissue reprocessing? Maybe there is no literature available to investigate that.

DR. DENIC:

A. I'm trying to run the laboratory on evidence-based literature and everything has to be supported. Whatever we do, we have to find that--even pathologists are now advised if you want something to be done in the lab, please come up with a paper stating that this is it, this has to be done, these are the consequences, these are the benefits, in order to do that.

MS. NEWBURY:

Q. Okay.

DR. DENIC:

Page 51

A. So --

MS. NEWBURY:

Q. And you don't see that in the circumstances, especially where there was no quality assurance or quality control, that something more than waiting for someone to provide evidence-based literature that there could be a problem, is enough to go back and do a larger sample?

DR. DENIC:

A. It's not that I don't contemplating that, Ms. Newbury. What we have done from 2005 never been done anywhere. So we went with the same thoughts, help the patients, as such. At this particular moment, as I stated, why I'm not contemplating further on, the various factors in my mind why we cannot do this at this particular--and that's why it was left to the patient as well to identify themself, to come down, we do them--if we have to do them by batch, you know, ten patients come forward as such, we do them. So this is the best--at this particular, the state that our lab is in, that we can do that. Otherwise, we have to stop all the processes, we won't be able to

Page 52

sustain a high workload and demand of the cancer--new cancer patients. So this is my answer really. I cannot shed any more light on this.

Page 53

MS. NEWBURY:
Q. So it's primarily the lack of resources, as opposed to your confidence that there isn't a problem. Is that correct?
DR. DENIC:
A. I mean, I cannot say that we won't find one or two patients as such.
MS. NEWBURY:
Q. Okay.
DR. DENIC:
A. I'm not stating that. I'm not absolutely certain, but I believe, it's my opinion. It's not even close as a problem as we had with the false negative.
MS. NEWBURY:
Q. What if it's somewhere in between a five percent error rate and the error rate that you have with the ER negatives, would that be worth--I'm just wondering where, at what point -
DR. DENIC:
A. I mean, that's purely speculation really. We are speculating what if it's a five, but what if it's a six percent? What if it's a four percent? It's still a number of patients, as such. I mean, I think I cannot speculate. I think this is not the venue to speculate. I think we should reconsider the stuff and try to do anything what's best for the patient, but it has to be somewhere, a certain way to put on the argument on the benefits, on expectations of such endeavour.
MS. NEWBURY:
Q. Thank you, Dr. Denic. Those are all the questions I have.
DR. DENIC:
A. You're welcome.
CROSBIE, Q.C.:
Q. I have two minutes worth of questions.
THE COMMISSIONER:
Q. Yes, but before you go ahead, since there are some changing of you this morning, Mr. Eaton, does your arrival indicate a change of position in respect of questions for this witness?
EATON, Q.C.:

Page 54

Q. No.
THE COMMISSIONER:
Q. Okay, Mr. Crosbie.
DR. NEBOJSA (NASH) DENIC, EXAMINATION BY CHESLEY CROSBIE, Q.C.
CROSBIE, Q.C.:
Q. Thank you, Commissioner. Dr. Denic, we've met. Ches Crosbie, I represent the members of the class action. One or two things that arose from your testimony on Friday afternoon. This business about the refrigerator and the cold section room, which is adjourning the OR. There's one now installed in St. Clare's, but not in the Health Sciences Centre. Is that right?
DR. DENIC:
A. No, both of them have refrigerators.
CROSBIE, Q.C.:
Q. Oh, they both do?
DR. DENIC:
A. Both.
CROSBIE, Q.C.:
Q. Okay, and the theory behind that is if you--ideally, you want to get the specimen down to the lab and in fixation in 15 minutes or so.

Page 55

Is that right?
DR. DENIC:
A. That's correct. You want tissue, actually tumour in particular, to be exposed within the 30 minutes.
CROSBIE, Q.C.:
Q. Sure. But if you can't do that, and occasionally it doesn't happen according to what you'd want to happen, then the ideal thing--the second best thing is to put it in the fridge, to try and slow decomposition?
DR. DENIC:
A. Mr. Crosbie, I would think so, you know, and I accepted Ms. Fergrofski's (sic) argument for that, we should have refrigerators, but when you start digging now through the literature, and I think that could have been a submission in regard to the influence of refrigeration on the tissue. One of the argument is that the tissue can even stay on the bench in formalin over weekend, and I think the other argument about the refrigeration, that refrigeration can have detrimental effect on fixation. Furthermore, to overcome this issue, what we do, we're still using the refrigeration -

Page 56

CROSBIE, Q.C.:
Q. I think we understand that, but the second best is to put it in the fridge, isn't it? Isn't that the reason for the recommendation? If you can't get it down and get it fixed?
THE COMMISSIONER:
Q. But isn't it in formalin in the refrigerator?
DR. DENIC:
A. Then in formalin. They have to be in formalin, and then if you put it in a refrigerator now and expose to the low temperature, penetration and fixation process is going to be retarded. It's going to be delayed, and at least that's the answer that CAP, which is College of American Pathologists, based on a QNA, said about it. But, to go furthermore, what we felt, and that's why we have pathology assistants, they're monitoring what's happening in the OR.
CROSBIE, Q.C.:
Q. We needn't dwell on it. I guess you're point is that you accepted the recommendation and you work with the fridge as a current best practice, I suppose.
DR. DENIC:

Page 57

A. That's correct, but we are still revisiting stuff and we're going to do some consults as such and then maybe that's one of the things that I'm going to be after Dr. Dabbs -
CROSBIE, Q.C.:
Q. I'm sure, and that's -
DR. DENIC:
A. - has the money to talk about it.
CROSBIE, Q.C.:
Q. That's all part of ongoing quality assurance.
DR. DENIC:
A. That's correct.
CROSBIE, Q.C.:
Q. My other question has to do with this issue of machine error. You have training in forensic pathology, I believe.
DR. DENIC:
A. I do.
CROSBIE, Q.C.:
Q. And you're familiar with the process of weighing evidence, I guess?
DR. DENIC:
A. That's correct.
CROSBIE, Q.C.:
Q. I think you mentioned on Friday, the

Page 58

possibility of clogging in the machine?
DR. DENIC:
A. That was my understanding from UK's experience.
CROSBIE, Q.C.:
Q. Have you read the 40, the 54-page DAKO troubleshooting manual to see if clogging is a recognized problem with the machine?
DR. DENIC:
A. No, I didn't.
CROSBIE, Q.C.:
Q. Have you inquired with the manufacturer to see if clogging is recognized as a machine failure issue?
DR. DENIC:
A. No, I didn't. This is the knowledge that I acquired recently.
CROSBIE, Q.C.:
Q. Did you review the maintenance records for the Eastern Health machine?
DR. DENIC:
A. No.
CROSBIE, Q.C.:
Q. And why is that?
DR. DENIC:

Page 59

A. The first--this is not something that I was in charge with.
CROSBIE, Q.C.:
Q. May I suggest there are no such records?
DR. DENIC:
A. I don't know what's in the -
CROSBIE, Q.C.:
Q. To the best of my knowledge, we have not found any such records in maintenance of machines, so that could be a reason why no one could review them.
DR. DENIC:
A. It's possible.
CROSBIE, Q.C.:
Q. You don't know anything to the contrary?
DR. DENIC:
A. It's possible. I heard that there's a lack of documentation and that would be the part of it. I agree with you.
CROSBIE, Q.C.:
Q. And you weren't able to perform a forensic examination of the DAKO autostainer in question, were you?
DR. DENIC:
A. That's correct.

Page 60

CROSBIE, Q.C.:
Q. And that's because Eastern Health disposed of it before you could do that?
DR. DENIC:
A. That's my understanding, and I was inquiring myself to track down where the instrument went, because I think before we put this puzzle together, you need all pieces, and one of the piece was missing and I was told that nobody knows where this machine went and what happened.
CROSBIE, Q.C.:
Q. And the reason the piece of the puzzle is missing is that Eastern Health disposed of it.
DR. DENIC:
A. I really don't know who disposed of it, but Eastern Health, did the managers do anything, lab director, somebody did it.
CROSBIE, Q.C.:
Q. Well, maybe we'll find out when Mr. Gulliver comes in. Thanks very much.
DR. DENIC:
A. You're welcome.
THE COMMISSIONER:
Q. Mr. Pike?

Page 61

MR. PIKE:
Q. No questions. Thank you very much.
THE COMMISSIONER:
Q. Mr. Browne?
DR. NEBOJSA (NASH) DENIC, EXAMINATION BY MR. PETER BROWNE
MR. BROWNE:
Q. Thank you, Commissioner. Good morning, Dr. Denic.
DR. DENIC:
A. Good morning.
MR. BROWNE:
Q. A few areas I wish to cover with you, and I'd ask you to cast your mind back to, I think, the first day unfortunately, you gave evidence here last week, and you were asked a question by Mr. Coffey about your exposure to immunohistochemistry during your residency and my question is, specifically, in terms of your understanding of who should understand about the test and how the test was performed, what was your understanding? Where did that knowledge base lie?
DR. DENIC:
A. Pathologists, they don't perform the test, and if you put me in the lab down there, I

Page 62

wouldn't know how to do it. You know, I know the basics, you know, you apply the antibodies, apply secondary antibodies. So these are basics of all the tests as such, but I wouldn't know how to perform.
MR. BROWNE:
Q. What is your understanding, who--what people or persons would have had that understanding?
DR. DENIC:
A. The technologists.
MR. BROWNE:
Q. Now we've heard about fixation problems and these have been mentioned by yourself, in terms of questioning from Commission counsel and others. Dr. Banerjee spoke to this. Dr. Mullen spoke to this, as well as others. Today, Doctor, if you noticed a problem with fixation of a particular tissue specimen, what would, if any, corrective action would you take?
DR. DENIC:
A. Today, we have check and balances and quality controls and assurance on every step, including pre-analytical, analytical and post analytical phase of this. So, on our

Page 63

requisition, the time when the tissue was put in the formalin is recorded and that time comes directly from the OR. So we can identify the time when the tissue is exposed to the formalin. We know the time when the tissue comes to the lab because the book has been signed and receive of the specimen is done because--and then it's been attended immediately by the pathology assistants who are on call all the time. Then the tissue, we know that the tissue, by protocol, is left in the formalin for 24 hours, at least, to be fixed. Been sliced at that time and left in the formalin to be fixed, so that's all recorded and we exactly know the time when the tissue was sampled, then we know the time when the tissue is submitted to the tissue processor. This is all pre-analytical phases, and the tissue processor, we know the times when the solutions were changed as well. So if anything can go wrong, you can go back and see when the solutions were changed. We even have the templates, because the various containers of the solutions sometimes can be confusing, there's even a picture of the

Page 64

tissue processor showing you the set up of the container, so that you can always cross check that. So we exactly know when processor starts, because by default it's always 6:00. So you know the time in the processor. You know the time how long tissue stays in the formalin, because the first chamber is still formalin and you can add that time to the time of fixation from the OR. Then we know the people who are cutting the tissue.

MR. BROWNE:

Q. So Doctor, just to--I guess, the focus of my question perhaps is a little bit more pointed. There are a number of steps that you have in place and where you can now go back and target if there's a particular problem which led to that fixation that you identified? Is that -

DR. DENIC:

A. Exactly. We can go to the root cause analysis with no time and check it out every single station.

MR. BROWNE:

Q. And that's through the policies that you have in place?

DR. DENIC:

Page 65

A. That's correct.

MR. BROWNE:

Q. In terms of the timing and so on, that allows you to pinpoint a particular problem, if you were to go look back to see where it may be, as you say, the root cause?

DR. DENIC:

A. That's correct.

MR. BROWNE:

Q. You were asked about educational memos and you referenced Dr. Khalifa's memo in 1998, Dr. Ejeckam's memo in May 2003. Going back to Dr. Khalifa, when you were--I think you were around during his time, you were doing your residency, do you recall Dr. Khalifa distributing any documentation surrounding synoptic reporting?

DR. DENIC:

A. Yes, he was actively working on synoptic reporting of various tumour sites, so he made, I think, he almost addressed all tumour sites.

MR. BROWNE:

Q. And he had documents that he distributed with that?

DR. DENIC:

Page 66

A. That's right, and they've been printed at Health Sciences at that time and distributed to the attending physicians, pathologists. So we all had that on our desk, so that we can fill up the synoptic reports.

MR. BROWNE:

Q. And the purpose of that was to move away from then the inconsistent narrative reporting to a detailed checklist that would have helped clinicians, oncologists, on the other end?

DR. DENIC:

A. That's correct, and these are CAP based practices.

MR. BROWNE:

Q. You were also asked about Dr. Fontaine's--there was a--you were shown an exhibit which had a meeting note where Dr. Fontaine suggested to his colleagues at the Health Sciences about availing of Dr. Carter's services for rereads of ER/PR. Was that type of approach necessary at St. Clare's, given that Dr. Carter shared the same offices?

DR. DENIC:

A. You didn't need any memo. I mean, Dr. Carter was there and we utilized her services

Page 67

whenever we could, you know, especially for the breast specimens.

MR. BROWNE:

Q. Okay. Now there are a number of articles, Doctor, that I just want to reference and I should enter right now. You had mentioned in your testimony last week a couple of Ackerman's, Rosai Ackerman's textbooks where you cited about the--I think a particular passage concerning, I guess, the relationship between--statistical relationship between ductal and lobular and I think you also mentioned Tavassoli. So perhaps we can, Registrar, enter those two exhibits, beginning first with P-2623 and 2624.

THE COMMISSIONER:

Q. 2623 and 2624? There are others as well, are there not?

MR. BROWNE:

Q. Yes, and perhaps why don't we just enter them all at this point, Commissioner. The next is the Tavassoli reference, which is P-2625, and the Royal College of Pathologists Recognition Roles of Specialists, Pathologists, P-2626. Requirements of a Specialist Breast Unit, P-

Page 68

2627, and then lastly, the UK NEQAS Journal, which is P-2628.

THE COMMISSIONER:

Q. All right, entered.

EXHIBITS ENTERED AND MARKED P-2623 THROUGH P-2628

MR. BROWNE:

Q. Thank you, Commissioner. Doctor, going back to the first of these exhibits, P-2623, and we can probably deal with 2623 and 2624 as a package, and I'll--one is, I think, if we look at--I'll follow down here. This is the 2004 version, it seems, and I think the other exhibit is a previous, an earlier version of that textbook, looks to be 1996. Nevertheless, Doctor, I think the passage remains the same in both. Is that, from your--and the passage you were referencing, if we could just look at it, is this one right here? It's under the section "Hormone Receptors." It's the second paragraph, and in terms of giving your evidence, the other day, you were referencing the comment "not much correlation exists between the cyto architectural type of breast cancer carcinoma and the presence of hormone receptor protein. Specifically no

Page 69

statistically significant difference has been found between ductal type and lobular type tumours." Is that the reference you were referring to?

DR. DENIC:

A. That's correct.

MR. BROWNE:

Q. Okay, and then, but nevertheless, you did qualify that later on by saying, look, there was Tavassoli which actually did give a reference, and Registrar, if we can have 2625? And again, this is under the section "Hormone Receptors" and is that ploidy?

DR. DENIC:

A. Yeah, ploidy.

MR. BROWNE:

Q. Just as a matter of curiosity, what is ploidy?

DR. DENIC:

A. This is a chromosomal abnormality. It's an abnormal number of chromosomes.

MR. BROWNE:

Q. Okay, and the reference again, Doctor, that you gave in evidence is at the first line. It says "about 70 to 92 percent of lobular carcinomas are ER positive."

Page 70

DR. DENIC:

A. That's correct.

MR. BROWNE:

Q. So the purpose of these articles, Doctor, is just to confirm the source of your evidence the other day?

DR. DENIC:

A. That's correct.

MR. BROWNE:

Q. Now there are a couple of other articles that you've asked to be entered, Doctor, and I'll begin with the first of these. Registrar, can we look at--and I think some of these come out of requests that Mr. Coffey made to you, I think, on Friday. Is that correct?

DR. DENIC:

A. That's correct.

MR. BROWNE:

Q. Okay. The first of these, Doctor, is P-2626, and this is from the United Kingdom. It's from the Royal College of Pathologists, and it talks about the recognition and roles of specialist cellular pathologists. Now is this from Dr. Makretzov?

DR. DENIC:

Page 71

A. No, this is something, that was a document that was shown to me by Mr. Coffey when explaining the benefit of subspecializing to pathologists, and some of their recommendation I took out. At the time I quoted, I thought that this article is really coming from the article from the Board of Directors in Anatomical Pathology, but it's rather the Royal College of -

MR. BROWNE:

Q. Okay, and -

DR. DENIC:

A. I think this is UK, Royal College.

MR. BROWNE:

Q. - the passage you're looking at, I think it may be at page four, and that's under Section 5, the attributes of a specialist pathologist. Is that the passage you were referencing there?

DR. DENIC:

A. That's correct.

MR. BROWNE:

Q. Okay.

DR. DENIC:

A. It could have been slight revisions, but this

Page 72

is the gist of it.
MR. BROWNE:
Q. The next document, Doctor, is P-2627, Registrar. And again, this is entitled the requirements of a specialist breast unit. Doctor, this is something Dr. Makretzov brought to your attention about the UK experience?
DR. DENIC:
A. That's correct. This is a position paper and they are working towards creation of the specialist breast unit.
MR. BROWNE:
Q. And this is, I think, so we're clear on this point, this was the notion of having like stations where the patient can go see a pathologist, oncologist, radiologist, all in sort of a one clear area?
DR. DENIC:
A. That's right. One unit or to be close by, so they could be available to the patient.
MR. BROWNE:
Q. Right, for immediate consultation?
DR. DENIC:
A. That's right, so that the people work together

Page 73

on the same patient really.
MR. BROWNE:
Q. And then lastly, Doctor, there is, Registrar, P-2628, and this is from the UK NEQAS publications and this is 154. There's a comment section at page 12. I'll just bring it to your attention now, Doctor, which is highlighted there, and it says, "as a very high ER positive case is likely to stain strongly, even when the sensitivity of the assay is relatively low. Similarly, due to the limited number of nuclei frequently staining in normal glands, it is difficult to detect changes in day-to-day assay sensitivity by relying totally on this material for quality control." There was a point, I think, you wanted to draw out to the Commissioner's attention from this commentary section?
DR. DENIC:
A. I mean, I think I stated, and that's why I was puzzled with some of these cases turning from zero to 100, you know, and it is quite clear that any kind of really of test, as they state here, with the low sensitivity test, while low sensitivity test can tell that probably the

Page 74

test wasn't optimized well. So that, so even then, some nuclear staining would have been positive, and in some of the cases that we've seen, they change from zero to 100. So even thinking that the test wasn't validated, the test wasn't optimized, you would think that applying at that time would pick up any type of staining in the nuclei, but it didn't. So that's why I also stated it could be something more. Is it something that the antibody never even touched the tissue.
MR. BROWNE:
Q. So you raise this as a possible contributing factor?
DR. DENIC:
A. That's right. Although there's another point there, although they're talking in regards of the controls here, if you can see they're talking about staining in normal glands, frequency of staining in normal glands. Normal glands we see as internal control as well. While they're probably referring, not quite certain, to the external control using normal glands, they tell you that the limited number of nuclei frequently staining in normal

Page 75

glands, it is difficult to detect changes in day-to-day assay sensitivity by relying totally on the material for the quality control. So while we recognize value of internal control today more than we did and we're putting stuff, that's why we still seeing the cases where internal control not going to be positive, but the stain's going to work based where the stain is present. If a stain is present in the nuclei and you have external control which is well done, then you know that the test works, and these are the experiences as well that you can pull out from Dr. Mullen's list that he also reported the cases where internal control was positive, but not staining.
MR. BROWNE:
Q. Now Doctor, Ms. Newbury asked you about an exhibit that was previously entered under Dr. Pritzker and that's P-1767. I just want to reference that. That's the recommendations from approved standardization of immunohistochemistry, and attached to that was another exhibit, P-1727, where these individuals, some of these individuals, Dr.

Page 76

Taylor, Dr. Hewitt, among some of the committee members, were asked several questions, and I'm going to ask you to--I think there's a particular section, page seven, that you want to offer or point out to the Commissioner. I think it's this one right here. It's the question "despite popularity of antigen retrieval AR techniques, the precise molecular mechanism underlying the process remains an enigmatic, what is the most AR technique?" and there's a couple comments there I think you wanted to draw out and potentially comment on?

DR. DENIC:

A. Where I see the complexity in all of this, and again, I'm just looking at this as a practising pathologist and again, I'm not the expert in immunohistochemistry, but the complexity and why that things are going as they're going, not only in our lab, but worldwide is as well, as it states here about antigen retrieval, which is the one of crucial part as well of this process. It states here that in three of them, even four, says--Dr. Taylor says about antigen retrieval, "don't

Page 77

think anyone is most understood." Dr. Yaziji said, like you said, "they are mostly speculations without solid evidence. The cross linking hypothesis is most popular one, but again, without solid proof." Dr. Hewitt said "it is certainly shrouded in mystery. I view it as a simple heat time and pressure, go back to physical chemistry." And Dr. Badve said "akin to fixation, the processes associated with antigen retrievals are poorly understood. All of the changes seems to be different for the various protein. The overall process seems to be relatively specific for the given protein." So we're applying pretty much science that we really don't know how it works.

MR. BROWNE:

Q. And you're referring both to the antigen retrieval and the fixation?

DR. DENIC:

A. Fixation. Actually, that's what they are referring to, and that's, to me, as a practising pathologist, again taking -

MR. BROWNE:

Q. An interesting observation?

Page 78

DR. DENIC:

A. Yes. Probably that's why we are manipulating to a certain degree, exposing to various temperatures, trying to standardize fixation, that we all control certain parameters in order to get certain colour. But we really don't know how that works on the tissue.

MR. BROWNE:

Q. Just following on that point, Dr. Denic, you were asked by Mr. Coffey, I'm not sure if it was Thursday or Friday, your reasons for test failure and you indicated there were no single factors. You recognized fixation. You recognized antigen retrieval. You recognized the possibility of the machine itself, and I think you made a statement, something to the effect, I made a note here, "fixation, to my opinion, not to a great extent" and you wanted to explain that, I think, but you didn't come back to it. Is there--do you want to explain that further now?

DR. DENIC:

A. I think I did touch bases with Ms. Newbury when she was asking about it. If fixation is the main culprit as was portrayed, fixation

Page 79

processing, then Mount Sinai wouldn't report them. You wouldn't think that results would come down reported. If the tissue is not readable, if the tissue is badly affected, I wouldn't report it.

MR. BROWNE:

Q. So that's the point you were trying to make on that?

DR. DENIC:

A. That's right. If I identify fixation, while I believe it did play a role, I seen the cases that were not well fixed, I seen those ones, but the question is obviously due to the various techniques they were using, they were satisfied that obtained results are real results and they can report on them.

MR. BROWNE:

Q. Registrar, P-1373, please? Doctor, I just want to ask you a question surrounding the retroconverters and the false positives. Ms. Newbury had a number of questions with respect to that. This is an e-mail from Ms. Predham to you and it says--and others. It says "Hi, Kara and I reviewed the retro list and here is the final list that will need to be reviewed

Page 80

at the panel next Thursday, given the results from Mount Sinai." We'll see here, Doctor, these are the numbers. Was this, just have a look at this, the situation here, is this one where these were patients where 30 percent or less became an issue and this question of coming under 30 percent and then one of these was, in fact, a weakly positive case. So is this sort of an interpretation issue, in terms of the application of the 30 percent?

DR. DENIC:

A. When I reviewed the slides, there were interpretation issues.

MR. BROWNE:

Q. Right. But in terms of the numbers here, are these all cases where 30 percent or less?

DR. DENIC:

A. Some of them were already, yes, 25 to 30, that's why that case came into the equation, and the other one was 30. There's one from Carbonear that were negative, negative from Gander, and from Gander, ten percent was a case, and from Western, we have a weak positive. So that was put as such, because without explanation what a weak positive

Page 81

means.

MR. BROWNE:

Q. Right. So it's a question of this 30 percent as opposed to, you know, an actual conversion because of potentially fixation issues or -

DR. DENIC:

A. That's right, that's right, because fixation was adequate in most, except of two cases.

MR. BROWNE:

Q. Doctor, you were shown an exhibit by Mr. Coffey and asked about your discussions. I think the particular exhibit involved a meeting you had with a husband of a deceased patient and what you recalled around that. I understand you've subsequently had a look at that slide of that particular patient and again, looking at the issues of fixation, was fixation an issue for that particular patient?

DR. DENIC:

A. No.

MR. BROWNE:

Q. Was internal control an issue, internal control as a an issue for that patient?

DR. DENIC:

A. Internal control was negative, but the tissue

Page 82

was staining positive, and fixation wasn't even recognized with Dr. Mullen as well. He put that the fixation was adequate. So she falls in the group of these patients that internal control could be negative, but a tumour is well positive and shown nuclear staining positivity, variability of the staining, so which tells me that the test did work and subsequently we know that it was retested and showed positivity. So she was positive then. She was positive now.

MR. BROWNE:

Q. And what would then be your opinion as to the likely reason behind the change in the result?

DR. DENIC:

A. The reason behind the change in her results is first, that it was different technology that the tissue had been done at Mount Sinai in 2005, in this case from '98, for different antibodies, different procedures, and also this is not the same level that the tissue was sectioned from. So I have the block here, just I don't know that Commissioner ever seen the block. It looks--you did see the block?

THE COMMISSIONER:

Page 83

Q. I have seen one.

DR. DENIC:

A. Okay, so this is the block where the tissue is embedded in paraffin. So what you see here, in the middle, is the piece of tissue embedded in paraffin. So when they're putting on a machine for cutting, they strip a surface with razor sharp blade and they take five micron of it. So not necessarily if when they are stripping, they're taking the ribbons off this and they're putting on a glass slide and produce the glass slides and so the Mount Sinai never got the surface that we got read, so that their section would be somewhere underneath. So it's not even the same section. So different procedures, different depth of the block. It could be some variability of the staining, and again, you have inter observer variability, so that pathologist said 10-15.

MR. BROWNE:

Q. So those would be the reasons you would see?

DR. DENIC:

A. Those would be the reasons as well.

MR. BROWNE:

Page 84

Q. Okay. Doctor, I want to--one last area I want to just cover with you. The Commissioner spent some time, I think, with you asking about lines of communications and today, Doctor, I just want to--and perhaps you'd give an example of each. Do you view that there are good lines of communication that exist between pathologists and technologists?

DR. DENIC:

A. I really think that good lines of communication exist. We work one on one more frequently, and we have worked one on one, you know, we were always at their disposal and it was just a telephone call away. We meet each other to these various rounds, such as breast disease site group, lymphoma tumour, where they will meet with the hematological oncologist as well.

MR. BROWNE:

Q. Now are you referring to technologists or oncologists?

DR. DENIC:

A. Oncologists.

MR. BROWNE:

Q. Oncologists, okay.

Page 85

DR. DENIC:

A. Then the other rounds, tumour rounds, chest tumour board, urology oncology rounds, and on top of this, when we have somebody coming for the workshop, we invite oncologists too. So the last time, in June, I already mentioned Dr. Srigley was invited. I invited Dr. Srigley to come and do the workshop for the group that does uropathology and we invited oncologists too. So they have attended from the oncology group.

MR. BROWNE:

Q. What about -

DR. DENIC:

A. And we were working together as a part of NAP, which is Newfoundland Association of Pathologists, on synoptic reporting.

MR. BROWNE:

Q. Okay, and that's with the oncologists as well, to try to develop a template for synoptic reporting?

DR. DENIC:

A. That's right.

MR. BROWNE:

Q. What about between pathologists and technologists, Doctor, again, your views on this?

DR. DENIC:

A. This is something, this is evolving. We start inviting them on our rounds, especially on our Friday rounds, which are pathology updates, and they are also presenters on those rounds. So the pathologists come and they present to the pathologists, especially the experiences they acquired for the meeting they attended recently, and other way around. So they are attending. Those rounds are attended by technologists, mostly from immunohistochemistry and pathology assistants.

MR. BROWNE:

Q. And lastly, Doctor, between pathologists?

DR. DENIC:

A. Between pathologists, of course, we are meeting, as we said, on our monthly meetings where every single issue of interest can be discussed. Pathologists, as a group, they are still meeting separately, pathologists from Health Sciences and pathologists from St. Clare's, but one of the reasons that I would like to have all the pathologists on the same

Page 86

Page 87

site is that we're going to be physically closer and going to be easier to organize all of this. So this is pretty much -

MR. BROWNE:

Q. Doctor, that's it in terms of the formal part of my questioning of you. As other witnesses have been given the opportunity, I'd offer you the opportunity to speak to the Commissioner on any comments, observations, or recommendations you may wish to make at this time.

DR. DENIC:

A. I brought -

MR. BROWNE:

Q. You have a statement, I understand.

DR. DENIC:

A. Right, I have a statement. I want to thank the Commission for the opportunity to participate in this important process, thank you. We have heard a lot about the issues affecting the laboratory medicine program during the course of this Inquiry and you have already heard from many of my colleagues. I want to echo the sentiments of my colleagues by saying that I and all the other

Page 88

pathologists care deeply about patient care. The issues around the problems with ER/PR testing had had a profound impact in raising public awareness of the importance of pathology to patient care. More importantly, I believe Government now appreciates the necessity to ensure that the laboratory medicine program is properly resourced and funded to ensure the circumstances which brought us to this point are never repeated.

When my colleagues became aware of problems with hormone receptor testing in 2005, their first thoughts were for our patients. The decision to undertake a retrospective review of ER/PR tests was necessary, but at the same time, unprecedented, certainly in Canada, and I believe unprecedented worldwide. We know about these problems today because physicians in our lab and in the Cancer Centre put patients first by going through this important and necessary process. Our only goal in launching the review was to improve patient care. Today, I believe we are playing an important role in moving toward the need for

Page 89

standardization of ER/PR and thereby reducing the potential for error. The complexity and scope of the issues around ER/PR testing problem are significant, as I am aware all realize through the process of this Inquiry.

It is important to know that the review encompassed the work of some 50 pathologists representing a broad cross section of our specialty North America wide, at least. When you review the literature, you realize that this is a worldwide problem. I hope that findings and recommendations from the Commission will have implications for labs around the globe who are paying close attention to this Inquiry. I am please with the steps and accomplishments that we have taken in the aftermath of discovering the problem with ER/PR. In my view, our lab has risen from the ashes.

I would also like to say that while there were deficiencies identified with ER/PR, our labs perform 11 million tests each year, tests that can be trusted.

Our goal now is to make our laboratory medicine program one of the best in Canada.

Page 90

As physicians our number one priority will always remain to ensure our patients receive the best possible care. That sometimes can prove to be frustrating when you feel constrained by a system that lack resources to do this important work. As we attempt to address the problems in our health care system, it is vital that we don't let laboratory medicine fall off the radar. We must ensure that we have the resources necessary for proper operation of our labs.

The laboratory leadership and Eastern Health are actively working to strengthen our program. We are also working to assist laboratory medicine problems in the other health regions to strengthen their programs. I know it will take time to accomplish our goals. It will also take resources and manpower to address any weaknesses in the system. Furthermore, I think these efforts can be strengthened by ensuring that certification and accreditation of laboratory services in Canada are pursued through appropriate provincial and national bodies.

I would like to conclude by saying that

Page 91

today we are dealing with the consequences of the ER/PR, but not the cause. I hope that the Commission will address these causes, starting with the lack of funding, under staffing, under resourcing for the laboratory medicine program and the lack of standardization of immunohistochemistry testing.

To our patients and their families, I would like to express my deepest sympathies for the pain that this caused them. We are committed to ensure that this never happens again and we are dedicated to ongoing care. Thank you.

MR. BROWNE:

Q. Thank you, Commissioner.

THE COMMISSIONER:

Q. Mr. Coffey, is there anything arising?

COFFEY, Q.C.:

Q. Yes, Commissioner, just a--things.

DR. NEBOJSA (NASH) DENIC RE-EXAMINATION BY BERNARD COFFEY, Q.C.

COFFEY, Q.C.:

Q. Doctor, Mr. Browne asked you about Dr. Khalifa's synoptic reporting guidelines back when he was here and introduced them here. In

Page 92

your experience was there anybody monitoring whether pathologists were actually following those?

DR. DENIC:

A. Not that I know of, but now we do.

COFFEY, Q.C.:

Q. Yeah. And, Doctor, you did, I believe, in answering the question from Mr. Simmons, make a reference to yourself and Dr. Carter having looked at all of the slides relating to the deceased patients?

DR. DENIC:

A. That's correct.

COFFEY, Q.C.:

Q. Did you and Dr. Carter, or Dr. Carter make any notes on those observations at the time?

DR. DENIC:

A. What we did, Mr. Coffey, is we had printouts of the patients' reports, and on the printouts I marked which block and I marked internal control present, staining, that kind of stuff, but not any general notes but those ones.

COFFEY, Q.C.:

Q. And you were, at that point, in making these notes, were noting your observations of the

Page 93

original slides?

DR. DENIC:

A. In terms of internal controls.

COFFEY, Q.C.:

Q. Yes. So -

DR. DENIC:

A. Yes, I did.

COFFEY, Q.C.:

Q. And do you know if they have been passed on, like, a photocopy of those sheets, do they -

DR. DENIC:

A. They're not sheets, they are just -

COFFEY, Q.C.:

Q. The pathology reports, yes.

DR. DENIC:

A. - the report itself.

COFFEY, Q.C.:

Q. Do the pathology reports that you wrote on still exist?

DR. DENIC:

A. They do.

COFFEY, Q.C.:

Q. And could I ask if that hasn't been done, that they be passed on to Mr. Browne?

DR. DENIC:

Page 94

A. Certainly.

COFFEY, Q.C.:

Q. A copy, okay, be passed on to the Commission, thank you. Doctor, as well, you in answering a question for Mr. Simmons you referred in the context of interlab variability, you were just talking, you were being asked about that, you referred to low expressors are known to be problematic in studies of interlab variability. In that context a low expressor is what percentage range?

DR. DENIC:

A. From one to ten.

COFFEY, Q.C.:

Q. One to ten, thank you. Clarify that. And, Doctor, there was--Ms. Newbury asked you about this whole issue about false positives, okay. So I just want to clarify something for the Commissioner here. The four retroconverters, okay, that were finally judged to be retroconverters, do I understand you correctly that it was your view or understanding that ultimately this was attributable to a mistaken interpretation of cytoplasmic staining for nuclei staining?

Page 95

DR. DENIC:

A. That's right. And some background staining.

COFFEY, Q.C.:

Q. Background staining.

DR. DENIC:

A. Yes.

COFFEY, Q.C.:

Q. Doctor, in relation to that, then, you arrived at that view or conclusion based upon an examination of the original slides for those four patients?

DR. DENIC:

A. That's correct.

COFFEY, Q.C.:

Q. So to your knowledge in this regard, okay, in this entire review to date, are you aware of any instance of nuclei staining that was false?

DR. DENIC:

A. No.

COFFEY, Q.C.:

Q. Okay. Just to be clear on that. And in the context of false positive in its pure sense, that would involve, in fact, a nuclei stain inappropriately, wouldn't it, a false

Page 96

positive? It could be a misinterpretation issue, but as well, a false positive in a sense of a nuclei has stained and it shouldn't have, you haven't seen a case of that here, have you?

DR. DENIC:

A. What I have seen in those cases is like you see it's almost like a blob of the antibodies covering this stuff, so then you know that's not just nucleus protruding from the background stain, no, this is -

COFFEY, Q.C.:

Q. Every, everything is staining?

DR. DENIC:

A. Just a smear.

COFFEY, Q.C.:

Q. In effect, everything is -

DR. DENIC:

A. And this came like partially like a blob.

COFFEY, Q.C.:

Q. Sure.

DR. DENIC:

A. And that's why I say that could have been misinterpreted.

COFFEY, Q.C.:

Page 97

Q. So, Doctor, in terms of this issue of false positives, okay, that Ms. Newbury has been asking you about, if someone was to identify all the positive patients, I think the figure you used, there'd be about 1500, roughly?

DR. DENIC:

A. Roughly.

COFFEY, Q.C.:

Q. Rough. And that would be the ER positive patients, 1500 of them. And if someone was to identify them and was to locate all of their original slides, then one could have a pathologist or pathologists simply review the 1500 slides, ER, 1500 PR and determine whether in those pathologists' view there was nuclei staining not attributable to background staining?

DR. DENIC:

A. They could.

COFFEY, Q.C.:

Q. Okay, could do that. Doctor, just again so the Commissioner understands or appreciates this, in a typical case how long does it actually take you to view an ER slide? You have the slide right here, and how long does

Page 98

it physically actually take you to do it?

DR. DENIC:

A. Again, depends on the slide to slide.

COFFEY, Q.C.:

Q. Yes, and that -

DR. DENIC:

A. And intensity of the staining, because as you heard, intensity of the staining could be very weak.

COFFEY, Q.C.:

Q. Yes.

DR. DENIC:

A. And still positive. So not necessarily have to be very bright in order--and this is experience if you seen from our new staining and as I reported on that particular case from Mount Sinai where the discrepancy, so every lab has a weak staining. So those cases require more time and thorough review, especially--and also you have to track down for the internal controls.

COFFEY, Q.C.:

Q. Yes.

DR. DENIC:

A. You know, so it might take you ten minutes per

Page 99

slide. And also to appreciate them, sometimes you need a second pair of eyes just to see what would be the percentage that somebody would call at the time.

COFFEY, Q.C.:

Q. So it would be ten minutes per slide or per pair of slides? And again, I appreciate, the Commissioner will -

DR. DENIC:

A. If the slides are--if the slides are pre-stained and you have a bright slide, it could take a couple of minutes.

COFFEY, Q.C.:

Q. Okay, so -

DR. DENIC:

A. You know, but depends on the intensity and you never know what you're going to find, okay, and how long you're going to spend on each slide.

COFFEY, Q.C.:

Q. And again, I want the Commissioner to have some sense of if the slides were all kind of stacked up in order for the pathologist or a group of pathologists, then one could go through them, some would be straightforward,

Page 100

take a minute or two minutes, and some you're saying could take ten or 15 or 20 minutes?

DR. DENIC:

A. Yes, but you have to put in equation what would be the comfortable level of somebody, how many slides per day should they read.

COFFEY, Q.C.:

Q. Yes, okay.

DR. DENIC:

A. Because it's very tiring, exhausting process looking for the nuclear staining and searching for the internal controls and making the assumptions of the percentage.

COFFEY, Q.C.:

Q. Has there been any estimate performed as to how much time might be required to do this, to look at the original slides, original positives?

DR. DENIC:

A. Not that I know of.

COFFEY, Q.C.:

Q. Thank you. Doctor, there are my questions. Thank you, Commissioner.

DR. NEBOJSA (NASH) DENIC, EXAMINATION BY THE COMMISSIONER

THE COMMISSIONER:

Page 101

Q. Dr. Denic, there were just a couple of points that I hope you can clarify for me. Mr. Simmons asked you a question on Friday and you've referred to it again and that's the matter of the intensity of the stain not being a question when you're trying to determine whether the percentages within the nuclei, is that correct?

DR. DENIC:

A. That's correct. What the pathologists, some of the pathologists use so called Allred Score.

THE COMMISSIONER:

Q. Um-hm.

DR. DENIC:

A. In Allred Score the score reflects to the intensity of the stain and the number of nuclei. And that has been utilized in some of the centres. And however, there's various schools these days and some people utilize intensity of the stains only if the case goes in a group of week expressors, from one to ten that you can say, okay, weak, five percent. But if you have a higher percentage of the cells, the intensity of the stains doesn't

Page 102

play a role. And I learned that from Dr. Carter, as well, and she would never report the intensity of the staining. And again, to our experiences seeing the slides and the reports coming in from Mount Sinai, the weak is taken off of the list, as such. Because one should wonder what intensity of the stain going to tell to the oncologist, what does it, weak 30 means for the oncologists.

THE COMMISSIONER:

Q. Okay.

DR. DENIC:

A. Is it positive or negative, are you claiming this patient is positive or negative. So while still again some of the people are using Allred Score, I think it's the various schools reflecting on this issue.

THE COMMISSIONER:

Q. What about intensity of the stain for controls, is that a question that should be examined?

DR. DENIC:

A. Intensity of the controls, we are using actually as external control we are using three types, one good expressor, intermediate

Page 103

expressor and low expressor or no expressors. I haven't read the meaning if your stain is moderately weak, what does that mean. You're trying to use your best expressor, best expressor you would expect intensity of the staining to be very good, should be brown.

THE COMMISSIONER:

Q. And would the normally the external control be in that classification?

DR. DENIC:

A. That's right.

THE COMMISSIONER:

Q. Okay.

DR. DENIC:

A. And about internal control, as such, I really don't know what does that mean. If you see a weak positivity in internal control and you see your positivity in your tumour, I think it's very safe to conclude that the tumour is expressor of estrogen receptors regardless of the intensity. These are only my experience that I seen working with Dr. Carter at that time.

THE COMMISSIONER:

Q. Okay.

Page 104

DR. DENIC:
A. And I don't know anything else.
THE COMMISSIONER:
Q. Now, you've mentioned, switching off to another topic now, and that is that the development of the new policies and procedures within the lab. And one of the things that I took from your evidence last week was that has been valuable in this process is that it has caused you, in effect, to review what you're doing and to concentrate on the details of what you're doing. Can you tell me whether or not the existence of or the development of policies and procedures has been beneficial or otherwise in other respects?
DR. DENIC:
A. Oh, definitely it was beneficial. You have your guidelines. You know, without guidelines difficult to practice. While we did practice based on professional standards of practice at that time, we realized and we know, really, that this is not enough and it's not the way to go. So that's why all of these policies and procedures, they should define in most instances every single step that's being done

Page 105

in the lab so that everybody can follow. And we are trying to put this on intranet and some of them already been lifted on intranet. So not everybody has to know about every single policy what's written, but at least you have the ability to go back and just search and find it. So it's very valuable thing that we have done.
THE COMMISSIONER:
Q. Well, one of the things that has occurred to me is that particularly in light of the description that I've gotten from you and other witnesses about a fairly good sized turnover in pathologists, as well as oncologists, as far as that's concerned, at Eastern Health is that the existence of policies and guidelines at least enables those who are new to your organization to know where to go to find the standard that you are using?
DR. DENIC:
A. That's absolutely correct.
THE COMMISSIONER:
Q. All right. And the final point is that you on a couple of occasions have referred to the fact that you view this as a worldwide

Page 106

problem?
DR. DENIC:
A. That's correct.
THE COMMISSIONER:
Q. Or a global problem I think was used, not by you, but by one of the other witnesses, at least, in communication. And when you say it's a worldwide problem, what aspect of this are you saying is worldwide problem?
DR. DENIC:
A. False negatives are worldwide problem.
THE COMMISSIONER:
Q. Okay.
DR. DENIC:
A. And no standardization--again I believe myself as well I'm going to learn more through Dr. Dabbs testimony. We read in the United States, and there the article is as well, in the United States this is a problem too. We seen that through the works of Dr. Rhodes and his paper as well because he was conducting the studies on over 200 labs across the world and he found the problem there. So that's what I'm referring to. I think this is not isolated one.

Page 107

THE COMMISSIONER:
Q. Uh-hm.
DR. DENIC:
A. What's isolated in this case, that we embarked on this process and went back to look in every single case that fell in that category. So that's what difference is. You know, there's one of the attempts as well through Dr. Banerjee's paper, they went--when they introduced the new antibody which was SP1, and they tested 4000 patients actually tissue, they found complete conversion from zero to the number using new antibody in 6 percent, it was 240 patients. So what we did, nobody has done to this extent to identify the patients, try to help them out, to go back regardless of the consequences that we were facing, and the public scrutiny that we were exposed to.
THE COMMISSIONER:
Q. Thank you, and thank you for participating and providing us with your insights into this issue. I very much appreciate it.
COFFEY, Q.C.:
Q. Commissioner, on the point of the remark involving Dr. Gown's report, I've told my

Page 108

fellow counsel that I'm going to defer that for now, and if I'm going to come back to it, I'll let them know.

THE COMMISSIONER:

Q. All right then. Why don't we take the morning break and then we can prepare for the next witness.

(BREAK)

THE COMMISSIONER:

Q. Mr. Coffey.

COFFEY, Q.C.:

Q. Dr. David Dabbs, Commissioner.

DR. DAVID JOSEPH DABBS (SWORN) EXAMINATION-IN-CHIEF BY BERNARD COFFEY, Q.C.:

REGISTRAR:

Q. Would you please state and spell your complete name for the Commission?

DR. DABBS:

A. David Joseph Dabbs, D-A-B-B-S.

COFFEY, Q.C.:

Q. Commissioner, there are three new exhibits I'm going to ask to be entered, and they are P-2621, 2622, and 2629.

THE COMMISSIONER:

Q. Entered.

Page 109

EXHIBIT ENTERED AND MARKED AS P- 2621

EXHIBIT ENTERED AND MARKED AS P- 2622

EXHIBIT ENTERED AND MARKED AS P- 2629

COFFEY, Q.C.:

Q. Thank you, Commissioner. Registrar, could we open, please, P-2622. Doctor, this is your curriculum vitae?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. Okay. Doctor, I'm going to ask you perhaps if you could just to take the Commissioner through your educational background and your professional background, okay.

DR. DABBS:

A. Yes, certainly. I attended the University of Pittsburg, Undergraduate School. I subsequently went to Graduate School and obtained a Masters Degree in Chemistry at Cleveland State University. I subsequently went to Medical School at the Medical College of Ohio in Toledo, Ohio. Did my residency training in anatomic pathology at the University of Washington in Seattle.

COFFEY, Q.C.:

Page 110

Q. That's your educational background, Doctor. What about your professional background, your career?

DR. DABBS:

A. In terms of the positions that I've held, my very first position was at East Carolina University, School of Medicine, in Greenville, North Carolina, where I was Assistant Professor. I subsequently spent a couple of years at the Case Western Reserve University Hospital at Metro Health and Medical Centre in Cleveland, and then I subsequently went into private practice for about four years in Oakland, California, at Samuel Merit Hospital. I subsequently moved back to Pennsylvania as Associate Professor of Pathology at Penn State University in Hershey, Pennsylvania, and then when the opportunity called to go to Pittsburg, I went to Allegheny General Hospital where I was Associate Professor in Pathology associated with the Medical College of Pennsylvania, Hahnemann School of Medicine there. Due to some issues that occurred in Pittsburg at that institution, I then moved on to private practice for a period of one year

Page 111

at St. Agnes Health Care in Baltimore, and when the opportunity came forward to move back to Pittsburg as Director of Anatomic Pathology at Magee Women's Hospital, and that was in September of 2001, I was subsequently named Chief of Pathology at the same institution in April of 2003 and have been there since.

COFFEY, Q.C.:

Q. Doctor, you're certified by?

DR. DABBS:

A. I'm certified by the American Board of Pathology and Anatomic Pathology.

COFFEY, Q.C.:

Q. And that was November 15th, 1983?

DR. DABBS:

A. Yes, sir.

COFFEY, Q.C.:

Q. And also by the American Board of Pathology, Anatomic Pathology, and Cytopathology, November 16th, 1989?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. Doctor, while we're on the point, what's the difference between cytopathology and anatomic

Page 112

pathology?
DR. DABBS:
A. Cytopathology basically is the study of individual cells as opposed to tissues. Surgical pathology is largely dealing with tissues, tissue sections. Cytology is mostly a specimen that is collected as a fluid or as an aspirate specimen and is composed of small bits of cells, if you will.
COFFEY, Q.C.:
Q. Doctor, as well on page three of your CV, professional society memberships are set out there, and editorial board appointments. Doctor, have you been involved in the preparation of any textbooks?
DR. DABBS:
A. Yes, I have. I have a textbook on diagnostic immunohistochemistry that was originally published by Elsevier as a first edition, I believe, in October, 2002. It went on to second edition about three or four years later and is currently in preparation for a third edition.
COFFEY, Q.C.:
Q. And, Doctor, your role in that is what?

Page 113

DR. DABBS:
A. My role in that book is as a contributor as well as an editor of the book.
COFFEY, Q.C.:
Q. And, Doctor, the book is intended for what audience?
DR. DABBS:
A. The book is intended for anyone who is involved in the practice of immunohistochemistry and that includes pathologists, pathologists in training, anyone in the laboratory who may be using immunohistochemistry for any reason, and as a reference book for oncologists as well.
COFFEY, Q.C.:
Q. And in relation to the two editions of the book that have been published to date, did you contribute any particular chapters to it?
DR. DABBS:
A. Yes, I did, I contributed the chapter on breast immunohistochemistry as well as the chapter on tumours of unknown primary, also known as metastatic carcinomas of unknown primary.
COFFEY, Q.C.:

Page 114

Q. Doctor, could you tell the Commissioner, please, about your own introduction to and experience with immunohistochemistry, perhaps going back to the beginning and take us through?
DR. DABBS:
A. Certainly. I was fortunate in being at the epicentre of immunohistochemistry at the University of Washington during my residency, and that was the period from 1980 to 1980. There was a fair amount of immunohistochemistry going on at that time that was pretty much avant garde, and that's where I acquired my interest in this discipline. When I moved to my first position in Eastern North Carolina, I developed an immunohistochemistry laboratory there as well, and basically continued my interest, attending meetings, societies, and all of these venues. When I subsequently ended up going up to the Hershey is when I developed a further interest in breast pathology. It wasn't until when I was in my position at Allegheny General Hospital in Pittsburg, beginning around 1997, that I was somewhat frustrated with the state

Page 115

of affairs of immunohistochemistry in that there really weren't many good references, a go to place, if you will, for information regarding immunohistochemistry. There were a few textbooks out at that time. They were quite dated, each more than five years old, and so it was my plan at that time to create a resource that I felt was badly needed, and I basically wrote a proposal to one of the publishers in the States and they accepted this and that's how the book became where it is.
COFFEY, Q.C.:
Q. Doctor, are there other immunohistochemistry - diagnostic immunohistochemistry text available in North America?
DR. DABBS:
A. In North America there are some other books that are available. One is written by Clive Taylor and Richard Coady. That book just came out, I believe, approximately two years ago. Dr. Taylor, of course, had published books previously on immunomicroscopy and immunohistochemistry, and--so his is a new edition about two years. Dr. Mehrdad Nadji

Page 116

from Miami also has a book out by the ASCP Press, that's very popular as well. Of course, there are other surgical pathology textbooks out that do have sections on immunohistochemistry and relevant chapters.

COFFEY, Q.C.:

Q. Doctor, I understand that you have prepared a presentation for the Commissioner. If we could open, please, Registrar, Exhibit P-2621 and I take it, Doctor, this is it. Perhaps then, Doctor, I'll leave you with control of the mouse for the slides, and if you could take the Commissioner and the rest of us through this. This is entitled "Best Practices for Hormone Receptor Testing by Immunohistochemistry".

DR. DABBS:

A. Yes, certainly. Basically, what I would like to discuss and ultimately get to is the consensus recommendations on estrogen receptor testing of breast cancer by immunohistochemistry. This is a paper that is in press in "Applied Immunohistochemistry and Molecular Morphology". This is a popular journal for our discipline. This paper

Page 117

basically was generated from ad hoc group of pathologists and laboratory scientists and technical experts representing academia community hospitals, industry and reference laboratories. We conducted a full day consensus meeting in Santa Barbara, California, January 27th of this year, to discuss these critically important issues in an effort to develop rational evidence-based guidelines for best practices in the assessment of estrogen receptor by immunohistochemistry. Now ultimately before I get to this paper, what I wanted to do is what steps led up to this particular meeting at Santa Barbara, and what does history of this topic tell us. Way back as far as 1889, there was a Dr. Schinzinger who suggested that endocrine ablation was important in the treatment of breast cancer, and this actually panned out further by Dr. Beatson in 1896, who performed the first operation to remove ovaries in a patient with inoperable breast cancer, and his quote was that "eight months after the operation the disease in this patient had disappeared". Boyd was the first

Page 118

actually to do a controlled study some four years later on 54 patients, and patients who had advanced breast cancer. He removed the ovaries and 35 percent of these patients went into complete remission of their disease. Now there was a large hiatus that subsequently ensued over the next several decades, and the question that was battered around at the time was should removal of ovaries in patients with breast cancer be prophylactic or therapeutic based on advanced stage. So up until 1960, this information was controversial in the oncology literature and it wasn't until 1960 that Jensen and Jacobsen actually demonstrated that radioisotopic or radioactive estrogen accumulates in target tissues in the body in the pituitary gland, in the vagina, and in the uterus, and that these radioisotopes were found in cytoplasm and nucleus of these targeted cells, and it suggested that ablation of the pituitary gland which controls the ovary or adrenal gland, which is also controlled by the pituitary, may be a treatment to eliminate these sources of estrogen in the body. So in essence then in

Page 119

the next few years, from 1965 to 1970, I chose these articles as sort of exemplifying what I called the prelude to estrogen receptor testing. Paper by Lewison entitled "Castration in the Treatment of Advanced Breast Cancer" that was published in Cancer in 1965, a paper by Sander, "The In Vitro Uptake of Oestradiol", that's also known as estrogen, and biopsies from 25 breast cancer patients, the first time that anyone ever demonstrated that, in fact, tumours from breast cancers can take up estrogen and bind to it. Then Korenman, from the Journal of Clinical Endocrinology and Metabolism, discussing specific estrogen binding sites of the cytoplasm of human breast cancer. So these were all really sentinel papers that demonstrated that breast cancers do, in fact, take up estrogen. Now McGuire in the early '70s, late '60s, demonstrated in his laboratory a method of showing, in fact, that the estrogen that binds in breast tumours can be measured. This test became known as the dextran coated charcoal ligand binding method. Initially it was the ligand binding method

Page 120

exclusively, and the principle of this test was the measurement of available cytoplasmic estrogen receptor binding proteins measured as a fraction of the total sample protein content of that cell. Just a couple of years later, Ferherdy showed that the dextran coated charcoal step in the ligand binding method aided in reducing non-specific estrogen receptor binding receptors. He discovered that there were more receptors found in post-menopausal women than in pre-menopausal women and suggested that it may have a prognostic value for treatment regiments. Now I wanted to take you through these steps in the DCC or dextran coated charcoal/ligand binding method that was initially published by McGuire. Basically you take a piece of tissue and you homogenize it and put it in a centrifuge and isolate what is called the cytosol, which presumably represents the cytoplasmic content of whatever cells that you have. You then take that and you fractionate it through what is called the sucrose density, and you then isolate those specific fractions and expose them to radioisotopic label estrogen, and the

Page 121

principle here is that that estrogen which is radioactive will bind to estrogen receptors that were present in the cytoplasm of that cell. Then this mixture is exposed to a slurry of charcoal, dextran coated charcoal, which removes unbound estrogen, and then what's left is estrogen receptors bound to radioactive estrogen and it is exposed or counted in a scintillation counter to see how much radioactivity there is. The exposure to estrogen--the specimen is then exposed to estrogen to determine non-specific binding. In other words, estrogen will displace anything that is binding to the remaining receptors in a much tighter fashion and knock out any non-specific binding, and then it's counted again. I know this is--you know, seems very laborious and time consuming and difficult to interpret, and it is. It's a very laborious procedure performed in few laboratories. The final result then was expressed in femtomoles of estrogen receptor protein per milligram of cytosol protein. Now to put this in perspective, femtomole means 10 to the minus 15. So that 14 zeros and a one.

Page 122

COFFEY, Q.C.:
Q. That's how small a quantity?
DR. DABBS:
A. That's how small a quantity, exactly, femtomoles.
COFFEY, Q.C.:
Q. Doctor, you just in passing referred to the fact this was done in relatively few or very few laboratories?
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. Why was that?
DR. DABBS:
A. Well, I was going to--the next slide here, is that this was a very high tech procedure that required very exotic, if you will, laboratory instrumentation that was very expensive and required very great technical skill, laboratory science type technologists, not the type of typical technologists that you find in a usual hospital laboratory.
COFFEY, Q.C.:
Q. And, Doctor, while I'm on the topic, this would have been--would this have been in use

Page 123

when you first started your residency?
DR. DABBS:
A. This was in use and was the preferred method of testing as I started my residency in the year 1980.
COFFEY, Q.C.:
Q. Doctor, at that time was there any certification regime or licensing regime related to who could perform this sort of test?
DR. DABBS:
A. Well, to my knowledge, the laboratories that performed this operated under the aegis of the usual kinds of quality control standards that were prevalent at the time. The issues regarding quality control and everything are pretty much the same as they are now for immunohistochemistry, the same issues, and, in fact, one of the first slides that I demonstrated there was the first step was homogenizing the tissue. Well, that became an issue when it became evident that laboratories were getting different results for ER by this method, depending on how tissue was homogenized. So every step of the way had to

Page 124

become somewhat standardized as time moved forward with this test.

COFFEY, Q.C.:

Q. Thank you, Doctor. Go ahead then, please.

DR. DABBS:

A. So the DCC ligand binding method required a large amount of fresh tissue. It required immediate freezing of fresh tissue when removed from the patient, and I can tell you that there was one evening when I was on call and I got called in and specifically for the reason of collecting tissue. What it meant was as soon as the breast tissue was devitalized at the time of surgery, that tissue was handed off. The pathologist took it right to the laboratory and froze it immediately. That's the kind of immediate care that was required for that, and I'll show you an important reference in a few moments as to why that is so. So this method required radioactive reagents. Some of those reagents were actually estrogen compounds, DES, diethylstilbestrol. Those are known carcinogens. Expensive laboratory equipment not usually found in hospitals. The other

Page 125

main issue with this test was what's referred to as blind sampling, and basically samples for the assay are largely independent of what is actually examined histologically, so while there was some attempt at taking a sample that was representative of the tumour, whatever tissue was sent for this assay was really blind, people really never knew 100 percent what was in it, how much normal tissue might have been in there. If the sample, for example, had only a few tumour cells and a lot of stroma, it could affect the result; if there was any devitalized totally dead tumour tissue in there, you really would never know. So that was considered to be one of the main drawbacks of that particular testing, non tumour area sampled and no direct visualization of the assay sample. Not to mention that if this testing, for example, were to be done in Toronto and wasn't being done in St. John's just for an example, you would have to transport, expense of sending this on dry ice in a very rapid way over to Toronto. Another issue associated with this that made it very tedious and mathematically

Page 126

challenging and again a test to the tedious and scientific method involved was so called "scatchard plot analysis" which basically was a determination of binding coefficients, how tight the estrogen receptor that was present bound to the estrogen that it was exposed to. That related to specificity issues for the actual test. So again the quality assurance issues were the same, quality control test results were standardized test specimens. It wasn't until 1978 when Pertschuk published in Cancer, "The immunofluorescent detection of estrogen receptors in breast cancer", and this is really a sentinel paper. One of the other authors on this was David J. Brigatti. He was the person who actually developed the very first automated immunostainer that was ever put together. He also was a faculty member at Hershey at Penn State. So basically what these authors did was using an estrogen polymer labelled with something that they could see under a special microscope called fluorescein. The principle here was that this polymer would bind to the estrogen receptor in the cell cytoplasm and you could see where it

Page 127

bound by putting these cells under a microscope, a fluorescence microscope. So this fluorescein that was present on the polymer would show up when placed under a fluorescent microscope. Receptors were found in the cytoplasm and in the nucleus, and what they did was they found that there was a 90 percent correlation with the dextran coated charcoal ligand binding method with this immunofluorescence procedure, and in this paper he comments that the technique can be performed by the average surgical pathology laboratory in general. Tumours with less than 10 percent positive cells were negative by DCC ligand binding and those with 11 to 20 percent positive were borderline by DCC ligand binding. I think this is one of the first mentions of this magic number, 10 percent, that tended to permeate the rest of ER testing literature if you follow that throughout time. Then just a year later, Antoniades in the American Journal of Clinical Pathology looked at correlation of estrogen receptor levels with histology and cytomorphology in breast carcinoma, and this really was the first paper

Page 128

that had a critical look at what did tumours look like when they expressed estrogen receptor. Prior to this, there were few papers that only tangentially addressed this issue, and in my opinion didn't really do a very good job of it because what they said was, you know, we're looking at these tumours and the way they look doesn't really correlate with what we're seeing by the dextran coated charcoal method. These authors, using strict criteria from what was then the National Surgical Adjuvant Breast Project, which has been and always was centred in Pittsburg, showed a very strong correlation with better differentiated tumours, especially tubular cancers and lobular cancers, so called high expressors. So this was the first paper that discerned what tumours looked like when they express high amounts of estrogen receptor. Then this paper by Eusebi and others actually increased sensitivity by showing that a two stage method for estrogen receptor analysis correlated with morphological parameters of breast cancer as well. It showed an enhanced sensitivity over direct methods. It

Page 129

demonstrated for the first time that nuclear expression dominates and not cytoplasmic, and that it correlates well with morphology. In other words, better differentiated tumours, those that do not look really aggressive under the microscope, have the most estrogen receptor content. This paper, you'll notice it's basically one--almost two pages by Hasson et al in Cancer from 1981 right when I was a resident. Comparison of estrogen receptor levels in breast cancer samples from mastectomy and frozen tissue samples, they demonstrated that devitalized tissue may give a false negative result, that the comparison of fresh frozen tissue in the subsequent mastectomy specimens was really the culprit here, that there were markedly lower ER results obtained in the mastectomy specimen as opposed to a fresh frozen tissue section. In other words, what they were saying was that if they were called for a consult to determine whether this was cancer and they got fresh tissue in their hands, they did a documented frozen section that it was cancer, and they stowed some away right away and froze it.

Page 130

Then what they did, they compared that result for estrogen receptor with the mastectomy specimen that came hours later and sat around in the laboratory, and showed that there were huge differences, that the estrogen receptor content markedly fell off with time, and suggested that, in fact, because of that a low expressor--if there was a patient who had a low expressing tumour initially, it may, in fact, become negative because of this issue with disconnecting the blood supply and causing this devitalized tissue lag time.

COFFEY, Q.C.:

Q. It may become negative in the sense that when it's analyzed as a mastectomy specimen --

DR. DABBS:

A. Correct.

COFFEY, Q.C.:

Q. It would be analyzed as negative?

DR. DABBS:

A. Yes, and this was the call to arms that it was critically important to be there when the surgery was done and the surgeon was actually disconnecting the blood supply from the tumour, that that's when you had to obtain the

Page 131

tissue and freeze it immediately to get an optimal result. Then Shimada and colleagues just a few years later showed that the immunocytochemical staining of estrogen receptor in paraffin sections by the use of monoclonal antibodies was comparable to that of frozen section, and this was one of the first papers that compared frozen section with paraffin and using two different methods, immunoperoxidase, and another more sensitive technique, the Avidin-Bioten method for immunohistology, and also corroborated previous other studies that all of these correlated very well with the results that were obtained by dextran coated charcoal ligand binding method. This paper by McCarty and others that described estrogen receptor analysis correlation of immunohistochemical and biochemical methods was the first paper to really quantitate, attempt to quantitate estrogenreceptorresultsby immunohistochemistry and actually compare them with dextran coated charcoal methods and patient outcome, and this had never been previously performed. What he devised was

Page 132

something called the H score, H simply meaning histochemical, and the H score which is still used today in some institutions including ours is the sum of the proportion of cells by immunohistology with nuclear staining times the intensity of staining. So, for example, if you have 100 percent of cells by this scheme that stained 4 plus, your H score is 400 and that's the maximum it can be. If you have a completely negative result, 100 percent of cells stain zero, your H score is zero. If you have 10 percent of cells that stain 1 plus, 10 percent of cells that stain 2 plus, 10 percent of cells that stain 3 plus, and 70 percent of the remainder stain 4 plus, what you do is you add up all those four numbers by taking 70 times 4, plus 10 times 3, plus 10 times 2, plus 10 times 1, and that's your H score, okay. It worked very well and correlated with outcome and the desirable aspect of this is that our clinician colleagues do like to have a quantitative measure put to this test result as opposed to it being reported just as positive or negative. It is possible to report it as just

Page 133

positive or negative, but what the quantitation does is it gives you a reasonable number for the clinician to tell the clinician how much impact the patient may get from antihormonal therapy. For example, a patient with a H score of 350, I can tell you several things about that; that patient almost certainly is also PR positive. We know that from our metrics. They're going to get a good impact out of anti-hormonal therapy. Whereas if someone has a H Score of 60, the patient may have an impact, but it will be substantially less than the patient who has one of 350. So the importance of this quantitation is is dialled into one of the other factors that deal with individual patients. Some patients may do well on therapy, some patients may be frail or have other relative contraindications to anti-hormonal therapy, so this is a judgment call. This number helps the clinician, the oncologist and the patient determine what course of action they want to take.

This same study actually compared the only antibody at the time, when I was a

Page 134

resident was used on frozen tissue H22 by Abbott. And you can see here that the quantitative comparison with the biochemical method had a sensitivity of 93 percent and a specificity of 89 percent based on clinical outcome alone. A very important paper.

Now, the quality issues with the dextran-coated charcoal and ligand binding method were exemplified very nicely in this paper by Thorpe from 1987. And I mentioned some of these already: the biopsy composition and inability to really distinguish normal from tumour in some instances, the concept of homogenization was a variable, incubation time with radioisotopes, there could be unwanted absorbtion of the Ligand on free surfaces of the glass tubes, there could be absorbtion of free steroid by Dextra-Coated Charcoal. In other words, this was not an easy test to do. There were far more steps and far more things that could go wrong, in actuality, than with immunohistology. And again, variation in scintillation counting.

So in 1989 Berger came out with the first introduction of an antibody to progesterone

Page 135

receptor. This was the first time that this actually allowed for routine analysis of progesterone receptor. Progesterone receptor was not routinely done with the dextran-coated charcoal method simply because it required so much tissue. Estrogen receptor itself required a great deal of tissue. And I can tell you that back then many times it was not difficult getting adequate tissue because back then mammographic screening was not what it is today and tumours were larger, regrettably, patients presented with larger tumours, so there was, in many instances, adequate tissue but progesterone receptor was not routinely done because it required so much additional tissue. So the introduction of the PR antibody was a very important step for immunohistology.

These authors then demonstrated that, in fact, PR is an independent prognostic factor and ER positive, PR positive patients respond better as a group to endocrine therapy. What it turns out is if you look at metrics, and by metrics I mean for any given laboratory the overall percent of patients who are ER

Page 136

positive and then you can break that down into the category that's ER positive and PR positive, there will be a category that's ER positive and PR negative, there will be a category that's ER negative and PR positive, and then there will be the double negatives, the ER negatives and PR negatives. What they demonstrated here was that when PR is also positive, these patients get the best bang out of the treatment, they get the most impact with anti-endocrine therapy, presumably indicating that the--at least some of the physiology of those tumour cells is still intact. Estrogen receptor in normal cells induces the presence of progesterone receptors, that's in normal cells. So it's heartening in this group of tumours to see that ER positive and PR positive, that physiology axis is still working and so these patients who are--and these are almost always strong positive ERs or moderate positive ERs who also have PR, they respond best. And these papers, each one of these four here document this, the paper by Thorpe, McGuire, the two papers by McGuire, and Pertschuk, as

Page 137

well. So this was an very important milestone to recognize that if someone needs to have this anti-hormonal therapy, which is not without side effects, it's nice to know that these are the patients who will get the most out of that treatment.

This paper then by Shousha in 1990 showed something that no one had ever demonstrated before and that was he took 60 cases that were negative for estrogen receptor by dextran-coated charcoal and he reassessed them by immunohistochemistry and found six to be weakly positive, that's ten percent of the cases. He also found three that were moderately positive, and these were tubular and lobular cancers and should have been positive by DCC. But again, with all of those items that I showed you and some of the weaknesses of the DCC Ligand Binding in that, sometimes you really never knew what sample you were submitting. And I can tell you that with lobular cancers are notorious in that they tend to be tumour cell poor and have a lot of stroma. So it's conceivable that on sampling and with the processing of tissue

Page 138

that there may be not sufficient number of tumour cells, for example, to actually be assayed by DCC, given what we know now about these tumours. So he nicely demonstrated that DCC was not necessarily the gold standard that everyone had previously thought.

One of the first calls to standardization of hormone receptor testing by immunohistochemistry was done by Jose Eseban et al. And actually, in the very same supplement Pertschuk, whose name I've mentioned several times now, did the same thing. Basically they've recognized that immunohistochemistry was rapidly becoming the test of choice for multiple reasons. It was it can be performed in any surgical pathology laboratory, it could be--it was much more cost effective, it was reproducible and the studies to date had demonstrated that, in fact, the outcomes were as good or better, in some instances, in some papers, than what was seen with the dextran-coated ligand binding method.

And, in fact, Pertschuk, in 1996, using the ER1D5 antibody demonstrated, in fact, that the endocrine response was better compared to

Page 139

cytosol methods, compared to the DCC method or the frozen tissue method. And he found, in fact, that the percent of cell staining, ten percent was as good as naming the intensity and percent of cells. So in other words he was saying if you just use the percent of cell staining, that seems to be as good as naming the percent of cells staining as well as the intensity of cells staining.

And at about this time there was another paper that I don't mention here, and the author was Mascarelli, that also used the 1D5 antibody and in doing so they used cutoffs of five percent and ten percent just arbitrarily and the outcomes were the same. The patients who had five percent did as well as the ten percent. So that was the 1D5 certainly had been worked out well with outcomes as low as five percent. So you can see where some of these numbers of ten percent keep popping up in the ER literature over time.

This sort of takes us up to about the year 2000. And I wanted to point this out because this had a significant impact on me. I had moved to Mcgee Women's Hospital in

Page 140

September of 2001, approximately a year after this statement came out. And in fact, I noticed this statement when it came out. I was in Baltimore at the time. And this statement did have an impact on me and the way we reported estrogen and progesterone receptors.

Basically this consensus conference stated the following, "The decision whether to recommend adjuvant hormonal therapy should be based on the presence of hormone receptors as assessed by immunohistochemical staining on breast cancer tissue. Adjuvant hormonal therapy should be recommended to women whose breast tumours contain hormone receptor protein, regardless of age, menopausal status, involvement of axillary nodes or tumour size. While the likelihood of benefit correlates with the amount of hormone receptor protein in tumour cells, patients with any extent of hormone receptor in their tumour cells may still benefit from hormonal therapy. Hormonal adjuvant therapy should not be recommended to women whose breast cancers do not express hormone receptor protein."

Page 141

So what I did when I--whenever this statement came out is we changed our method of reporting at St. Agnes to the following: we would semi-quantitate it as we did before, but we also considered any result--rather, a positive result as any nuclear expression. And the way--the reason why we did that is if you read, you know, from here, "Patients with any extent of hormone receptor in their tumour cells may still benefit." Okay. My reading of that is that you want to report that because a patient may benefit from it and therefore my threshold became zero for a negative result.

And when I moved to Magee Women's Hospital a year later, I brought this up at one of our interdisciplinary tumour boards, you know, presented this same information to our tumour board and oncologists and they concluded that this would be an acceptable method of expressing the reports, but they were also very much interested in continuing semi-quantitation. And the reason for that was, as I mentioned before, they want to know how much hormone there is so that they can

Page 142

assess on an individual patient how much efficacy they will get from the medicine, because these medicines are not all 100 percent benign, they have side effects.

So I thought that this was an absolutely key event, a sentinel event, this statement. It has not changed since. If you Google this statement, it will say something to the effect that the statement is seven years old and if you Google further, you will find nothing else follow up, it hasn't been changed, it hasn't been amended, it hasn't been struck down, so it's still there. And I think as I move on to other items you will be able to put this into perspective in that I still strongly believe that this does have a role in hormone receptor reporting.

COFFEY, Q.C.:

Q. Doctor, when you say semi-quantification, why -

DR. DABBS:

A. When I say semi-quantitation, basically at a minimum you report the percent of cells that stain weakly, moderately and intensely, so that they all add up to 100 percent.

Page 143

COFFEY, Q.C.:

Q. Okay.

DR. DABBS:

A. The following paper by Fisher, actually, I was a co-author on this, and we reported this in Cancer in 2005. This paper is titled, "Solving the Dilemma of the Immunohistochemical and Other Methods Used for Scoring ER and PR in Patients With Invasive Breast Cancer." We basically compared the ER and PR results by comparing the dextran-coated charcoal method to immunohistochemistry in an NSABP group, that's the National Surgical Adjuvant Breast Project, protocol B09, that basically had 402 patients. This was a randomized study in which patients were treated with Tamoxifen or not treated based on hormone receptor status. Our criteria, when we went into this, was we were going to report any or none and we were going to then attempt to assess the intensity and percent of cell staining. And we also had a separate group of people who did a fancy computer assisted image analysis of this same material. And basically what we concluded was that the presence of any

Page 144

nuclear estrogen receptor expression is related significantly to overall survival of five and ten years regardless of the scoring method. In other words, if there was any expression whatsoever, that was clinically important, it was important because those people responded to therapy and it was prognostically significant at five and ten years regardless of the scoring method. In other words if there was any expression whatsoever, that was clinically important. It was important because those people responded to therapy and it was prognostically significant at five and ten years regardless of the scoring method. So you could analyze your tissue section by a fancy computer assisted image analysis, you could eyeball it by percentages, you could create an H Score or you can say present or absent, all those were equivalent.

This does not diminish the semi-quantitation in my mind because when you report the semi-quantitation, you're talking about the care of an individual patient and because no two patients are alike, some

Page 145

patients may have relative contraindications to the medicine, if you want to give them that medicine, in my mind and I think most oncologists, you'll want to have a real good reason to give it to them. You wouldn't want to give it to someone who has relative contraindications and has a very weak expression, for example.

So this takes us right now to recommendations for improved standardization in immunohistochemistry. This paper was published in 2007 in The Applied Immunohistochemistry and Molecular Morphology Journal. This was, this paper represents the fruits of the same ad hoc group committee meeting, we even had met the year before somewhere in the Florida Keys and came upon these recommendations.

First what I'll--my first line here is to basically reference that all of these items were taken into account by this committee. There was a person present at this meeting who was a representative of the CLSI, Clinical and Laboratory Standards Institute, people from the College of American Pathologist and of

Page 146

course, we were all mindful of our CLIA 88 responsibilities, the Clinical Laboratory Improvement Act of 1988 regarding items that were necessary to be present in our laboratory reports.

And basically to sort of start off with, these are the items that we all grappled with, that there are different procedures intra and intra laboratory items that can potentially compromise standardization. If you just consider the preparation phase or the pre-analytic phase, the IHC staining as part of the pre-analytic and analytic phase and the interpretation phase as part of the analytic phase, you'll notice that for the preparation phase there's biopsying, fixation, preparation, sectioning and drying. In the second step there's deparaffination, pretreatment, meaning antigen retrieval, the antibody, the detection method and the counterstain. And then for the interpretation phase, what are your controls, what are your cutoff values, what is the tumour entity, and what sort of things do you need to put on your report? If you take these three steps and

Page 147

these 14, you can see that there can be a myriad of potential items competing with each other that are not in the best interest of standardization. So what we have tried to do in this meeting was standardize these items the best way that we could.

I take this chart from this paper by Dr. Clive Taylor, from Archives Pathology of Laboratory Medicine, where he talks about the total test for immunohistochemistry, and this is immunohistochemistry in general, the elements of testing in this column, the QA issues and the responsibility. You know, the clinical question, what test is ordered? That's the pathologist, the clinician and the technologist all have responsibility. The QA issues are getting the specimen properly fixed, collected and fixed. What are the indications for the stain? What are the stains? This is within the realm of the pathologist. What is the technology and methodology? What are the reagents and the protocols? What's your specificity and sensitivity? What are the qualifications? What are the proficiency testing profiles for

Page 148

that particular test? What about your results? What are your criteria for a positive and negative report? What should be in your report content? And then finally, the interpretations, what are the qualifications of the person who is doing this, and how does this all integrate into everything else? And overtime, I think that Dr. Taylor's total test concept was really critically important in pushing us all in the right direction of standardization.

So basically, the items that we discussed in Florida were tissue acquisition. Again, this harkens back to the paper by Hassen. We need to get the tissue as soon as possible and into our fixative, which our preferred fixative is ten percent neutral buffered formalin. Tissue sections need to be cut thin, and this is especially important for breast tissue, where you obtain the specimens fresh from the operating room. They have to be cut as thin as humanly possible and placed into fixative. Tissue fixation, there should be minimum and maximum fixation times for predictive and prognostic markers, and this

Page 149

actually came up as a first time ever, if you will, recommendation by the College of American Pathologists and ASCO where they addressed HER2 testing. Where, for the very first time, there was an organization that said "you must fix your tissue a minimum of six hours if you're going to do HER2 testing, and not longer than 48 hours," and this was completely new to anatomic pathologists worldwide. Never before had anyone drawn a line in the sand and said this is what you have to do if you're going to do this type of testing.

COFFEY, Q.C.:

Q. That was HER2?

DR. DABBS:

A. HER2, yes, that was the CAP ASCO guidelines that came out just a couple of years ago.

COFFEY, Q.C.:

Q. So it was just a couple of years ago?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. For the first time, to your knowledge, anywhere -

Page 150

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. - there was, as you put it, such a line in the sand drawn?

DR. DABBS:

A. Correct.

COFFEY, Q.C.:

Q. In terms of regarding tissue minimum fixation time for particular type of tissue?

DR. DABBS:

A. Exactly, and this was referring to breast tissue, in fact. So the other items in best practice, you want to make sure that your tissue gross sections are nice and thin and that your tissues for microtomy are, you know, in the realm of four to five millimetres thick, which has been the standard of tissue sectioning ever since immunohistochemistry has been practised.

The pre-analytical factors, in your tissue processor, it has to be formalin. There's a need to change the solutions, especially for formalin, daily, and probably weekly, and replenish solutions as necessary.

Page 151

No solutions in the processor should exceed 37 degrees Celsius. Paraffin should not exceed 60 degrees Celsius. Blocks should not be allowed to sit in heated paraffin for any extended period of time, and embedding has to be done in accordance with standard embedding procedures without using excessively hot paraffin.

Tissue microtomy, tissues are sectioned to four to five microns. Tissue has to be able to adhere to the slide and it sounds like a sticking point here, but just simply having your tissue sections adhere to a slide is critically important. You can do this several ways. You can have it sit on the slide and dry overnight or you can have it in a controlled oven to bake so that all of the water is taken out of that section. It's critical, because if you have water in that section and you put it through an immunohistochemistry stainer, what will happen is that the tissue will lift off and it will partially degenerate, so that you can end up with holes and folds and part of it will be missing. Okay, if it's not--just something

Page 152

this simple can kill the whole system. Everything has to be done very methodical. You cannot short change any method.

Tissue has to be de-waxed, de-paraffinized appropriately, and if you--so if you change anything in your system, if you change a paraffin, for example, you need to re-optimize your process and make sure that the result that you have been getting is the same result that you got before. Probably no two paraffins have the same melting point. Some paraffins come nowadays with polymers in it to help histotechs cut tissue better. So you have to make sure that you're properly de-waxing your paraffin. You have to make sure that the technical--the person who is performing the immunohistochemistry knows what they're doing, knows the fine detail that has to be attended to every one of these steps, because without proper preparation, putting it in the machine is--if you don't have it properly prepared, putting it in any automated machine is not going to fix it. It's sort of like if you're going to have your house painted, you don't just paint over big peeling

Page 153

areas and whatnot. You have to properly prepare the surface in order for the new paint to stick, and it's critically important for the technician to have the competence to recognize that and also recognize when things aren't working quite well.

Additional pre-analytic processes is antibody optimization. The antibody package insert is there for a reason. It comes with every product, whether it be an item approved by FDA for use or in vitro device or analyte specific reagent. They tell you what the item is, how it was generated, what it has reacted to and their experience, and in some instances, on those package inserts, it will recommend a dilution, and what you want to do is, at the minimum, work one above and below that dilution, and just a word about dilutions. Many antibodies nowadays come as ready-to-use reagents. You open a vile and you take that reagent and you put it on the machine. But in the early days of immunohistochemistry, and even nowadays, there are laboratories that basically make their own dilutions. They buy antibody as a

Page 154

concentrate. It's cheaper and as effective, but the critical item there is being able to make those dilutions and titre those dilutions appropriately. It requires intricate equipment, pipettes that are properly calibrated. One slight mistake in a dilution can give you a false negative or a false positive result. So these are items that are highly dependent on the skill of the tech who is doing the procedure, and the fact of the matter is, at least in the States nowadays, it's difficult to find technical people with that kind of skill from scientific laboratories. They're difficult to find and they don't usually work in hospitals. They work in other scientific laboratories. So this is one reason why there's a move towards automation and standardization, the development of ready-to-use reagents has come about, reagent that's already predetermined how it will react and the dilution is already made, so that that step is just another step that doesn't have to be dealt with. So dilutions, again, it's an absolute critical item that one has to deal with.

Page 155

In working up antibody optimization, AR stands for antigen retrieval, you have to work this up at at least a low and a high pH and see how the characteristic of your test responds to each. You should use at least two different detection systems, and for categorical results, for example, something that's just positive or negative like a keratin stain, it's recommended that one use 25 samples. Ten are expressors, ten are intermediate expressors and at least five are negative for the item in question. So this is the overall high view of antibody optimization, according to the recommendations of this committee.

One test battery that you might, you know, consider just the complexity of it is you have your buffer here and you have various temperatures, and different pHs and you have different slides holding the same tissue. So that, for example, in this one, 100 degrees C, you have this breast tumour at pH one to two. This one is seven to eight, this 10 to 11, and you run it through these antigen retrieval processes and then continue with your test and

Page 156

see which works best for your antibody and your tissue in question. This is just one of many ways, and this actually was taken from the same paper on the total test by Dr. Clive Taylor.

So what about antibodies that come into the laboratory. New antibody lots come in all the time. Do you have to do this all over again? Not really. If you just take three samples, one a high expressor, one an intermediate and one a negative, and just test the new lot that comes in and make sure that you're getting the same result as you were before. If you're not, you might want to call the company and say "you know what, I used this the same way. Is there something new about this lot?" You might have to change the conditions of your test. The lot might have changed to some degree. So this is another item that should be carried out with new antibody lots.

In addition, in the pre-analytic step, if one is using a technique that is crucial for blocking endogenous peroxidase, and most of them are, and antigen retrieval, these items

Page 157

have to be worked out.

The IHC procedure itself requires primary antibody application to a slide, followed by a secondary antibody, usually with a detection agent. Then there's a localization with a colour reaction that's developed and the slide is then counterstained and cover slipped.

So what about the analytic process then? It's recommended that pathologists document the immunostaining, where it is, what is positive and what is negative, semi-quantitate it to some degree, comment on appropriate positive and negative internal controls, and comment on appropriate positive and negative external controls.

Post-analytic. On your report, you should tell how you've dealt with the tissue. You should document the fixative and the fixation. You should document the antibody source, the clone and targeted antigen, and by the way, these are items which are on the College of American Pathologists checklist. When they come to inspect your laboratory, they will look at your reports to see if these items are present.

Page 158

One of the items that I pushed for with this most recent meeting, which I'll go over in a few minutes, is the need for metrics for predictive and prognostic markers. Now by metrics, I mean what are the results for estrogen and progesterone receptor or whatever other antibody, for HER2, in our laboratory? How many positive--what's our percent positive? What does the literature say? What's the percent positive for estrogen receptor? If you look at the literature, it says 70 to 80 percent. If you look at your metrics and see that you're 45 percent, you have a real problem. If you have 95 percent positive, you have a real problem. If you're between 70 and 80 percent, that's a bird's eye view that you're probably doing okay. So in my opinion, and I pushed for this in the new paper, right now that's in press, that metrics are highly desirable for any laboratory. That way, if something is changing--and things do change, some vendors disappear and you have to pick up new antibodies or new detection systems or whatever. You want to know where you have been and what has happened in your

Page 159

metrics so that you have a complete grip on what this test is doing in your laboratory, because the impact is incredible. You're talking about therapies that affect patients' lives. So you want to be able to compare to other testing methods outside the laboratory, whether it be for HER2, immunohistochemistry, HER2 by fluorescent in situ hybridization, ER/PR, or whatever.

And then, this brings us lastly to the section that I was going to talk about on the current consensus recommendations on ER testing for the paper that's in press.

COFFEY, Q.C.:

Q. Doctor, I should apprise the Commissioner, there are some things in some of these slides that we've looked at that I will be taking you back to this afternoon, but if we could continue then.

DR. DABBS:

A. Okay. Tissue fixation.

COFFEY, Q.C.:

Q. Or perhaps -

THE COMMISSIONER:

Q. I'm just going to raise the question of

Page 160

whether you want to do the luncheon break -
DR. DABBS:
A. Yeah, perhaps we -
THE COMMISSIONER:
Q. - at this point, which perhaps is a good point to make the break, since we normally break at quarter to one.
COFFEY, Q.C.:
Q. Yes, because otherwise we'll be only three or four slides into it. Thanks, Doctor.
THE COMMISSIONER:
Q. All right then. We'll break until two.
COFFEY, Q.C.:
Q. Thank you.
(LUNCH BREAK)
THE COMMISSIONER:
Q. Please be seated. Mr. Coffey.
COFFEY, Q.C.:
Q. Thank you, Commissioner. Exhibit P-2621, which is up here on the screen. Doctor, we had gotten as far as the consensus recommendations, I believe, on estrogen receptor testing in breast cancer by immunohistochemistry, and the first slide is tissue fixation. You go ahead, Doctor.

Page 161

DR. DABBS:
A. Yes, thank you. So the critical issues began with communication and coordination with operating room personnel and clinics, where tissue is acquired, be they biopsies on core biopsies or actual resection specimens. The first recommendation is that resection specimens must be sectioned fresh, as soon as possible, preferably 0.5 centimetres, placed in fixative as quickly as possible, in less than one hour, and that the time in formalin should be recorded. Tissue sections must be immersed in an adequate volume of fixative with a ratio of tissue to fixative of about one to 20, within a maximum of one hour from removal and acquisition. It's desirable to include normal tissue with tumour in the same cassette, if that is possible, from resection specimens.

The second recommendation is that breast core biopsy should be fixed and processed in an identical manner to excision specimens. Specimen acquisition time into formalin should be recorded.

The third recommendation is that only ten

Page 162

percent aqueous phosphate buffered formalin, ph7 to 7.4, ten percent, should be used as fixative for breast tissue samples. The reason for this is that accrued data on hormone receptor testing and clinical outcomes have been performed on formalin fixed paraffin embedded tissues. Alternative fixatives are possible, but a formal cross validation study requires a minimum of 100 samples that are fixed in both the alternative fixative and ten percent neutral phosphate buffered formalin.

The fourth recommendation is that the time that the samples spend in ten percent phosphate buffered formalin should be standardized for all breast specimens, to help ensure adequate and uniform fixation. Minimum fixation times of at least eight hours, not to exceed 72 hours, unless validated by the medical director, and what this does is it avoids alcohol fixation and promotes antigen retrieval standardization. The key reference for this is the paper by Goldstein, which is mentioned here.

Recommendation number five, alcohol fixed fine needle aspirates, if there is a clinical

Page 163

suspicion of breast cancer that may need ER analysis and an FNA is performed, then all efforts should be made to collect a portion of the cytology specimens in formalin. Validation is required with appropriate alcohol fixed cytology specimens. In other words, it's okay to use alcohol as a primary fixative, but it must be validated within the laboratory with appropriate positive controls, and that is not easy to do. At our institution, we mandate that if we know we're going to be collecting a sample on a patient with a history of breast cancer, specifically for hormone receptors or HER2 analysis, that we go equipped with formalin to collect the specimen as such, so that it's properly fixed.

Tissue processing. Breast cancer specimens should be processed in conventional processors. If alternative processors, such as those that are microwaved enhanced, need to be validated by the medical director on 100 samples.

Number seven, the first formalin containers in the tissue processor should always be newly replenished, and that means on

Page 164

a daily basis. The time in formalin includes the processor exposure to formalin. So that eight-hour minimum time that I was talking about, that would include the time in formalin on the tissue processors. Formalin normally is the first liquid that the tissue is exposed to on a tissue processor and that may vary from minutes to an hour, hour and a half. That can be included as the eight-hour minimum exposure time.

COFFEY, Q.C.:

Q. So I take it, Doctor, implicit in what you've just said, that tissue processors at times, in terms of the first step, the first fluid into which the sample is placed, can vary with time, from minutes to up to an hour and a half?

DR. DABBS:

A. It depends on what the tissue processor program is and probably would vary at each possible laboratory.

COFFEY, Q.C.:

Q. Okay.

DR. DABBS:

A. Recommendation number eight, it is strongly

Page 165

recommended that none of the tissue processor solutions, excluding paraffins, should exceed 37 degrees Celsius if the processor contains breast tissue for potential ER and other biomarker testing. Revalidation is required if significant changes in processor solutions or paraffin types are changed.

COFFEY, Q.C.:

Q. Doctor, in relation to that, what is the potential downside or negative effects of exceeding that temperature?

DR. DABBS:

A. Exceeding temperature basically, we're going into the realm of the unknown, and so what we do not want to do is damage the tissue by exceeding temperatures that we are already known and comfortable with, in terms of the way specimens have been handled in the past. So rather than go into an unknown zone, it was felt that these temperatures would be best controlled as to what is usual standard and operating procedure in hospital laboratories.

COFFEY, Q.C.:

Q. And here, you noted that revalidation is required if significant changes in processor

Page 166

solutions, so I take it if for some reason the solutions, solution or solutions to be used in the tissue processor are changed?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. I don't mean like changed routinely, but as in the type of solution used?

DR. DABBS:

A. The type of solution. For example, as far as processor solutions go, some people may be using xylene substitutes. They would need to make sure that those xylene substitutes give the same result as xylene. There are also some green initiatives that involve recycling of solutions that are characteristically found on a tissue processor, including alcohols and xylenes. They're basically stills that recover the native solution, and so, if one is going to use recycled materials in a tissue processor, you have to make sure that they are pure solutions and make sure that you're getting the same results as you had previously.

COFFEY, Q.C.:

Page 167

Q. Which is the revalidation process?

DR. DABBS:

A. Exactly.

COFFEY, Q.C.:

Q. Doctor, you've referred to this already, the paraffin types. So I think--don't know if the Commissioner has really heard this before, and if she has, I apologize. Not all paraffin, I take it, are the same?

DR. DABBS:

A. Correct.

COFFEY, Q.C.:

Q. Here, routinely, there have been just references to paraffin embedded tissue.

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. I take it, there are different types of paraffin?

DR. DABBS:

A. There are different types of paraffin and they're marketed differently, and some of them have polymers in them to enhance tissue structure, so that when the technician cuts them on a microtome that they are easier to

Page 168

handle and easier to handle on their water bath and whatnot, and that's fine, but it has to be made clear that because of the complex testing requirements that we need to make sure that if there's a change in the paraffin, that there's not going to be a change in the immunohistochemistry result, because of melting point change or something of that manner. I would imagine that if you put the polymer in with plain wax, that it will change the melting point. So if you're going to try to de-paraffinize and clear it with xylene, it may have an effect there as well. Basically, what we're saying is if you're introducing an unknown, it's the responsibility of the medical director of the laboratory to make sure that the results that they're getting with the changed solutions or paraffins are the same as what happened before the change.

COFFEY, Q.C.:

Q. Thank you, Doctor. Go ahead, recommendation nine, I believe.

DR. DABBS:

A. Yes. Paraffin and tissue processors or embedding centres should not be warmed over 60

Page 169

degrees Celsius and the tissue should not be kept in heated paraffin for extended periods of time.

COFFEY, Q.C.:

Q. I take it that's because the effect of doing so is unknown?

DR. DABBS:

A. Exactly, exactly. Documentation, recommendation number ten. It is recommended to include a designated field on the requisition sheet for recording time into formalin and time out. Time in formalin can be dictated into a gross description, a surrogate marker of time out of formalin is when the tissue processor begins. Time of collection recording is encouraged at clinic sites where biopsies are performed. A surrogate marker could be the date of collection.

So let me give you some examples here. Our people who do the gross descriptions, whenever they handle the specimens, they dictate--this is the late item, the time in formalin, and then that's placed. The time out of formalin for us is the--our surrogate

Page 170

marker for that is when the tissue processor begins. In our laboratory, we have seven or eight tissue processors and they all pretty much start at designated times and we have those records. So that if someone wanted to track down a specific case and say, okay, how long was this in formalin, we will look at the gross description to determine the time in formalin and look at the tissue processor log from which that was processed to find the time out of formalin. Because in our tissue processor, I think the first hour and 30 minutes is in formalin.

Granted, there are going to be biopsies that are done in doctors' offices and our surrogate marker for the time in formalin with those is currently the date of which the biopsy is performed. We have a quality initiative that we're going back to those medical groups and asking that they place on the requisitions that they send to us, the time that the specimen goes into fixative. So that'll tighten that up for us.

We recognize that there are a lot of players in acquiring tissue at various sites

Page 171

all over and they're not necessarily geographically close to us, and I'm sure you have sites that aren't necessarily geographically close to you. So communication and coordination with surgical supplied tissues and the rationale for doing this are essential. I think if you tell people why you're doing this, they'll understand and they'll be cooperative.

Standardizing the analytical variables, as far as testing. We have to realize that for any immunohistochemical reaction to take place, all of the components, it should be properly functioning, namely the primary antibody, the detection system and the chromogen. A drop of sensitivity of any of these components will lead to an inadequate assay with potentially false negative results.

The original biochemical assay and appropriately optimized immunohistochemical assays will show a spectrum of ER or PR content in individual cells, and we recognize that for immunohistochemistry for ER or PR, this means that some of the cells will be negative, have no staining. Some of them will

Page 172

be strongly positive, what we call three plus. Some of them will be moderately positive, we call two plus, and some of them will be weakly positive, we call one plus.

So recommendation number 11. The immunohistochemistry estrogen receptor assay should be performed with one of three antibody clones, 1D5, 6F11, SP1, and the reason for this is because there have been published outcome studies with each one of these antibodies. Now with the 1D5 antibody, the studies that initially came out in the mid 90s were by Perchuck. I might have mentioned this paper earlier, where they looked at five percent and ten percent cut offs and both of them were adequate in terms of outcome. What has subsequently happened is that for 6F11, there was a paper by Harvey in 1999 and Dr. Craig Allred is also on that paper. That outcome study used a one percent cut off, one percent or greater, and those people had a positive outcome with respect to therapy. So as little as one percent staining cells was considered to be a positive result.

And I will then make a comment about SP1.

Page 173

There was a study published in the Journal of Clinical Oncology in December of 2006 in which they used this antibody and compared it to the 1D5 using the one percent cut off. One percent was a good number, in terms of outcome, once again. What they did show is that the SP1 antibody did have about eight percent, eight to ten percent more positive cases in terms of the presence of estrogen receptor. However, those cases were generally weakly positive with estrogen receptor for the SP1.

So most institutions right now have these validated studies with 1D5, 6F11, and SP1 as one percent. There are many institutions that use that as a cut off, one percent or more. Some institutions still use ten percent.

Another issue here, ER assay should be performed by standardized methods, preferably using FDA approved test kits, and for your information, I was the person who actually pushed this at this consensus conference. I felt that since we were making--taking great pains to analyze or standardize all of the pre-analytic variables, that we also needed to

Page 174

standardize the platform upon which testing was taking place, and the ultimate standardized test is one that has gone through FDA approval. It is a recipe for doing a test and deviation at all from that testing procedure makes it invalid as an FDA test. So this is one testing procedure that is completely prescribed. It's a cookbook, if you will, and everyone pretty much agreed with this concept, that it was an attempt at standardizing the actual testing platform.

Recommendation number 12, positive and negative test controls should be included with every estrogen receptor IHC batch run. Internal positive controls. In this context, an internal control consists of tissue or cells in the same section or a separate section from the same patient specimen as the test section. External positive control and normal tissue from the same patient or from a different patient. Other external positive controls that can be used for ER and PR include benign gynecologic tissues such as endometrium, endomyometrium, cervix, endocervix and even ovarian tissue.

Page 175

Metrics. This is something that I also pushed for. It is highly desirable to maintain laboratory metrics for each prognostic predictive test results in order to monitor for potential analytic drift. For example, published literature indicates that 70 to 80 percent of breast cancers are ER positive. This should be a benchmark for each laboratory to monitor.

Standardizing interpretation. The NIH consensus statement that I mentioned before, published in 2001, recommended any nuclear ER as allowing a patient to be eligible for endocrine therapy. Harvey, in 1999, these investigators, using the 6F11 clone for ER IHC recorded that proportionate positive cells and the intensity of staining, known as the Allred score, then correlated the results with clinical outcomes in a large cohort of breast cancer patients treated with adjuvant therapy, almost 2,000 patients.

Cheang, this is the paper I referred to from the Journal of Clinical Oncology, December 2006, using SP1 clone and compared it to 1D5. One percent positive cells was a cut

Page 176

off for a positive result, more than 4,000 patients.

So recommendation number 13, a commonly employed threshold for positive results for ER IHC assays, in terms of the potential benefit from adjuvant endocrine therapy is one percent positive tumour cells, with a one plus or greater signal. So this differs from the NIH consensus statement of the year 2000 by a very small amount actually. It's between zero and one percent. There are some institutions that may still include this as a statement on the report, and we are one of them. We do that for two reasons. Number one, that statement has been made. It has never been changed. It has never been -

COFFEY, Q.C.:

Q. That's the NIH?

DR. DABBS:

A. That's the NIH consensus statement, that's correct. And the other issue is that patients, cancer patients in particular, are very savvy when it comes to investigating their own disease, especially on the internet, and if one were to find that statement, one

Page 177

might question as to why a test is--their test is called negative when they have 0.5 percent cell staining with estrogen receptor. So we certainly know, and I've pointed out before, and the NIH consensus statement says itself that there is a gradation of response, depending on the amount of hormone receptor present. So with someone with less than one percent, the predicted response would be low, but nevertheless, there could be a finite response and so we report as--we report the positive, percent of cells and this actually would translate into an H Score, as I said before, one percent, one plus the H Score would be one, okay.

Recommendation No. 14, "The interpretation of ER assay should include an evaluation of both the percent of positive tumour cell nuclei and the intensity of the staining reaction." I think the intensity of the staining reaction is important and I'll show you how we report it. Actually, this group adopted what we have been reporting at Magee-Women's Hospital since the year 2002 where the percent of cell staining one plus,

Page 178

the percent of cell staining two plus and the percent of cells staining three plus are recorded. That's raw data. What we have recently begun to do as of about six months ago is we convert this into the H Score so that there's a numerical value that comes out of this. Again, if 100 percent of cells are three plus, the H Score is 300. If ten percent of cells are two plus, the H Score is ten times two, it's 20, okay. If 40 percent of cells are one plus, the H Score is 40 times one or 40. And you add up all these. This plus this, plus that equals the H Score, okay.

And lastly I show this slide. I think that the intensity of staining is one of the items that one needs to look at in their assay as not just because it's there, but because it tells you something about your assay. This, the ER assay and no matter what paper you look at, whether it's by dextran-coated charcoal assay or by the early immunofluorescent studies where they were looking at cells with immunofluorescence or by immunoperoxidase. All of the papers talk about a heterogeneity of immunostaining of tumour cell nuclei. If

Page 179

you look here, these cells that are opaque brown, that's a three plus result. You cannot see any nuclear staining through it. This is negative, okay, that's a negative result, that's just pure counterstain, this blue, pale blue. This is opaque. So, what's in between? Well, we have some that are pale, which would probably be considered to be one plus. Then we have some that are kind of granular but you can still see a little bit of blue through it. That's a two plus. So we have zero, one plus, two plus, three plus. And you should see that in a certain proportion, probably 20 percent of all ER cases that you do, you will see this kind of heterogeneity. If you don't, it suggests that the method you're using is too sensitive and what has been described in that situation, actually, is all the cells looking like this right here or like this and nothing in between. And that, in my opinion, is not an optimized assay. If you're trying to translate the results of one testing into another and you're not getting the same results, it's not an optimized test. So that's what this committee is recommending,

Page 180

that the reason they're recommending this reporting is because they recognize that this is the way that tumour cell nuclei should stain. And in words, the IHC assay for ER should be optimized so that the staining can capture this dynamic range in terms of the distribution and intensity of staining and the level of expression should therefore be a part of the interpretative results of these tests.

And lastly, negative ER results on a needle core biopsy should be repeated on the surgical excision. At Magee-Women's Hospital, and I think at probably most laboratories in the States hormone receptors and HER2 are performed initially on the core biopsy that show cancer. These are specimens that are small, they are readily fixed, there is no cutting of them required, they still need a minimum exposure time to formalin of eight hours, the results are usually out the next day and the oncologists want to know what those results are so that they can begin planning therapy even before definitive surgery takes place. So if we have a negative result for hormone receptors, completely

Page 181

negative for ER and PR on core biopsy, we want to repeat that, even with adequate controls, we want to repeat that on the excision specimen to make sure that we're not dealing with a sampling issue. Because in weakly positive cases for hormone receptors you can have significant variability from place to place in the tumour so that you can have wide areas that are completely negative and you can have some areas that are weakly positive and even some areas that are moderately positive. So that's why we will repeat it on a representative tumour block from the actual surgical excision specimen or mastectomy or whatever that would be to make it air tight that we're not missing a positive case, okay.

COFFEY, Q.C.:

Q. So, Doctor, in relation to that, I take it that on a needle core biopsy if it's positive, the ER result is positive?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. In your institution is it repeated then on the surgical excision or is it just treated as

Page 182

positive?

DR. DABBS:

A. It's treated as positive.

COFFEY, Q.C.:

Q. Positive.

DR. DABBS:

A. We don't repeat it.

COFFEY, Q.C.:

Q. And why is that?

DR. DABBS:

A. Well, because there's a body of literature that clearly shows that there's a very high correlation, better than 95 percent, of results with what you find on core biopsy compared to excision specimens. The few percent that don't correlate are usually the ones that are negative on a core biopsy that end up being focally positive on a resection specimen. And so because of that difference, that's why we repeat them on negative cores.

COFFEY, Q.C.:

Q. Doctor, if we could, please, Doctor, I'd like to take you to the section of your presentation dealing with "Best Practices, IHC, Pre-Analytic Antibody Optimization."

Page 183

Perhaps you could just kind of look through that. It's--and then--okay. Doctor, okay, if we just--if you could, please, there's a slide entitled "Best Practices, IHC, Pre-Analytic."

THE COMMISSIONER:

Q. That would be on page -

COFFEY, Q.C.:

Q. No, that's -

THE COMMISSIONER:

Q. Page 9 of the exhibit.

COFFEY, Q.C.:

Q. Page 9 of the exhibit. No, it's further along in the slide presentation itself.

THE COMMISSIONER:

Q. Down--there we go.

COFFEY, Q.C.:

Q. There we are. Doctor, here if you just go on to the next page then--I wanted to ask you, and then one more. Wait now, back, I apologize, right here. Doctor, looking at the matter of "Best Practices, IHC, Pre-Analytic Antibody Optimization."

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Page 184

Q. I have some questions for you about, you know, kind of what might happen or what are the potential effects of failing to optimize. And, for example, and you've referred to this already, the idea of diluting antibodies?
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. And so in this whole process antibodies, if they're not premixed, are diluted at some point?
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. And what else, what other types of materials are diluted in this whole process?
DR. DABBS:
A. Well the other items that could be diluted would be the secondary antibody that is put on the primary antibody. So it's critically important that the primary antibody be diluted properly and it's obviously critically important that the person who is doing those dilutions knows how to do them and to do them with consistent accuracy.

Page 185

COFFEY, Q.C.:
Q. Doctor, if something is--the primary antibody is over diluted, what can the effect be?
DR. DABBS:
A. The effect can be that there is a false negative result.
COFFEY, Q.C.:
Q. And if it's not diluted enough?
DR. DABBS:
A. If it's not diluted enough, the same result can occur, because there is usually a narrow window of opportunity for the antibodies to be properly diluted so that they bind with the antigen in the tissue properly so that there's enough antibody and it's distributed equally. If you don't have enough antibody, that could be an issue; if you have too much antibody, that also can be--lead to a false negative result.
COFFEY, Q.C.:
Q. And we've also heard reference, you've referred to it and we've heard reference from others to antigen retrieval times?
DR. DABBS:
A. Yes.

Page 186

COFFEY, Q.C.:
Q. What can the effect be of leaving--I take it an optimized antigen retrieval time is a particular amount of time a particular lab adopts?
DR. DABBS:
A. It is, it's a particular pH buffer, it's a particular time of exposure in whatever heating apparatus. There are lots of heating apparatuses that are equivalent, so it really comes down to time, you know, the duration and intensity, duration and intensity.
COFFEY, Q.C.:
Q. And, Doctor, then I take it then that within that antigen retrieval process with these multiple factors involved, that if too much of something is there, it can create false negatives?
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. Like too much time?
DR. DABBS:
A. Yes, too much antigen retrieval certainly can cause a false negative.

Page 187

COFFEY, Q.C.:
Q. And how about not enough?
DR. DABBS:
A. The same.
COFFEY, Q.C.:
Q. So there are numerous different ways of arriving at false negatives?
DR. DABBS:
A. Yes. And even with--there are instances where if you have too much antigen retrieval and there's a biotin reaction product as a result of the chromogen, that can actually yield false nuclear staining that may be interpreted by the unwary as a positive result for a nuclear antigen.
COFFEY, Q.C.:
Q. I'm sorry, could you just run that past us again, Doctor?
DR. DABBS:
A. Sure. If there's--by the ABC, the Abidin-Biotin Method, if the tissue is over antigen retrieved, that can give--that can result in the appearance of these biotin inclusions within the nucleus. And so if you're looking for a reaction product such as ER in the

Page 188

nucleus, this will give a false positive result. It's not ER and it's not PR, it's biotin. That can be unleashed in the nuclei with over antigen retrieval.

COFFEY, Q.C.:

Q. But it can appear then through the microscope?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. As if it's interpretable staining?

DR. DABBS:

A. It'll look just like it.

COFFEY, Q.C.:

Q. How do you guard against that, Doctor?

DR. DABBS:

A. Well, you have to be cognisant of the fact that if you're using an ABC protocol and making sure that the antigen retrieval duration and intensity falls within the realm of what's recommended by the manufacturer, and also during your optimization process that you obviously won't be seeing that kind of--that artifact as false positives in your tissues which should be negative, your negative controls.

Page 189

COFFEY, Q.C.:

Q. Doctor, the issue of calibration of equipment in this whole process, how important is it?

DR. DABBS:

A. The calibration of equipment is critically important. It's just, you know, every component is critically important and especially if one is doing manual procedures in terms of making optimal dilutions. Calibration of the devices that you use, be they pipettes or whatever, has to be done on a periodic basis according to prescribed protocols and most manufacturers have ways of doing that.

COFFEY, Q.C.:

Q. Now, Doctor--just a moment, please, Commissioner.

THE COMMISSIONER:

Q. If you are purchasing premixed from a manufacturer, solutions, does--would the lab check on the manufacturer's stated solutions or do you accept, would it be acceptable in the--in your world to accept what is stated on the bottle which comes in from the manufacturer?

Page 190

DR. DABBS:

A. I think that it's within the realm to accept what is stated on the bottle, but the way of testing for that is looking at the protocols which are suggested, because that's where you should be getting appropriate immuno reactivity, and if you're not, then that's when you need to take it up with the manufacturer. I'm not aware of any way that you can go into a pre-dilute and figure out what the actual titration concentration is. But rather, the proof in the pudding, so to speak, would be in the optimization process and making sure that what is positive is positive and what is negative is negative.

THE COMMISSIONER:

Q. Okay.

COFFEY, Q.C.:

Q. Doctor, in this matter I'm going to ask you about the idea of using in external controls for ER and PR testing.

DR. DABBS:

A. Um-hm.

COFFEY, Q.C.:

Q. I think you referred to already in the slide

Page 191

presentation that there can be various sources for external controls?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. And could you just elaborate a bit on that, what's appropriate from your perspective in terms of deciding what sorts of external controls to use?

DR. DABBS:

A. I think from my perspective and what I strive to do is choose external controls that are going to show a dynamic range of reactivity with the antibody in question. And so there's a variety of tissues that are acceptable for that, there cervical, normal cervix, exocervix, endocervix, there are ovarian tumours, there are even breast tumours. And so what I like to use, actually, once I have the assay optimized, is I like to find a breast cancer that we have in abundance and one that shows a dynamic range. And as I--with reference to the one slide that I showed, I like to choose a tissue section that has strong positive, intermediate positive and

Page 192

weakly positive and some negative, because that way it tells me that each time that it's placed on the patient's test control slide and you look at that, it assures you that your dynamic range is operating, that everything is operating and hitting on all cylinders, that what is strongly positive is decorated, what is negative is not and everything in between is, you know, variably expressed. That in my opinion is the ultimate external control where you have the luxury of putting that on every slide. And you can find tissue -

COFFEY, Q.C.:

Q. Put on every slide, and that is on the same slide as the patient tissue?

DR. DABBS:

A. Exactly.

COFFEY, Q.C.:

Q. Okay.

DR. DABBS:

A. Correct.

COFFEY, Q.C.:

Q. So it goes through the same process, I take it?

DR. DABBS:

Page 193

A. Correct.

COFFEY, Q.C.:

Q. So it goes through the same process, I take it, as -

DR. DABBS:

A. Goes through the same process, exactly. It also acts as a dispense control, if you will. No machine or device is 100 percent infallible, so there may be an instance where whenever the slide is in a certain position on the machine and the antibody is supposed to be squirted out, that for some reason it doesn't squirt out. Well, you'll know that from your external controls when it's negative as your test control. And you'll look at that and you'll say that's most likely a dispense issue, let's repeat it, okay. So it acts in that fashion, as well.

COFFEY, Q.C.:

Q. And, Doctor, in terms of then the phrase you used is variable--sorry, variable--not variable intensity, variable -

MR. SIMMONS:

Q. Expression.

COFFEY, Q.C.:

Page 194

Q. Expression, that's it, thank you, variable expression, thank you. For the external tissue, external control tissue, this sort of variable expression in external control tissue, Doctor, I take it, though, that the people reading the controls have to understand --would have to in order to understand what it is they're supposed to be looking for, would have to be familiar with the external control tissues?

DR. DABBS:

A. Yes, most certainly.

COFFEY, Q.C.:

Q. In order to understand the sorts of variable expression I should be looking for --

DR. DABBS:

A. Correct.

COFFEY, Q.C.:

Q. They have to be able to identify it in the first place?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. How do you--I take it as well, Doctor, though, that external control tissue has a finite--

Page 195

there's only a finite amount of it, am I correct on that?

DR. DABBS:

A. Well, to some degree. I think that every pathology laboratory can go through the effort of collecting tissues. There are always different kinds of tumours available, and even if you just look at breast cancers, you know, routine processing of tissue, one almost never puts the entire tumour through for diagnostic purposes. It's just not necessary. So you have, you know, a fair amount of left over tissue that either go to disposal or you can begin to create your own internal tissue bank so that you have these paraffin blocks set aside for use whenever, and it does take an effort on the part of the laboratory, but, you know, it's just like controlling for anything else, it's like, you know, controls for chemistry, for hematology, and what not. It's just part of the usual operating procedure for the laboratory. So my answer to that is that it's really minimal effort, the tissue is there, it's just a matter of setting it aside and having it available for sectioning and

Page 196

made into controls.

COFFEY, Q.C.:

Q. Doctor, during the time frame that the Commissioner is dealing with, certainly during most of it, there's been evidence that she's heard to the effect that for much of it there were just external controls, one external control slide being used, certainly no more than one per ER test, and sometimes there was one external control slide for two or three patients ER slides, for example, and she's also heard that generally the external controls in that context, they weren't variable, didn't involve any variety generally, the approach was to adopt an external control that stained positive, strongly positive, in fact, okay.

DR. DABBS:

A. Uh-hm.

COFFEY, Q.C.:

Q. If an external control was expected to stain strongly positive, but it actually stained weakly, that was the way the pathologist interpreted it, what, if anything, would that suggest?

Page 197

DR. DABBS:

A. That would suggest to me that the actual staining process or pre-analytic phase for that tissue is probably not optimal because if you're not getting the proper strong staining of that, one has to look at the entire process, and if it's an external control that previously had been strongly staining, then you need to look at the actual technicalities of the staining process itself.

COFFEY, Q.C.:

Q. And technicalities in this context mean what?

DR. DABBS:

A. That would mean the antibody concentrations, the secondary antibody concentrations, the development process, and everything that we talked about before.

COFFEY, Q.C.:

Q. So it would be a signal to make further inquiries?

DR. DABBS:

A. Definitely.

COFFEY, Q.C.:

Q. In a technical sense?

DR. DABBS:

Page 198

A. Yes, it is.

COFFEY, Q.C.:

Q. Doctor, you've referred to in your presentation internal controls for ER and PR testing. Can you tell the Commissioner, please, about when you first were exposed to the idea of utilizing internal controls for ER and PR testing in IHC?

DR. DABBS:

A. Uh-hm.

COFFEY, Q.C.:

Q. When were you first exposed to that?

DR. DABBS:

A. Well, I think I was exposed to that early on, and by that, I mean probably as far as my residency goes, I think it was later in my residency whenever this started to come onto the scene for immunohistochemistry for ER. It would have been '84, 1984, 1983.

COFFEY, Q.C.:

Q. So sometime in the mid 80s?

DR. DABBS:

A. Mid 80s, yes, and further--and the issue there was that if there are normal breast elements present, they will stain to some degree, and

Page 199

we do know that normal breast elements will generally have a heterogeneous expression as well. There will be some that are weak and some that are very strong, and generally it's a minority of normal breast epithelium that is ER positive. So the issue there was if you--if it was entirely negative, that was generally a concern about the process and in the absence of appropriate externa controls, would be a cause to look into why--basically query as to why these elements should be staining or not staining, so you have to look at the staining process.

COFFEY, Q.C.:

Q. And that was in the early days. As time went on, Doctor, within your profession where you practised, did the role of--did the importance or significance of internal controls to the whole process change over time or has it generally remained the same?

DR. DABBS:

A. I think it's generally remained pretty much the same. No matter what antigen you're looking for, if you're looking for--you know, if you have a tumour and you do a keratin

Page 200

stain because you want to determine whether it's a carcinoma, and there are normal epithelial elements there, the normal epithelial elements, depending on what keratin you're using, should stain to some degree, and if they're not, then that becomes an issue. Then your internal controls are not working properly which suggests that there's something inherently wrong with either the pre-analytic or analytic phases.

COFFEY, Q.C.:

Q. Now today, Doctor, in your laboratory at Magee, how are then--how do you understand then pathologists handle the issue of internal controls for ER/PR, how is it handled now?

DR. DABBS:

A. Well, the ER/PR is performed on core biopsies, and as I'm sure you're aware, sometimes core biopsies of the breast may not contain normal elements because the radiologist might hit the tumour dead centre and get entirely tumour. We do not reject the specimen because of that. That's where our external controls become as important as an internal control. Our policy would be whether or not we had internal

Page 201

controls, if the tumour is negative, we repeat it, and I mentioned that, but in the instance where --

COFFEY, Q.C.:

Q. I'm sorry, just tell us that again.

DR. DABBS:

A. Sure. If we have a core biopsy that is entirely negative for ER and PR, that we will repeat that regardless of whether there's an internal control present or not, positive, for purposes of sampling, but for the internal control itself, if we have a tissue, a core biopsy that does not have an internal control on it, we will not reject that specimen. We will do the testing and if the result is entirely negative, we will just default and repeat that on the specimen. If it's positive, and we have an adequate external control, then we're good, we report that test as a test result.

COFFEY, Q.C.:

Q. And, of course, in this context that you're speaking about now in the present day, these external control tissue is actually on the patient slide?

Page 202

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. So it's gone through the same process --

DR. DABBS:

A. Right.

COFFEY, Q.C.:

Q. As the patient tissue has on the slide. Doctor, you're the author of a text relating to diagnostic immunohistochemistry. Can you tell the Commissioner, please, from your perspective--contributing author and editor, to get it entirely correct. From your perspective, what's your view as to the reproducibility and reliability of immunohistochemistry if done appropriately?

DR. DABBS:

A. Speaking of hormone receptors or in general?

COFFEY, Q.C.:

Q. In general first, and then hormone receptors.

DR. DABBS:

A. In general, I think that immunohistochemistry is a robust test that when performed optimally will give very consistent, accurate, and high reproducible results. The nature of

Page 203

immunohistochemistry is that there is a literature base upon which there are published studies that talk about various clones of antibodies and sometimes different techniques, detection techniques, some of which are more sensitive than others. So there will be some variability, though small, if you're looking at specific tests, and if you look, for example, at estrogen receptor, generally speaking those three antibodies that I mentioned, the SF11, 1D5, and SP1, are very robust antibodies and will give very comparable results. Depending on what your cutoffs are, that's where you will see your variability in determining how much is a positive result, but the way that most people result them, there's really very little variability. I would say, you know, just for ER and maybe immunohistochemistry in general, is somewhere between 1 in 5 percent if you look at variation because of different clones of antibody, different detection systems. Everything else being equal, the results are really small.

COFFEY, Q.C.:

Page 204

Q. You mean the differences in results?
DR. DABBS:
A. The differences are small and probably no different than other very well controlled rigorous studies for testing in clinical pathology.
COFFEY, Q.C.:
Q. Doctor --
THE COMMISSIONER:
Q. Sorry, I just want to make sure I got that last point. When you're saying that, in effect, for IHC in general, and certainly for --and for ER, the variation in result which you would expect to be observed is really no different than the rest of the pathology lab?
DR. DABBS:
A. Generally speaking, any process where all of these variables that I'm talking about, the pre-analytic or highly controlled, yes.
THE COMMISSIONER:
Q. So done right?
DR. DABBS:
A. Done right,yes.
THE COMMISSIONER:
Q. Done right, you really wouldn't put IHC in a

Page 205

class that was any different than the rest of the pathology lab?
DR. DABBS:
A. No, I would not. In fact, right now there are some commercial tests available in the States in which they're studied to look at a different testing method, RTPCR, Reverse Transcriptase PCR reactions for hormone receptor testing, and basically what they're doing is they're using immunohistochemistry as the benchmark, and they're finding very tight correlation with that, and these are very highly sensitive techniques that rely predominantly on messenger RNA, which is present in cell cytoplasm. So I think that's an attestation to tests that are well done will pretty much speak for themselves, and just recently we have some data where we looked at our results of ER/PR compared to external laboratory RTPCR and there's a 100 percent concordance between test results for estrogen receptor. If you plot them out on a graphic form, I think the P value was something like .51, which means that you can draw a line through it. The data points are

Page 206

moderately distributed, but nevertheless the concordance between what you call positive and a negative result is 100 percent. So I think that immunohistochemistry has come a long way, I think that the results are highly robust, reproducible and accurate and becoming more so for these really critically important predictive and prognostic factors, and the CAP ASCO guidelines addressed this a couple of years ago, and now we're trying to tighten it up on this end with the hormone receptor testing.
COFFEY, Q.C.:
Q. Doctor, from your perspective, is there what could be termed an acceptable error rate in ER/PR testing, you know, assuming that the testing procedure has been optimized? Is there any such thing as kind of an acceptable error rate, and I say error--one might not know which of two tests is the correct?
DR. DABBS:
A. I can look at that question in several ways, I would think. What threshold would I want in our laboratory if we had a repeat rate. I would want that to be less than 2 percent, and

Page 207

we monitor that. WE have a monthly meeting where we look at all of those issues, and I know that that's what we monitor for immunohistochemistry in general. So if we starting bumping up to 2 percent or more, then we're going to go in and find out exactly where it is that the issues are, and it may not be IHC itself, it may be tissue lifting off a slide or something like that.
COFFEY, Q.C.:
Q. I'm sorry, so what is this up to 2 percent, what does this refer to, Doctor?
DR. DABBS:
A. Well, that's our threshold for looking at repeats for immunohistochemistry in our laboratory. So if we have--our expected repeat rate for immunohistochemistry, in general, is under 2 percent.
COFFEY, Q.C.:
Q. So the Commissioner understands this, of all the tests done in, say, any one month in your laboratory --
DR. DABBS:
A. Correct.
COFFEY, Q.C.:

Page 208

Q. I take it, it is not unknown to have to repeat an IHC test?
DR. DABBS:
A. Correct.
COFFEY, Q.C.:
Q. That's not unusual.
DR. DABBS:
A. Right.
COFFEY, Q.C.:
Q. But you keep track of--your laboratory does, how often that happens per month?
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. And if the rate of repeating hits 2 percent, there are questions asked and inquiries made?
DR. DABBS:
A. Exactly.
COFFEY, Q.C.:
Q. Why is that, Doctor?
DR. DABBS:
A. Well, I think it's a reflection of good practice and that you want to know what it is that's being repeated and what it is that's starting to bump up against that figure. It

Page 209

may be something that's actually unrelated to the immunohistochemistry staining procedure itself, it might be related to tissue lifting off a slide for some reason. You know, perhaps a new histo technician who is on board who may not have followed procedure completely and didn't bake the slides long enough. I'm just making up an example.
COFFEY, Q.C.:
Q. Sure.
DR. DABBS:
A. So you're wondering why that is, and so that's how we get to keep our finger on the pulse of testing and issues related to it because we want to be able to act proactively as soon as we can if we see something like that happening. I think it's good practice.
COFFEY, Q.C.:
Q. Doctor, the Commissioner has heard evidence, of course, about--much evidence about what has been phrased "conversions" here in this matter. Doctor, several questions in this regard. From your perspective, what could account for or what might potentially account for a patient who is originally--upon

Page 210

originally being tested, okay, for ER and PR, in particular we'll use ER right now, a patient who when originally tested for ER was classified as a no expressor, zero percent, and on retesting became what I'll refer to as a high expressor, which as I say, I'll pick a figure, 80 percent or 90 percent, using the same block, albeit done in two different laboratories, what could account for the difference?
DR. DABBS:
A. I think it's probably safe to say that the one thing that would not account for it would be tissue fixation because if you're going from a negative to--would be a strong positive result, it's most certainly--almost certainly not tissue fixation, but rather technique, and by technique, I mean, this could be anywhere from all the steps that I demonstrated. Initial--the concentration, the tighter of antibody, if that is not done correctly, you're going to end up with a negative result.
COFFEY, Q.C.:
Q. In the original --
DR. DABBS:

Page 211

A. In the original lab, which when properly done in another lab will show up as strongly positive. It's not going to be related to antibody clone because the clones that are out there now are all fairly robust and very comparable, so that's not going to be a cause for it.
COFFEY, Q.C.:
Q. It wouldn't account for a low--no expressor to a high expressor?
DR. DABBS:
A. Correct, no, it would not. So here we're talking about technique. We're talking about probably, you know, tighter of antibody would be a crucial component, again a proper dehydration and de-paraffinization, and, of course, antigen retrieval is crucial there as well. If antigen retrieval techniques are comparable in this situation that you're telling me, then we're really pointing all fingers towards the initial antibody dilution being improper and giving an original negative result, which then when properly diluted, will give you the appropriate result.
COFFEY, Q.C.:

Page 212

Q. And that, of course, assumes that the antigen retrieval methods --
DR. DABBS:
A. Are comparable.
COFFEY, Q.C.:
Q. And if they're not, if there are any one or more deficiencies in that in the original laboratory, it could account for a no expressor to a high expressor in the retest lab?
DR. DABBS:
A. Yes, correct, and, you know, it's not just about heat and duration, but it's also about buffers. So if the buffer calls for basic pH, but it's being done in an acetic pH, that would obviously be a huge defect that could account for a false negative.
THE COMMISSIONER:
Q. Doctor, you said it's not going to be related to antibody clone?
DR. DABBS:
A. Right.
THE COMMISSIONER:
Q. Because these things are quite standard, as I understand?

Page 213

DR. DABBS:
A. They're fairly standard and fairly robust, and typically what does not happen is you go from a negative to a strong positive with any one antibody.
THE COMMISSIONER:
Q. And would you say that same thing of the full time period between 1997 and 2005?
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. And the clones you're talking about are 125 --
DR. DABBS:
A. 25 and 6F11 were the prevalent clones at that time, yes.
COFFEY, Q.C.:
Q. And the Commissioner has heard evidence that they are the two really in play here, I understand.
DR. DABBS:
A. Right, the SP1 is a more recent addition within the last two to three years.
COFFEY, Q.C.:
Q. Doctor, in effect, the same question, but in relation to going from a no expressor, zero

Page 214

percent, to what I'll refer to as a low expressor, say, you know, somewhere in the low 1 to 10.
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. Or perhaps 1 to 15, and going from zero to a relatively low number --
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. Zero in the original lab, St. John's in this case, and Mount Sinai perhaps reports it at 5 or 10.
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. What could account for that?
DR. DABBS:
A. I think the exact same issues that I just mentioned could account for it. There's another--this particular scenario also raises the possibility of primary poor fixation as well that may not have been picked up with as sensitive a technique locally, and--but when

Page 215

properly performed assay comes out to a weakly positive result elsewhere, so I could not exclude primary tissue fixation as being another variable. That might be an issue there.
COFFEY, Q.C.:
Q. And this would be under or over fixation?
DR. DABBS:
A. Under.
COFFEY, Q.C.:
Q. Under fixation initially?
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. So what is a less than optimally fixed tissue, an under fixed tissue, particularly if the tissue inherently is a low expressor --
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. Then using less than optimized techniques in the initial hospital, you might get zero --
DR. DABBS:
A. Yes.
COFFEY, Q.C.:

Page 216

Q. At least available to what the pathologist could see --
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. But run under optimal conditions using an optimal process on retest, the low expressor would properly low express despite the fixation problems?
DR. DABBS:
A. Correct, and even then the tissue may, in fact, be properly fixed, but it's a matter again of the technique. So it may just be a technique, it may be antibody dilution, antigen retrieval.
COFFEY, Q.C.:
Q. Doctor, what about a situation where the initial test showed the result was interpreted of what would fall into the category of being a low expressor, between 1 and 10, for example, becoming a high expressor on retesting, what could account for that, for example, something was originally reported as five and on repeat is--on retesting is reported as 95?

Page 217

DR. DABBS:
A. Again I think it's safe to say in that situation that the primary tissue fixation is probably all right, that it's about technique and once again falls back to antibody dilution or second antibody dilution antigen retrieval, the technique.
COFFEY, Q.C.:
Q. Doctor, I'll ask you this, and I point out, Commissioner, that just listening recently to Dr. Denic on the four retro converters, he attributed those to interpretation issues, but leaving that aside for the moment, Doctor, I'm going to ask you about particular potentiality, okay. What is in the original test reported as a low expressor, or fall into the low expressor category between 1 and 10, upon retesting going to zero, retesting in a second laboratory, the same block, is there anything that could account for that that you can think of, assuming that the second laboratory's technique is optimized, and perhaps the first laboratory may or may not be optimized?
DR. DABBS:

Page 218

A. Yes, yes.
COFFEY, Q.C.:
Q. Is there anything that could account for it?
DR. DABBS:
A. Well, I think that there are two issues that come to mind. One would be, of course, whenever you start cutting through a paraffin block, two things happen. One is you get somewhat of a different sample. So if you're going from something which is weak to begin with, you may cut into--cut a little deeper and get an area that's entirely negative, okay. Another would be if that tissue were not optimally fixed up front and, say, perceived far less than what is considered to be the best nowadays, the tissue up front gave a weak result, and with time with that tissue sitting in paraffin, it degenerated even further, okay. So when you cut it, you will get a negative result. So this is the two chief things that I can think of.
COFFEY, Q.C.:
Q. Just on that point, Doctor, because that's something that I stand to be corrected, I don't believe it's been raised here before,

Page 219

before the Commissioner, less than optimal fixing, under fixing --
DR. DABBS:
A. Uh-hm.
COFFEY, Q.C.:
Q. What happens after--what can potentially happen to that tissue after it goes into the paraffin block? What, if anything, is still going on or potentially could go on within the tissue itself in the paraffin block?
DR. DABBS:
A. Yes, I think that with--well, with something that's been less than optimally fixed, probably the initial sections that come off that block are probably going to be the best fixed of the lot, if you will, because generally formalin penetrates into tissue at the rate of 1 millimetre per hour. So if you're assuming that it got at least, you know, an hour or one to three hours, somewhere around two to three millimetres of that whole thickness will have seen some formalin, and be less than optimally fixed. So it wouldn't be surprising if you got a weak result there, but as you penetrate deeper into that, it's even

Page 220

more poorly fixed or not fixed at all. So once you start slicing into that tissue further, you're going to be getting tissue that's even less well fixed, and material that's not fixed. So it's not going to be an easy--it's not going to be easy to obtain any result from that. What actually happens to it, it's in paraffin, it's likely to--it's not likely to degenerate any further, but if it's only been in formalin for a very short period of time, it's not raw tissue, but it's not fixed tissue either.

COFFEY, Q.C.:

Q. Doctor, can you tell the Commissioner, please --I'm going to ask you some things from your perspective kind of the practicalities of the ER/PR testing process as a pathologist. Doctor, if you are asked for a particular--I appreciate in your institution you're using generally needle core biopsies.

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. So--but as well I'll ask you to address your mind to the idea of using excisional biopsies

Page 221

as well. When you go looking for which block to utilize for a patient, how do you go about it, what thought process do you bring to it?

DR. DABBS:

A. Sure. I think first of all you have to look at the entire case, so you will have looked at all of the slides, and then you will want to pull one that is representative of the tumour, and generally the morphology of breast cancer is they tend to have a reasonable fidelity from block to block, although on occasion you will find tumours that are mixed, that are in some areas better differentiated or less differentiated, so you want to try to choose a block that has something that representative of the entire tumour, especially areas that are of better differentiation, if you will, because we know by morphology the tumours that have better differentiation are more likely to be ER positive, and that's what it's really all about, trying to determine if the patient is ER positive. So that's the basis you would choose the block.

COFFEY, Q.C.:

Q. And, Doctor, in relation to that, is the

Page 222

presence of tissue that could form an internal control have any relevance?

DR. DABBS:

A. Yes, it would. You would want to choose a block that has normal tissue on it, and--along with tumour. One of the new recommendations is that if you want to--it might be optimal to take a portion of normal breast and put it in the same block so that you're assured of having that somewhere, but in most cases where there's tumour, you can always take a section that has adjacent normal breast tissue.

COFFEY, Q.C.:

Q. Doctor, so you've chosen the most appropriate block in your approach and it's sent off to be processed and you get your slides back. Doctor, can you tell us, please, in what sorts of circumstances in relation to an ER and PR test would you cause them to be repeated--what would cause you -

DR. DABBS:

A. To be repeated?

COFFEY, Q.C.:

Q. Yes.

Page 223

DR. DABBS:

A. Well, given that there's normal control tissue on it, if the normal control tissue was not staining at all, if the tissue was somehow lost anywhere as a result of either poor dehydration or poor drying such that the tissue was lifted up or part of it was missing, if there was any aberrant staining that couldn't be explained, and by that, I mean, cytoplasm or other components of tissue staining that shouldn't be staining. In other words, tissues that should be negative that is staining positive, like fat and stroma, inflammatory cells, things like that. And of course, if the morphology is such that given everything else is working and the tumour which may be well differentiated, typical high expressor is negative, that would be cause for concern and for a repeat.

COFFEY, Q.C.:

Q. Now in this context then, a typical high expressor, what are you referring to there?

DR. DABBS:

A. Well, those are tumours that would be well differentiated adenocarcinomas. They

Page 224

typically are Nottingham score, Nottingham grade one. They would include things like tubular cancers, classical papillary cancers, and other tumours such as lobular carcinomas, invasive lobular carcinomas. Also, looking at the comparable in situ components of these diseases, ductal carcinoma in situ of low grade, lobular carcinoma in situ of classical type. They're all high expressors uniformly, and so if something like that came negative, especially in the face of a negative control, that would be a red alert to stop and have this repeated and in fact, look at the entire batch run for that day, and then go from there and process that problem.

COFFEY, Q.C.:

Q. So bearing in mind the histology then, from having--and by that point determined, from your perspective, what the histology was suggesting to you about the type of tumour it was, you'd bear that in mind and compare that to--and your expectations accordingly, with what you were seeing on the slide?

DR. DABBS:

A. Yes, indeed.

Page 225

COFFEY, Q.C.:

Q. And lobular invasive, invasive lobular should be, more often than not, most often, positive?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. And if it doesn't, the slide is not appearing positive to you, the tissue, patient's tumour tissue, you would cause questions to be asked about that?

DR. DABBS:

A. That would be a deep concern, yes.

COFFEY, Q.C.:

Q. Doctor, in relation to that, I mean, these various classes, what you've referred to as typically high expressors, Doctor, when did you first become aware of that, and adopt that sort of approach? How far back in your career does that go?

DR. DABBS:

A. Well, I think, as a resident, when we were looking at these cases, it became clear that there were subsets of tumours which we call special types and these include tubular, papillary, mucinous variance. These were all

Page 226

well differentiated and in their classic form, they're virtually all positive. Lobular carcinomas, while not being classified as a special type of ductal cancer, because it's not a ductal cancer, is virtually always positive for ER and that's--you know, that's part of what I learned in my residency, and so given the correlation of studies that subsequently occurred with that, the histology and whatnot, it all sort of made sense that there's tumours that are well differentiated or most like normal are in fact going to express ER.

COFFEY, Q.C.:

Q. Doctor, I'd like to show you some documents. Doctor, before I do that, there was another exhibit that we'd entered today. It's Exhibit P-2629. It's entitled "Comparison of Evaluations for Hormone Receptors in Breast Carcinoma using two manual and three automated immunohistochemical assays." Go into this, Doctor, I believe you had asked that this be entered.

DR. DABBS:

A. Yes.

Page 227

COFFEY, Q.C.:

Q. Can you tell us, please, about this?

DR. DABBS:

A. Yes, actually, I was a reviewer for this paper. This paper came to me because I'm on the editorial board of American Journal of Clinical Pathology, and I was intrigued by it because it was really the first paper that I had run across that actually compared different platforms, very different platforms, including manual assays, and this paper included products by Biogenics, by DAKO and by Ventana, and you know, basically, if you scroll down the paper to the point of the tables that are illustrated here, I think you'll get a feel for the general robustness of the immunostaining platforms and, you know, they have here, for example, over here, the Biogenics, manual versus automated, and over here, the codes for the DAKO manual, DAKO automated and Ventana automated, and the charts are really very comparable, if you look at the incidents of positive cases, the intensity of cases.

So while they did demonstrate some

Page 228

variations in intensity and proportion of cell staining, they really were not dramatically different. These--I thought that one thing that this paper showed and the reason why it caught my eye, as a reviewer, is because it does attest to the antibodies that are used and their robustness. You can use different platforms. When properly assayed, they turn out to be very favourable and there really aren't going to be any disastrous differences in the way the test results are reported. So the concordance between the different groups were good and, you know, if you look at this table here, the distribution of total scores, you know, as you just sort of glance across those, you'll see while there are differences in scores, but they're not dramatically different. There's nothing that's really turning out to be negative where the other methods are showing positive and whatnot. So that an attestation in my view of the robustness of this antibody group, and I think the clones that they use in here for DAKO is 1D5, for the Ventana was 6F11, and the Biogenics, I forget exactly what clone it is.

Page 229

It's in here somewhere. But that was the key point that I wanted to make, that it's generally a robust assay and it's hard to-- when properly performed, it's hard to steer it wrong.

COFFEY, Q.C.:

Q. Doctor, if we could bring up, please, Exhibit P-1850? Now Doctor, this is a memorandum that was written back on February, dated February 16th, 1998, by Dr. Mahmoud Khalifa. The reference is "reporting of estrogen and progesterone receptor immunohistochemical results" and the Commissioner has heard evidence that, in fact, this was sent to all pathologists throughout Newfoundland at the time, and in effect, at the time, if you read down through it, he says "it has been suggested that assessment of ER/PR status, invasive breast carcinoma, be performed IHC on formalin fixed paraffin embedded tissues," and before that, and in fact, up to that point in time, there'd been a biochemical assay approach utilized in Newfoundland, Doctor.

So Doctor, I believe you've had an opportunity to at least review this in a

Page 230

cursory way, and as well here, Doctor, there is, on page four of this, there's--and into page five and six, immunohistochemical staining of steroid receptors. It reads "correlation with biochemistry" and then five, and then goes on down into page six, at that time, and actually, I believe, 19 ER cases and 17 PR looked at.

Doctor, and again, just perhaps to try and put this in perspective for the Commissioner, your own practice as a pathologist covers the bridge time between biochemical assay being routinely used and IHC ER/PR?

DR. DABBS:

A. Yes, it does.

COFFEY, Q.C.:

Q. Okay. Doctor, based upon your observations, and I think most, if not all your professional life, has been in the United States?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. What, if anything, did you observe about what would happen when institutions moved from

Page 231

biochemical assays to ER/PR IHC testing? Was there correlations done? How extensive, or correlations not done, and just started up? What were your observations?

DR. DABBS:

A. Yes. Well, I think some of the early papers, in fact beginning with that very first paper by Perchuck where they looked at immunofluorescence, they made a comment. It was almost--it wasn't quite an afterthought, but they obviously did compare their results with the DCC and said that cases that were deemed to be positive by DCC were ten percent or more positive cells by immunofluorescence, and that was sort of like the shot across the bow, the first time that that had ever been reported as such. But as you look at the papers throughout that were subsequently reported, every one seemed to gravitate to ten percent. Some of them clearly stated that they used ten percent as an arbitrary cut off.

The other paper by Perchuck, in 1996, where they looked at the 1D5, actually with outcome, and they used a ten percent cut off and said that this, you know, the patient

Page 232

correlation with outcome was better with the 1D5 antibody at ten percent than it was with the dextran-coated charcoal method. One year before that, Mascarelli, using the 1D5, did an outcome study where they arbitrarily chose five percent and ten percent, and both of the outcomes of those patients was very good with endocrine therapy, suggesting that you could even go below ten percent with that. So if you look throughout the history, you will find that ten percent seemed to be a gravitational number by which people gravitated to.

Now one of the things that pathologists need to be aware of is whenever they see results like this, they need to pay attention to what is the paper exactly talking about? What's the clone that they're using? What's the method they're using? Because if you're using their clone and their method, then you have the right to use their cut off. But if you're using a different clone and a different method, you have no right to use that cut off, because it hasn't been validated with an outcome.

COFFEY, Q.C.:

Page 233

Q. So in terms of then the idea of, for example, here, there's 30 percent. Dr. Khalifa does not say he advocates 30 percent. There's no--he's actually testified here and when you read this carefully, he says it's only a suggestion, suggestion in terms of even the manner in which it was to be reported. But here, on page three, there are examples. Example two says comment, "evidence from the available literature indicates that estrogen receptor immuno-reactivity detected in less than 30 percent of neoplastic cells would most likely correspond to a negative result in a biochemical assay of the same specimen. American Journal of Surgical Pathology in 1990." Okay. Have you had a chance to look at that, Doctor?

DR. DABBS:

A. I did look at that paper and, you know, the issue--I don't believe that what is actually stated here accurately reflects the content of that paper. The paper actually looked at--it looked at the dextran-coated charcoal method and the immunohistochemistry assay of a monoclonal antibody against 17 beta estradiol,

Page 234

which is not an estrogen receptor protein. It's the hormone that's number one that they were looking at. Number two, what they looked at, this 30 percent number, was something that they obtained by looking at what is called a receiver operator curve and what that is, it's an ordinate and abscissa, okay, an X and a Y axis, and depending on how this curve looks, it tells you about the sensitivity and specificity. Simply put, the best test, a best test theoretically, would be 100 percent sensitive and 100 percent specific, okay, and that curve, you wouldn't be able to see because it would be the ordinate and the abscissa, okay. For something that's less than that, it's going to sort of be like a parabolic arc, okay, and the worse the sensitivity and specificity, the smaller that curve will be. The better the sensitivity and specificity, the highest area under that curve, the higher area of that curve.

So what they looked at was they compared the percent of cells that are positive by immunohistochemistry with the ROC for the assay. There was no outcome related in that

Page 235

study whatsoever. And they said whenever we consider, if we consider the 30 percent positive immunohistochemistry cells are, then our test has this sensitivity and specificity. That's all that said. It didn't recommend it as a cutoff, there was no outcome associated with it. So I have trouble with this statement saying that "Immunoreactivity detected in less than 30 percent of neoplastic cells most like correspond to a negative result." That's not what the paper said. The paper said at 30 percent cutoff, that they had optimal sensitivity and specificity for the test is what it meant. And again, there was no outcome associated with that.

COFFEY, Q.C.:

Q. Doctor, here Dr. Khalifa does--I'll just go back up here, refer to, on the first page here of the memo, the second paragraph, he said, "The division of pathology of the Health Care Corporation of St. John's has been applying this technology" which would be the IHC formalin fixed paraffin embedded tissue, "for over a year. Recent audits correlating IHC with biochemical results in selected specimens

Page 236

for both techniques have been running parallel have shown high accuracy in the introduced IHC detection. The results of these audits have been discussed in several meetings and are available for review." And we understand that the fourth, fifth, and sixth pages of this particular document reflect that review. Doctor, in introducing something, like in moving, for example, from a biochemical assay to IHC on paraffin embedded tissue, comparing the results, like, for example, 17 and 19 cases, I mean, what kind of--in your view would that be enough, could you do with fewer or should you use more?

DR. DABBS:

A. Between 17 and 19 case?

COFFEY, Q.C.:

Q. Well, yes, here is--because if you actually look at them, you count them up.

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. Okay. He says these are ones with 100 percent concordant cases, agreement on both receptors, which has those there. And you can kind of

Page 237

look down through them, it's -

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. And on through them. And then on to the next page, and 100 percent concordant cases agree on both receptors and then 50 percent concordant cases, agreement in only one receptor. And then when you look at kind of--this is a table.

DR. DABBS:

A. Right.

COFFEY, Q.C.:

Q. A spreadsheet, in effect. And what I wanted to ask you about really is is the idea of utilizing, for example, a sampling of by comparison correlation of less than 20 in introducing such a new technique, you know, in comparison to one that you've been utilizing and relying upon?

DR. DABBS:

A. Sure.

COFFEY, Q.C.:

Q. Would 20 be enough?

DR. DABBS:

Page 238

A. For something like this, no, I think that you'd probably want to get at least something like 50. And again, I don't know that there really were any good guidelines back at that time to, you know, say how many do I need to do a correlational study. But this is a pretty small sample. The other aspect, I think, that as I recall from this, is that the cutoffs for the Ligand Binding assay were a little on the--were higher than what I was used to seeing. I think it was this, that was the immunohis--biochemical assay, yeah. The biochemical reporting typically in the States was reported at less than three was a negative result, above that was a positive result. So these are probably comparable here. But they really weren't broken down as from three to 20 is equivocal, that was considered to be a positive result, is positive.

COFFEY, Q.C.:

Q. Okay, just in your experience in the US?

DR. DABBS:

A. Yes. And all--yes. And in the early papers and the papers on DCC following the paper by McGuire where he used a three cutoff, everyone

Page 239

else pretty much adopted what he did, as well. On occasional paper it might go up to ten, but generally it was for most, probably the most frequent number that I had seen was three for the DCC.

THE COMMISSIONER:

Q. Mr. Coffey, wherever you can find a convenient spot, we'll take the afternoon break.

COFFEY, Q.C.:

Q. Now would be convenient, actually, Commissioner.

THE COMMISSIONER:

Q. All right, then.

COFFEY, Q.C.:

Q. Then we'll go on to something.

THE COMMISSIONER:

Q. We'll take a few moments.

(RECESS)

COFFEY, Q.C.:

Q. Doctor, before we leave Exhibit P-1850, Doctor, now the paper reference by O'Keane, by Doctor, I'm sorry, Khalifa, O'Keane's paper of 1990, Doctor, by 1998 in terms of the idea of cutoffs for ER and PR results, percentage of results, by 1998 what other papers existed in

Page 240

terms of the percentages that they suggested, you know, had appropriate relevance to, for example, clinical outcomes in comparison to other sorts of testing? Could you just tell us, you referred to them, some of them already, but I'd like you to kind of list them for the Commissioner again?

DR. DABBS:

A. Sure. Well, the--sure. Two of the key papers that came out in the mid '90s, one was by Mascarelli in 1995 where they used the 1D5 clone and looked at arbitrary cutoffs of five and ten percent. And then the other paper was by Perchuck showing that the 1D5 was superior to DCC in predicting outcome in patients treated with endocrine therapy, again, using the 1D5 and with a ten percent cutoff. And so that became -

COFFEY, Q.C.:

Q. So do you recall what year Perchuck's paper was?

DR. DABBS:

A. That was '96.

COFFEY, Q.C.:

Q. '96, okay.

Page 241

DR. DABBS:

A. Yes. And so, you know, even before that there were several papers that weren't necessarily tightly honed in to outcomes, but people were using the ten percent number. And then once these two papers came out I think that became pretty much probably the most common cutoff that people were using, at least in the States at the time. And then subsequently, you know, as I mentioned, three years later Harvey's paper and things went down to one percent. And but as I mentioned, the paper by O'Keane is not really associated with an outcome, per se, but what he is comparing is he's looking at the sensitivity and specificity of the dextran-coated charcoal test compared to IHC and concludes that if you choose 30 percent of positive cells by IHC as a positive result, then you maximize your sensitivity and specificity by the DCC test.

COFFEY, Q.C.:

Q. If you choose?

DR. DABBS:

A. If you choose. But there's no outcome associated with it. I saw that paper as comparing two different tests and nothing more. There was no outcome associated with that.

COFFEY, Q.C.:

Q. Doctor, by 1997 in your experience was IHC being utilized in terms of using paraffin embedded tissue, was it being widely utilized in North America?

DR. DABBS:

A. Yes, it was being widely utilized.

COFFEY, Q.C.:

Q. And at that time would there have been a number of tertiary care laboratories, at least to your knowledge, utilizing it?

DR. DABBS:

A. Yes, indeed.

COFFEY, Q.C.:

Q. And would it have been possible, you know, at the time, if a particular medical centre was going to adopt ER/PR by IHC as a new approach, replacing the biochemical assay approach, was it possible at the time then to avail of or utilize, compare your IHC results internally with IHC results obtained elsewhere, could you have done that kind of comparison?

Page 243

DR. DABBS:

A. Well, if one is going to adopt IHC having used DCC, then the best way to do this, and Battifora actually wrote about this, that the proper way to do that would be to look at your outcomes with the DCC and then compare your IHC with that. That way you're not just comparing test to test, but you're comparing the outcomes and that's what Perchuck and Mascarelli had done. If one wants to do IHC and adopt an external method, a method from another laboratory that had been validated, that would be an acceptable way. But as I mentioned earlier, if you're going to do that, you have to use precisely the same clone and the same methodology to make sure that you're recapitulating the exact conditions that the other investigator or the other laboratory used, and that would be an acceptable way. So for example, nowadays when people use one percent cutoffs or one percent or more, that has been, those outcome studies have been done for 6F11, 1D5 and SP1, the only--the devil is in the details, you have to look at those and see how those particular assays were performed

Page 244

and whether your metrics then are on par with what those people reported.

COFFEY, Q.C.:

Q. I believe in the '96 paper by Perchuck?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. You refer to--I'm sorry, would you just repeat again, what was it he found?

DR. DABBS:

A. He found that the 1D5, using the 1D5 antibody was better than the DCC method of predicting response to endocrine therapy.

COFFEY, Q.C.:

Q. So the 1D5 IHC approach?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. Was, in fact, better than?

DR. DABBS:

A. Better than.

COFFEY, Q.C.:

Q. From the old biochemical?

DR. DABBS:

A. Correct.

Page 245

COFFEY, Q.C.:

Q. Assay approach.

THE COMMISSIONER:

Q. Do I--I just make sure I understand this correctly. But the way that is determined is by looking at outcome for patients?

DR. DABBS:

A. Yes.

THE COMMISSIONER:

Q. And the method chosen to determine the use of IHC for ER/PR in this province, as I understood the evidence of Dr. Khalifa, was to try to reproduce, using IHC, the result that you would have gotten with the bioassay method?

DR. DABBS:

A. Yes.

THE COMMISSIONER:

Q. Now, it just seemed to me then, I've said it to another witness, that that's kind of like putting--that's putting your foundation as your foundation, a house of cards, because unless you are confident that the bioassay method was producing a great result, then you might, in fact, be establishing an IHC method

Page 246

which gives you less than the optimal result?

DR. DABBS:

A. Um-hm.

THE COMMISSIONER:

Q. Am I reasoning that correctly or missing something?

DR. DABBS:

A. Yes, I understand what you're thinking there. And I guess the best way I would describe this would be that based on the cutoffs that were used in the study by Perchuck in looking at the endocrine response of patients, they found that the results that they used from ten percent cells and greater was a better predictor of outcome, they could better predict the outcome of response to therapy than using those cutoff values for the DCC test.

THE COMMISSIONER:

Q. Okay.

DR. DABBS:

A. Now, what that suggested to me was that there were more likely a number of false negatives in that DCC test. And for the issues that I described earlier in terms of sampling

Page 247

predominantly -

THE COMMISSIONER:

Q. Um-hm.

DR. DABBS:

A. - I think that what Dr. Khalifa described in, or actually referred to in the paper by O'Keane is something very different. O'Keane's paper said, here's our results on DCC and here's what we have on IHC. If I want to--what he, basically what he did was he said, if I want to maximize my sensitivity and specificity for the DCC test, that translates into 30 percent positive cells by IHC, okay.

THE COMMISSIONER:

Q. Yeah.

DR. DABBS:

A. And to me that's comparing apples and oranges, because you're trying to take the sensitivity and specificity of one test and translate it to a different test, which is impossible to do in reality. So that's what that paper was about. It was not about outcomes. It basically said if you want to have the same sensitivity and specificity of DCC, then you have to call 30 percent positive IHC cells.

Page 248

Well, when I read that paper, I actually skipped over it and didn't even include it in the papers that I took seriously because I saw the flaw in that, that apparent attempted translation, because if you notice, no one before that or after that advocated those numbers.

THE COMMISSIONER:

Q. Um-hm.

DR. DABBS:

A. This paper, I think, was largely ignored in the literature, and for good reason. It compared--it tried to compare a sensitivity of the test with a totally different methodology with one that was in place.

THE COMMISSIONER:

Q. Okay. So if we come back to the lab in 1997 or, you know, a little--in the mid '90s, put it that way, which was deciding to switch to, for what would seem to me to be valid reasons, IHC, less cost, perhaps potentially better reliability?

DR. DABBS:

A. Yes.

THE COMMISSIONER:

Page 249

Q. Then if one wanted to do that, what in your view would be the optimal way of determining what IHC was going to look like within your lab, the cutoffs, what solutions you would be using, that kind of thing?

DR. DABBS:

A. Right.

THE COMMISSIONER:

Q. How would you go about doing that?

DR. DABBS:

A. I think the way to go about that would have been to look at the papers by Perchuck and Mascarelli, in particular, because what they did was they showed that the methodology worked, that it correlated with the outcome response to therapy and that's really a clinical validation, and that's what needs to be done. And they did it with that particular clone, so by adopting that clone and that methodology, one would be on safe grounds, including the cutoffs, one would be on safe grounds in terms of making that step from the biochemical to the IHC assay, okay. Now, we know, you know, 20 years later that the cutoff can go down to one percent and it still has a

Page 250

pretty good, you know, lesser but still pretty good link to outcome response to therapy, so those were the steps that would have taken over time. But I think that still as I look at the paper by O'Keane, you know, they have--it's very different. I don't think that people in this area were using an anti-estradiol antibody. If you're going to adopt a procedure, you have to adopt the antibody, the procedure and everything. And anti-estradiol, that's an antibody to the hormone, not the hormone receptor. So that's why this paper, I don't think, really went anywhere. It was an interesting sort of study and the metrics that were done on it, comparing, you know, with the ROC curves and whatnot, were valid for this particular study, but there were not outcomes and no one uses an anti-estradiol antibody, okay.

COFFEY, Q.C.:

Q. Doctor, look, please, at Exhibit P-0113? Doctor, this is a series of, this exhibit is a series of memorandums by Dr. Gershon Ejeckam to pathologists. Well, the first two memos we're going to look at are to pathologists, in

Page 251

effect, the pathologists in Newfoundland and Labrador. And the subject is "Immunohistochemical Stains." The first one is dated April 4, 2003. And he writes, "Kindly note that immunohistochemical stains for the following antibodies, CK34, CD3, CD5, CD20, CD79A, CEA, ER and PR have remained unreliable, erratic and therefore unhelpful for diagnostic purposes. Consequent on the above, staining with these antibodies shall stop forthwith until we can solve the reliability, sensitivity and specificity problems. Efforts are under way and hopefully a solution will be found within the next four to six weeks. You will be duly informed when such stains can resume." And it's copied to Barry Dyer, who was the manager in the laboratory at the time, he's a technologist, and all other technical staff on immunohistochemistry. Now, Doctor, in relation to circumstances where you had, well, are listed here, eight antibodies, that one had to in the lab here locally, felt, or at least Dr. Ejeckam felt that he had to stop them being used, I'll just ask you first of

Page 252

all, have you ever encountered any situation like that before in terms of being told about it?

DR. DABBS:

A. I personally have not encountered anything where anyone, you mean, suggested to me that they were having problems with antibodies and like that, no. I can make, you know, some general comments about 34 Beta E12. That antibody requires specific enzyme digestion. The other antibodies are pretty much, you know, what we would call meat and potatoes of immunohistochemistry laboratory. So when I see a memo like this, it suggests to me that there is something globally wrong with the laboratory, the IHC laboratory in general and probably is time for an SOS to obtain some expertise, technical, to come in and try to right things.

COFFEY, Q.C.:

Q. Doctor, and that was April 4th, 2003, dated. This is another memo of May 2nd, 2003. And again, it's in effect to all pathologists within the province from Dr. Ejeckam. The subject is "ER/PR Immunohistochemical Stains"

Page 253

here. And he is informing the pathologists that "We have rectified the difficulties related to the immunohistostain of ER/PR. Therefore, we can now resume regular requests for these antibody stains." And he goes on to provide a certain amount of information then about ER and PR. And this memo has been described as a, at the time, an educative memo. Have you had the opportunity to have a look at this?

DR. DABBS:

A. I have seen parts of this. There was a part that just went by, I think it was point No. 2.

COFFEY, Q.C.:

Q. Yes.

DR. DABBS:

A. ER/PR false negative results increased in core biopsies, therefore were possible"--yeah, that's actually, I think actually the reverse is true.

COFFEY, Q.C.:

Q. The reverse is true.

DR. DABBS:

A. It would be held by, I would say, the vast majority of people who do this, yes.

Page 254

COFFEY, Q.C.:

Q. And why is that, Doctor?

DR. DABBS:

A. Well, you see, there for some time and without evidence, evidence based, I think that pathologists feel that smaller amounts of tissue fix quickly. And for a short period of time I think actually one of the CAP recommendations came out implying that you only needed one hour of fixation for core biopsies, which was, I think, rapidly retracted once they came out and people found out that there really wasn't any evidence based on that. Because I can see how if one is treating core biopsies as small portions of tissue that don't require much fixation, false negatives will be rampant, there's no question about that. In fact, in the States the vast majority of literature will say, you know what, core biopsies we find to be the best because larger specimens tend not to be fixed with attention to detail, okay, so they have chose core biopsies, in fact, to be the test method of choice. But that's actually clinician driven in our institution. But I

Page 255

can see how one can make a statement like that, because if core biopsies are not adequately fixed, false negatives will be rampant and there would be a tendency to under fix it thinking it's a small biopsy, it only needs two hours.

COFFEY, Q.C.:

Q. I take it, Doctor, that the fixation process not only involves penetration of the tissue by formalin, but as well, actual time in the formalin after the penetration has occurred?

DR. DABBS:

A. Exactly. And what it is is, you know, penetration of the tissue by formalin does not equal fixation. Fixation continues once the tissue is taken out of formalin and it's a chemical process that involves cross linking of a lot of proteins. And so some of it clearly still remains a mystery, but fixation does continue beyond exposure to formalin and that's why that minimum exposure time is so critical, to get enough formalin on board and enough fixation to occur for the proper immunohistochemistry to work.

COFFEY, Q.C.:

Page 256

Q. And there's a reference in paragraph 5 to the consensus statement?
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. Or at least there's a reference to it, I'm not going to say that the actual--it's attributed to be quote is in fact a quote, I'm not going to suggest that.
DR. DABBS:
A. Right.
COFFEY, Q.C.:
Q. But that consensus statement of 2000 is referred to in paragraph 5. And he says in paragraph 6, "Higher staining intensity does not reflect better results. This is a function of staining procedure and may alter all cytoplasmic staining in ER/PR immunostain are to be considered as negative." I gather that the last sentence, no one takes any issue with that?
DR. DABBS:
A. Correct.
COFFEY, Q.C.:
Q. And the idea of "Higher staining intensity

Page 257

does not reflect better results." What does staining intensity, from your perspective, how is it properly considered here a factor, in here?
DR. DABBS:
A. Well, I think that staining intensity is important because if all of your results are either strongly positive, you know, the three plus nuclei and the rest are all negative, your test is not really optimized properly. So staining intensity is important with respect to just an internal quality assurance measure. And secondly, it does reflect the amount of hormone receptor in a given nucleus, because if you have something that's three plus brown and opaque and something that's one plus or two plus, you know, it is quantitative. And you can get into fancy machines, you can use your eyeball, you can use an H Score, but that difference is there and so that kind of difference in expression is to be--is not to be taken lightly.
THE COMMISSIONER:
Q. While we're on the subject, we've heard over the course of the past few months, I was going

Page 258

to say weeks, but it's been months, reference to the Allred score.
DR. DABBS:
A. Yes.
THE COMMISSIONER:
Q. And how does that relate to your H Score?
DR. DABBS:
A. The Allred score is a different scoring method that basically does the same thing. It takes into account the proportion and intensity of staining in any given tumour. And the proportion basically there is a little cartoon that comes with showing the proportion of cells of something like greater than 75 percent and 50 to 74 percent and, you know, it gets down to one percent, and then the intensity is pretty much the same, zero, one, two and three. So you have, on the intensity side, zero, one, two and three, and on the proportion side, one through five, so a top Allred score would be eight, five plus three, you add those together. And Dr. Allred used that with Harvey in that paper from 1999 where they looked at outcomes and used the one percent, greater than one percent cutoff which

Page 259

corresponds to an Allred score of, I think, of three. So it is a form of an H Score, if you will, it's just dialled up differently, it's a big different. And the, I think the H Score has a broader dynamic range associated to it as opposed to the Allred score and that's probably the only benefit of using an H Score over an Allred score. But there is nothing wrong with using an Allred score. It does adhere to the prescription that's suggested by the committee in that proportion and intensity should be scored. And Allreds method is validated in that paper by Harvey from 1999.
THE COMMISSIONER:
Q. So then what is in common between the method used in your institution and Allred is that both intensity and the percentage -
DR. DABBS:
A. Yes.
THE COMMISSIONER:
Q. - are factored into the information which is provided?
DR. DABBS:
A. Yes, they are. And, in fact, for cases where we have DCIS only, ductal carcinoma in situ

Page 260

only and we have a request for hormone receptors, we score that according to Allred because it's just easier matching up those cartoons with that, so.

THE COMMISSIONER:

Q. Thank you.

COFFEY, Q.C.:

Q. I was going to ask you about that, Doctor, now that you've raised it. The DCIS cases, what approach does your institution take to, in a case, a DCIS case or I suppose the same thing would apply to a lobular carcinoma, too, they can be in situ too, I gather? Am I correct or incorrect?

DR. DABBS:

A. That's an interesting question, but no one, to my knowledge, has ever requested hormone receptors on a lobular carcinoma in situ, and I would dissuade them from it because I would say you're wasting your money, it's 100 percent positive.

COFFEY, Q.C.:

Q. And in terms then of DCIS cases, what is the approach in Magee to whether or not ER and PR tests are done on DCIS and could you explain

Page 261

why?

DR. DABBS:

A. Sure. I think the rationale for doing hormone receptor testing on DCIS is laudable and it basically is, you know, DCIS, the presence of DCIS is certainly a risk factor for subsequent invasive breast cancer. And so oncologists, if they're going to give therapy, at least a group of them believe that it's important to know whether it's receptor positive or negative. And so the rationale for doing that is if it's positive, then they're going to treat the patient with therapy that causes a reduction, and that means either Tamoxifen or Arimidex or something along those lines if it's ER positive. Our policy is if it's pure DCIS, we will wait--from the core biopsy, if we have a core biopsy of DCIS, we don't do the hormone receptors, we wait for the resection. If it's pure DCIS and then we get a prescription from the doc that says do hormone receptors, then we will do them, we don't reflex them. So that's the situation where we will do those hormone receptors. We report them with the Allred score. And I have

Page 262

queried our docs in our interdisciplinary tumour board asking what the rationale for that was because that is assuming, you know, the argument, my argument is that you're assuming that that patient who has a negative ER/PR in her DCIS will develop--or not require hormone therapy. You're assuming that she could never, ever develop an ER positive DCIS. And while that sends a shudder through them, they will point to at least one study, and it's not that comprehensive a study, suggesting that and it's only suggestive evidence, that a patient who has a ER positive DCIS develops an ER positive invasive tumour and vice versa, those that are negative, okay. But still, in my way of thinking of biology, not everyone follows the rules and so my suggestion to them was I didn't see where that kind of testing really had a great deal of science behind it, that what if a patient who was ER negative, PR negative for DCIS developed an ER positive invasive cancer and were never on anti-hormonal therapy, then what. And that's still a bone of contention, if you will, hasn't been resolved. But that's

Page 263

their rationale for the testing in DCIS.

COFFEY, Q.C.:

Q. And, Doctor, here at P-0113 is a reference to ER positive tumours and the ones you'd referred to earlier are there. And you've indicated that--when you were speaking about these, in fact, these are the ones you'd named, tubular, mucinous, papillary?

DR. DABBS:

A. Papillary.

COFFEY, Q.C.:

Q. Papillary, I'm sorry, and you said though, invasive wouldn't--I'm sorry, lobular wouldn't fall into--it would tend to be ER positive, but it wouldn't be classified in the same way these were?

DR. DABBS:

A. Correct. These are--this group here is considered to be the so-called special type of invasive ductal cancers.

COFFEY, Q.C.:

Q. Ductal, okay.

DR. DABBS:

A. And lobular, by definition, is not a ductal cancer. So that's the only semantic different

Page 264

there.
COFFEY, Q.C.:
Q. And the reference to "low nuclear grade tumours are usually positive for ER/PR and negative for HER2/neu, while high grade tumours tend to be positive for HER2/neu and negative for ER/PR," do you agree with that?
DR. DABBS:
A. Correct, yes.
COFFEY, Q.C.:
Q. And now, Doctor, this happened, as you can see from the dates, in the main, in April of 2003, suspension of testing. If we could look, please, at Exhibit, just bring it up here, C-0228? Now Doctor, and go to, please--well, first of all, Doctor, that's just an immunoperoxidase request form that was in use here in St. John's or in Newfoundland, in fact, in 2003. This particular one is dated February 10th, 2003, and you see there, requesting ER/PR. Page two of the exhibit is the pathology report, or at least a portion of the pathology report, and then if you look at page four of the exhibit, you'll see there are two addendums there. One is March 17th, 2003,

Page 265

addendum number one, "estrogen progesterone immunoperoxidase method, ER occasional positive cells less than one percent, PR 15 percent positivity," and Doctor, it's noted "no controls available" and then addendum number two, which is May 28th, 2003. The first one was reported before the April 4th memo, the second one is entered after the May 2nd memo advising of the test being reinstituted. May 28th, 2003, and it says "as requested, repeat estrogen and progesterone receptors by immunoperoxidase staining. Estrogen receptors 40 percent positivity and progesterone receptors 73 percent positivity."
DR. DABBS:
A. Yes.
COFFEY, Q.C.:
Q. So Doctor, now we don't know, in particular, why this test was repeated, but if--let me see, March, April, May, well it's in effect just over two months. If an ER/PR test were conducted just over two months apart on the same surgical specimen--now again, we don't know even if it's the same block, because you can't tell the way they report these.

Page 266

Assuming for the moment it's the same block, would you expect to get, if the test were being carried out properly, going from an ER of less than one to 40 percent and a PR of 15 percent to 73 percent, would you expect that kind of swing?
DR. DABBS:
A. No, that kind of swing, assuming that the slides have been properly interpreted, would be a red flag for an event that needed to have serious inquiry by lab administration.
COFFEY, Q.C.:
Q. Doctor, if we could look, please, at Exhibit C-0175? Now Doctor, this is again a pathology report. The surgical specimen number is a different surgical--different patient, different surgical specimen number, is 03:SU4821, '03 being the year. The surgical specimen number being 4821. If we could go, please, to the second page, Doctor, you'll see here, addendum number one is dated May 6th, 2003, and it reads here "when compared to controls, the specimen is negative for HER2/neu, ER and PR," the rest of it relates to the HER2/neu test below this, and then

Page 267

addendum number two, three days later, on May 9th, 2003, the pathologist reports "the ER and PR were repeated due to quality assurance issues. The repeated stains show the following: ER positive in 80 percent of the cells. PR positive in ten percent of the cells" and the doctor notes "this replaces a previous report" and she has contacted the Cancer Clinic to advise them of that.
Doctor, now here, this first entry of addendum number one indicates that it was entered May 6th. If that entry of May 6th reflects a test after the testing resumed, if it does, after it resumed on May 2nd or thereabouts, if after the testing was resumed, one got a negative ER and PR and then three days later had the test rerun because one had concerns about quality assurance issues and got 80 and 10, would that, do you think, cause or should it have caused some inquiry to be made further?
DR. DABBS:
A. Yes, definitely. Again, this is a sort of red flag or a sentinel event, whatever language you want to use, to suggest that the process

Page 268

for immunohistochemistry is erratic and needs to be really thoroughly investigated.

COFFEY, Q.C.:

Q. If we could look, please, at Exhibit C-0174? Here, Doctor, again this is a pathology report. It relates to specimen number 02:SS5231. You'll see that in the top left-hand side there.

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. If we could go, please, to page four? Thank you, Registrar. Doctor, there are three addendums here. The first of them doesn't relate to ER/PR, but the second addendum, addendum number two, is dated August 29th, 2002, which is in effect eight or nine months before the ER and PR testing were suspended in April of 2003. On August 29th, 2002, the pathologist reported, addendum number two, "immunohistochemical staining for estrogen receptors is positive in approximately 15 percent of lesional cells. Immunohistochemical staining for estrogen receptors is negative," and then addendum

Page 269

number three, see that at the top of the page there, on June 11th, 2003, the same pathologist reported the following: "at the request of Dr. Zaidi," and I will tell you that Dr. Zaidi is an oncologist, okay, used to practice in St. John's, "immunohistochemical staining for estrogen and progesterone receptors has been repeated. Estrogen receptors show faint positivity in approximately 10 to 15 percent of lesional cells. Progesterone receptors are unequivocally positive in approximately 75 percent of lesional cells" and it's entered the same day.

Now Doctor, I appreciate the tests were done, in effect, ten months apart, or almost ten months apart, but if an ER is reported as negative, and I take it in this context, negative probably meant zero and PR 15, and then on repeat, ten months, or just over ten months later, it goes 10 to 15 ER and 75 percent PR, would that cause any questions to be raised about what was going on, for example, back in August of 2002?

DR. DABBS:

Page 270

A. Yes, again, assuming that this is performed on the same block, this would be a cause for concern, and again, the methodology of IHC would need to be looked at very closely.

THE COMMISSIONER:

Q. I'm sorry, Mr. Coffey, but these things are just running through my head. The point you make about being performed on the same block -

DR. DABBS:

A. Yes.

THE COMMISSIONER:

Q. - I'm assuming that sort of nature being what it is, in fact, there might be a variation as you move through the block, the result that you would get, or in theory, in any event, and that's what I'm wondering is whether or not there are studies done as to the sort of normal variation in results that one might expect from the same block?

DR. DABBS:

A. Well, I think that there can be some variation. What strikes me is an ER that goes from completely negative to up to 15 percent of cells, that would be more variation than I would be accustomed to, and especially going

Page 271

from 15 percent PR to 75 percent. That's a big difference, and I think those two taken together are most indicative that there's something wrong with the process, that whatever was done the first time was probably improper. There's too much variation there.

THE COMMISSIONER:

Q. I guess what I'm asking is what would be acceptable or considered normal variation if you were running a test again for some reason, and you would say essentially that's the same result?

DR. DABBS:

A. I think a well performed ER test that is negative and re-performed and found to have less than one percent of cells would be acceptable.

THE COMMISSIONER:

Q. Okay.

DR. DABBS:

A. On a deeper level, for example.

COFFEY, Q.C.:

Q. Doctor, if we bring up, please--sorry, before we do that, so Doctor, here, in the context, if you were just handed this and kind of asked

Page 272

to read it, and looking at the space in time, first report August of 2002, second report June of 2003, and you pointed out from your perspective there's a difference in the results of some significance, if you were faced with that, for example, in your own laboratory, say you were to be, would that cause you to do anything, and it was now 2003, the second result, would that cause, do you think, would it cause someone to question what had been going on in 2002?

DR. DABBS:

A. Yes, very definitely.

COFFEY, Q.C.:

Q. Yes, and assuming, for example, for the moment, that the 2003 test is the correct result?

DR. DABBS:

A. Right.

COFFEY, Q.C.:

Q. That you're satisfied that it has been optimized, as it were.

DR. DABBS:

A. Correct.

COFFEY, Q.C.:

Page 273

Q. That nothing happened in your lab in the meantime, you might want to then question, well, this earlier result, what does that mean?

DR. DABBS:

A. Correct.

COFFEY, Q.C.:

Q. What was going on back then.

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. Doctor, I'm going to ask you now to look at, please, Exhibit P-0046. Now, Doctor, this is--well, it's the first page, it's the cover letter. It's dated October 17th, 2005. Second page is the report by Dr. Diponkar Banerjee of the British Columbia Cancer Agency, dated October 17th, 2005, entitled "External Quality Review of the Health Care Corporation of St. John's, Laboratory Medicine Programs, Immunohistochemistry Service," and Doctor, have you had an opportunity to read this?

DR. DABBS:

A. Yes.

Page 274

COFFEY, Q.C.:

Q. And Doctor, I'm just going to ask you, perhaps I'll ask you, from your perspective, when you read this, what caught your attention? What was it, if anything?

DR. DABBS:

A. Well, I think that what caught my attention was the sort of multi-factorial items that led to failure and resultant false negative results, beginning with, you know, as Dr. Banerjee says here in the review of cases, poor fixation, negative internal controls and absent internal controls and so forth, and you know, I think he did a very thorough review. I was impressed with the review, and everything here, as best I can tell, makes sense, based on evidence.

COFFEY, Q.C.:

Q. And -

DR. DABBS:

A. The one item that I am looking at right now, at the bottom of the page, where he says "fixation time in formalin does not affect ER results as long as two millimetre thick slices of tissue are placed in fixative within 15

Page 275

minutes of surgical excision and adequate heat-induced antigen retrieval is performed." That's about the only thing that I disagree with there. Section thickness, while desiring to be two millimetres, fixation time in formalin does affect results and no matter what the section thickness is and this is the only thing that sort of stood out that I took issue with.

COFFEY, Q.C.:

Q. And if I recall correctly, Commissioner, Dr. Banerjee, when he testified, he -

THE COMMISSIONER:

Q. He agreed with you.

COFFEY, Q.C.:

Q. Pardon me? He actually agreed with you, yes.

DR. DABBS:

A. Okay.

COFFEY, Q.C.:

Q. It's just the way it was phrased at the time. He certainly pointed it out, my memory of it, if my memory is correct, when he came and testified. Now Doctor, that's the kind of summary of the review of cases that had converted up to that point in time, and this

Page 276

is in the summer and fall of--early fall of 2005. Then the conclusions about the reasons for test failure is spelled out here, and in effect, other than referring to the two systems, DAKO and Ventana, he repeats the reference to tissue fixation as concerns he expressed earlier and refers to internal controls, and his observations in relation to them.

Doctor, what I wanted to ask you, in relation to this--but as well, before I do, I also want to refer you to "other system flaws observed." The idea of lack of dedicated immunohistochemistry technologists, he points out "a rotation system is used," was being utilized at the time. Doctor, at your own institution, are there dedicated immunohistochemistry technologists?

DR. DABBS:

A. There are some--there's a dedicated group of histotechs, which are also well trained in immunohistochemistry and we have, I think, four or five of them, and they will rotate onto that service, in addition to doing other duties. But not all histotechs do

Page 277

immunohistochemistry. It's a dedicated subgroup who have the interest and skill and desire and are also very critically appraised in terms of their abilities and they sign off on a checklist, you know, for duties that they're competencies.

COFFEY, Q.C.:

Q. Number two here, he refers to "a lack of an officially designated pathologist as director of immunohistochemistry service," and he notes, in his view, "the technologists thus get conflicting feedback from a large number of pathologists." He notes "there's no accountability for the quality of the service." Doctor, at least at your institution, is there a director of immunohistochemistry service?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. Is that a pathologist?

DR. DABBS:

A. Yes, and that is very important, in my opinion, because what can happen is if you have a lot of people chatting up results, it's

Page 278

difficult and the techs can get mixed messages and there's no one in control. There's no one at the helm. There has to be that person so that--and there should be, professionally, so that they can share these ideas among themselves and there's a lightning rod person for issues that come up, so they can talk about it among themselves professionally and then take things to the technicians.

COFFEY, Q.C.:

Q. Now, such a director of immunohistochemistry, a pathologist, what sort of background do you think is desirable anyway in such a pathologist? Like how would one go about choosing or recruiting such a pathologist and/or training them?

DR. DABBS:

A. I think that there probably are several ways of doing that. First and foremost, one would have to rely on the abilities that can be documented from that person, either from past experience, either in writing from references or telephone calls or whatever. This person is, you know, trying to forward themselves as having the abilities. Of course, it's always

Page 279

desirable to have someone who is published in the field as well, to show that competency, ask them what sort of meetings that they've attended, what sort of groups they've been involved with and so forth. So there really aren't immunohistochemistry fellowships per se. That's a very rare breed, and so fellowship training would not be an expected route of getting someone like that. It's all about experience, publishing, references to date.

COFFEY, Q.C.:

Q. He also refers to a lack of standard operating procedures for grossing, fixation, tissue processing, block selection, positive control block selection, method optimization through systematic titration, incubation time, and antigen retrieval time for each analyte. Doctor, first of all from your perspective, should a pathology laboratory have standard operating procedures for all of those things?

DR. DABBS:

A. Very much so.

COFFEY, Q.C.:

Q. And what are the risks associated or potential

Page 280

consequences associated with not having SOPs from your perspective?

DR. DABBS:

A. Well, without having SOPs, I don't see how actually a laboratory, at least in the States, could survive a CAP inspection or whatever governing body would be doing the inspection because all of these things are expectations, these things are no checklists, all of the manuals that we're talking about with standard operating procedures, they have to exist. They were viewed by inspectors and so forth. Without them, I think there's really no accountability for assurance--for quality being assured, yet alone quality control. So it's a recipe for disaster.

COFFEY, Q.C.:

Q. Doctor, can you tell us, please, about how in the United States, clinical laboratories--at least the pathology end of the clinical laboratory is accredited or inspected or licensed? How does that work in the United States?

DR. DABBS:

A. The governing bodies basically is the federal

Page 281

government and there are multiple venues by which one can choose to be inspected. There's a joint commission accreditation of hospitals is one, there's CMS, Centre for Medicare Service, is another, and then there are other agencies which have been permitted to inspect under the aegis of the aforementioned entities, and that includes the CAP, College of American Pathologists. So those are the entities which inspect. They do so on a two year cycle. They have a checklist which everyone is very familiar with, and, in fact, every year one is supposed to do a self-inspection, to do through these to look at any potential deficiencies. There are quarterly updates for the checklists in various areas that should be gone over by people in the quality assurance part of the laboratory, and in essence, one should be ready for an inspection at any time. Now in the States, the laboratory inspections are unannounced. Even though, you know when you're going to have a window of opportunity. I suspect the future is going to be it's going to happen whenever it happens, they won't give you a window, it's

Page 282

just going to happen. I think we're moving in that direction, but right now they're unannounced inspections.

COFFEY, Q.C.:

Q. I'm sorry, right now they're --

DR. DABBS:

A. They're unannounced inspections, but you know what window--if they say you're going to be between July and December of 2008, you'd have some time --

COFFEY, Q.C.:

Q. During that time frame.

DR. DABBS:

A. If someone is going to show up unannounced, right, but I think the way it's evolving is that at one point in time no one is going to give you a window, they're going to say we're going to inspect when we want to.

COFFEY, Q.C.:

Q. We just accredited or licensed you, we'll be back in due course?

DR. DABBS:

A. Correct.

COFFEY, Q.C.:

Q. Doctor, can one, to your knowledge in the

Page 283

United States, run a tertiary care clinical laboratory pathology division without being accredited or licensed in some manner?

DR. DABBS:

A. Not at all.

COFFEY, Q.C.:

Q. Why is that?

DR. DABBS:

A. That's because the federal government has set guidelines for accreditation of hospitals, and this is also under the aegis of the old Clinical Laboratory Improvement Act, which sets guidelines for laboratories and this is an evolving area as well. For hospitals to be accredited, they have to have accreditation at the laboratory as well. If the laboratory is not accredited, the hospital would not have the laboratory to function.

COFFEY, Q.C.:

Q. Doctor, he refers to a lack of subspecialization amongst pathologists leading to a lack of in-depth knowledge of IHC technical and interpretation details and pitfalls. Doctor, your career spans well over two decades now. Doctor, in the area of

Page 284

pathology, and again I'll just ask you generally about your experience in the United States, subspecialization within pathologists, how far has that gone?

DR. DABBS:

A. That's--subspecialization in pathology is a - it's an evolving concept. There are more and more large medical centres that have moved to subspecialty sign out. For example, Mass. General Hospital, Cleveland Clinic, our institution--if you look at our institution, we have gone to basically a product line sign out where there is a bench for every subspecialty area. So there is a group of pathologists entirely dedicated to head and neck cancer, a group to GI cancer, thoracic pathology, cardiac pathology, neuropathology, renal medical biopsy, genital urinary pathology, GYN and breast, and at Magee, Magee is actually a separate hospital, it's been a women's hospital for many decades and there was at one time--Magee used to be a general hospital, but what's happened over time is that all of the general surgery that is performed there, those specimens are shipped

Page 285

to our centres of excellence where those pathologists sit. So, for example, if someone does GU surgery or GI surgery, we package up those specimens, fix them appropriately and ship them to the hospitals. They're not very far away. They're within a half an hour drive. Subsequently if there's breast or GYN surgery done at the other UPMC hospitals in our vicinity, they ship those specimens to us. So that's how the subspecialty works.

COFFEY, Q.C.:

Q. And why is that?

DR. DABBS:

A. Well, it works that way because there's a limited number of pathologists with subspecialty expertise, and rather than having, you know, six centres with breast pathologists which is going to be difficult to come by, you put them all in one place and have them do all that work. So it's worked out pretty well.

COFFEY, Q.C.:

Q. I take it, that's all with a view to the principle that all things being equal, subspecialists will do or can be expected to

Page 286

do a more accurate job?

DR. DABBS:

A. More efficient, more accurate, yes, and even some commercial laboratories in the States are now picking up on this concept as well. So it's--with the amount of knowledge and complexity of specimens and expectations, subspecialty sign out is increasing.

THE COMMISSIONER:

Q. Do I take it then there are no generalists in your institution?

DR. DABBS:

A. At the UPMC core hospitals, that includes Presbyterian Shadyside Hospital and Magee, if you want to look at general pathologists, I would say that they're sprinkled throughout on one of them. I did a lot of general pathology that still sticks with me, but I do a lot of specialty sign out now. So what's happening in terms of recruiting for pathologists who come out of training and go directly into a subspecialty area, they probably--their general capabilities will probably atrophy over time, but part of the UPMC system there are, I think, ten other community hospitals

Page 287

where there are general pathologists, and the funnel complex cases to the centres of excellence. So that's our workload sort of flows.

COFFEY, Q.C.:

Q. Doctor, I'm still on the topic in paragraph five and the idea of subspecialization. We've heard, Doctor, that immunohistochemistry as a subject matter has grown considerably over the past 20/25 years.

DR. DABBS:

A. Yes, very much so.

COFFEY, Q.C.:

Q. And I gather that it's expected to grow again or at least to continue to grow?

DR. DABBS:

A. Yes.

COFFEY, Q.C.:

Q. Doctor, from your perspective, how practical is it for a general pathologist to be able to expect today--for example, coming out of school today, coming out of a residency today, to be able to perform all the different types of analysis that might be required or might be done, for example, in your institution by a

Page 288

number of different types of subspecialists, how practical is that in today's world?

DR. DABBS:

A. I think for a community pathologist, that they do a good job, and I think that the expectations are there. I think that a good deal of it depends on the residency training that they have undergone. There are a number of good, very good and excellent resources for whatever area that we're talking about, and, you know, immunohistochemistry is sort of a meat and potatoes part of general surgical pathology. So a good trainee is going to make the effort to learn as much as they can from their everyday practice, specimens that they're exposed to, textbooks, and online resources that they have, meetings that they go to, and it's the expectation that they will do that as well as they can because that's the kind of thing that they will be dealing with in their general practice. Many community hospitals will rely actually on immunohistochemistry, they'll send tests out to commercial laboratories in the States, and sometimes they'll get results back on line

Page 289

where they have a screen just like this, and they can look at the images there. The tests are all done by an outside laboratory. Of course, the burden on them is knowing what they're looking at, and the burden on the laboratory is making sure that they have all the adequate quality assurance methods in place so that the product that they're giving to their clients, the pathologists, is a good quality.

COFFEY, Q.C.:

Q. Is there, for example, in relation to ER and PR tests, is there any such thing to your knowledge as sort of a minimum number, for example, that a laboratory should be involved in producing over--I'll just pick an arbitrary period like a year.

DR. DABBS:

A. Uh-hm.

COFFEY, Q.C.:

Q. In order to kind of keep the technologist or technician's skills sharp, where there be any minimum number--are there any studies done on that, do you know, first of all?

DR. DABBS:

Page 290

A. I'm not aware of --

THE COMMISSIONER:

Q. Are you talking about technologists?

COFFEY, Q.C.:

Q. Technologists first of all, technologists first, yeah, in the laboratory.

DR. DABBS:

A. As far as technologists, I'm not aware of any number in that regard, but what I can tell you now is that with the move to automation that adding another stain on the stainer is not a feat that is difficult for a technologist who is used to doing immunohistochemistry in the first place. They certainly would have to be accredited or signed off as being competent in dealing with the appropriate controls and handling those results appropriately. As I think about this, I mean, what I see is arbitrary and just my feeling, that if you're seeing somewhere around one of these a day, that would seem to be a reasonable number to keep up competence of pathologists who's looking at it, as well as the technologist. So you know--even if they did a batch run, say, once a week, if, you know--that might be

Page 291

doable as well. Our oncologists are pretty demanding. They always want results yesterday, so we run them as soon as we get them and we usually have enough to do that because we have, I think, about between 1500 and 1800 new cancers per year, so we have--we always have multiple numbers per week of ER/PR, but I would say roughly one a day would seem to be reasonable.

THE COMMISSIONER:

Q. Mr .Coffey, whenever you can find a convenient spot, we'll break for the day.

COFFEY, Q.C.:

Q. Perhaps I'll come back then tomorrow and pick up at paragraph five, Doctor.

DR. DABBS:

A. Okay. Just before we do go, Commissioner, there is another exhibit. I'll refer to this, and I expect it'll be referred to tomorrow. It's P-2630.

THE COMMISSIONER:

Q. Entered.

COFFEY, Q.C.:

Q. Thank you, Commissioner.

EXHIBIT ENTERED AND MARKED AS P-2630.

Page 292

THE COMMISSIONER:

Q. 9:30 in the morning. Thank you.

Page 293

CERTIFICATE

I, Judy Moss, hereby certify that the foregoing is a true and correct transcript in the matter of the Commission of Inquiry on Hormone Receptor Testing, heard on the 15th day of September, A.D., 2008 before the Honourable Justice Margaret A. Cameron, Commissioner, at the Commission of Inquiry, St. John's, Newfoundland and Labrador and was transcribed by me to the best of my ability by means of a sound apparatus.

Dated at St. John's, Newfoundland and Labrador this 15th day of September, A.D., 2008

Judy Moss

-F-

-N-

-T-