COMMISSION OF INQUIRY
ON HORMONE RECEPTOR TESTING

BEFORE THE HONOURABLE JUSTICE CAMERON - COMMISSIONER

June 24, 2008

Appearances:

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

Chesley Crosbie, Q.C. . . . . Members of the Breast Cancer
. . . . . Testing Class Action

Mark Pike . . . . . NL Medical Association
Jennifer Newbury . . . . . Canadian Cancer Society (NL Division)
Stacey O'Dea. . . . . Central, Western and Labrador-Grenfell
Regional Integrated Health Authorities

LIST OF EXHIBITS

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COMMISSIONER:
Q. Please be seated. Ms. Chaytor.
CHAYTOR, Q.C.:
Q. Good morning, Commissioner. Our next witness is Patricia Wegrynowski, and I would ask, please, that she be sworn or affirmed.
MS. TRISH WEGRYNOWSKI (SWORN) EXAMINATION BY SANDRA CHAYTOR, Q.C.
REGISTRAR:
Q. And would you please state and spell your complete name for the Commission?
MS. WEGRYNOWSKI:
A. My name is Trish Wegrynowski. It's spelled T-r-i-s-h, and my surname is W-e-g-r-y-n-o-w-s-k-i.
REGISTRAR:
Q. Thank you.
MS. WEGRYNOWSKI:
A. You're welcome.
CHAYTOR, Q.C.:
Q. Good morning, Ms. Wegrynowski.
MS. WEGRYNOWSKI:
A. Good morning.
CHAYTOR, Q.C.:
Q. I'm sorry, I called you Patricia, but your

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name is actually Trish?
MS. WEGRYNOWSKI:
A. It is Patricia, but I go by Trish.
CHAYTOR, Q.C.:
Q. By Trish, okay. Thank you. We have a number of new exhibits this morning, Commissioner, I would ask to have entered. And they are P-1730 through to P-1766, inclusive.
COMMISSIONER:
Q. Entered.
EXHIBITS P-1730 THROUGH P-1766, INCLUSIVE, ENTERED INTO EVIDENCE.
CHAYTOR, Q.C.:
Q. Thank you. If I could have, please, P-1730? Ms. Wegrynowski, we'll begin with, if you could take us through the highlights of your educational and professional background.
MS. WEGRYNOWSKI:
A. Sure.
CHAYTOR, Q.C.:
Q. And 1730 is your curriculum vitae. And we see here at--we could start at the education and licensure. It indicates that in 1977 you graduated from Toronto Institute of Medical Technology with an RT?

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MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Perhaps you could tell us what that is?
MS. WEGRYNOWSKI:
A. That's a two-year program that was run out of the Institute of Medical Technology. In that program the first year is 10 months of learning based on the five areas of the laboratory, that would be chemistry, hematology, microbiology, blood bank and pathology. The second year is 12 months in hospital, so that's your clinical year. When you complete that and your examinations in those two years you then sit your national examinations.
CHAYTOR, Q.C.:
Q. Okay. And I take it RT is registered technologist?
MS. WEGRYNOWSKI:
A. Yes. The nomenclature changed to medical laboratory technologist many years later.
CHAYTOR, Q.C.:
Q. Okay. And then in 1983 you're at Humber College and you have a business communications

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diploma?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. 1986 Michener Institute, OHA Management, Series 1 certificate?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And in 1998 you have a BAS with merit from York University?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And in your licensure it shows--perhaps you could tell us what a BAS is?
MS. WEGRYNOWSKI:
A. It's a Bachelor of Administrative Studies.
CHAYTOR, Q.C.:
Q. Thank you.
MS. WEGRYNOWSKI:
A. You're welcome.
CHAYTOR, Q.C.:
Q. And what's involved in a Bachelor of Administrative Studies?

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MS. WEGRYNOWSKI:
A. I took that program specifically because I believe that laboratories need to be run like businesses; there's different aspects to it. It's the training of organization, finance, the whole gamut of what went well with science.
CHAYTOR, Q.C.:
Q. Okay. And your licensure, from 1977 to 1993 was through the Canadian Society of Laboratory Technologists?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And then 1994 through the present the College of Medical Laboratory Technologists of Ontario?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And so why is there the switch from '93 to '94?
MS. WEGRYNOWSKI:
A. The CSLT stopped and became then the Canadian Society for Medical Laboratory Science. The

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laboratory technologists then became registered with the government and that's when we received our own college, so I have been in the college. We are required to be members of the college to practice in Ontario.

CHAYTOR, Q.C.:

Q. And in Ontario you're subject to legislation?

MS. WEGRYNOWSKI:

A. Yes. I have to ensure that my education is kept up and I have a number of continuing education hours and we are tested--I should rephrase that. We are given an opportunity provide our information to the college, it's almost on a roulette wheel, your number gets chosen and it's your time up. So what you need to do is they will go through your education for the previous two years to ensure that you're keeping your scope of practice up.

CHAYTOR, Q.C.:

Q. Okay. And under your professional affiliations and activities you have "Canadian Society for Medical" -

MS. WEGRYNOWSKI:

A. "Laboratory Science."

CHAYTOR, Q.C.:

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Q. - "Laboratory Science."

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. So what would that be?

MS. WEGRYNOWSKI:

A. That's our national organization.

CHAYTOR, Q.C.:

Q. Okay. And CMLTO Pathology Practice Guidelines Committee, what -

MS. WEGRYNOWSKI:

A. Yes, I worked on that committee. We were determining what guidelines we could use for the histology laboratory in the Province of Ontario. We were looking what expectations would be for benchmarking.

CHAYTOR, Q.C.:

Q. Okay. And you are or have been an invigilator for MLA examinations?

MS. WEGRYNOWSKI:

A. Yes, I am.

CHAYTOR, Q.C.:

Q. Okay. And what does that mean?

MS. WEGRYNOWSKI:

A. There are presently the CSMLS are looking at

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including the MLAs in their umbrella as are the CMLTO. There are a number of organizations that are out there that are providing education, the OSMT at this point is the only one providing, to my knowledge, providing an examination.

CHAYTOR, Q.C.:

Q. And so MLA, the Medical Laboratory?

MS. WEGRYNOWSKI:

A. Technician.

CHAYTOR, Q.C.:

Q. Technician, okay. And the National Society of Histotechnology, you've been involved with that?

MS. WEGRYNOWSKI:

A. For many years. They are an American organization. Their whole mandate is in the area of histotechnology, so they do histology and they have a tremendous amount of information in immunohistochemistry. And I have the pleasure of sitting on the executive for a number of years and have attended several of their conventions and I'm also a member of their immunohistochemistry research group.

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CHAYTOR, Q.C.:

Q. Okay. And you're also part of Immunohistochemistry Users Group, Toronto. What is that?

MS. WEGRYNOWSKI:

A. There will be several meetings during the year at different hospitals where members of the immunohistochemistry community will get together and we will have a key note speaker and different suppliers will be there.

CHAYTOR, Q.C.:

Q. And is that just technologists or does that also include pathologists?

MS. WEGRYNOWSKI:

A. Technologists. It's -

CHAYTOR, Q.C.:

Q. Technologists?

MS. WEGRYNOWSKI:

A. Yeah.

CHAYTOR, Q.C.:

Q. Okay. And the College of American Pathologists Inspector, what does that involve?

MS. WEGRYNOWSKI:

A. I have inspected several laboratories from the

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CAP, using the CAP guidelines.
CHAYTOR, Q.C.:
Q. Ontario Accreditation Assessor's Certificate?
MS. WEGRYNOWSKI:
A. I originally sat my exam for that particular certificate, that's when QMPLS was coming out and the Ontario Lab Accreditation.
CHAYTOR, Q.C.:
Q. Okay. And technical advisor, Canadian Consensus for Her2/neu Testing Guidelines in Breast Cancer?
MS. WEGRYNOWSKI:
A. Those guidelines were published last year. Frances O'Malley and Wedad Hanna, I believe, are co-authors of that and I was approached to be the technical advisor.
CHAYTOR, Q.C.:
Q. Okay. And your employment, if we just start at the next page.
MS. WEGRYNOWSKI:
A. Okay.
CHAYTOR, Q.C.:
Q. You began in 1978 through to 1983 with Women's College Hospital in Toronto?
MS. WEGRYNOWSKI:

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A. Yes. That was my first job, and I worked in histology. And it was around 1983 that immunohistochemistry was coming into being and I was asked if I would like to lead that area, which I did. And I remained in that position until 1990.
CHAYTOR, Q.C.:
Q. Okay. So you moved to immunohistochemistry in 1983 and you've been involved with immunohistochemistry ever since then?
MS. WEGRYNOWSKI:
A. Except between '90 and '99 when I went to Credit Valley, that was my hiatus.
CHAYTOR, Q.C.:
Q. Okay. Yes, and we see that here. So from 1990 to 1999 you're back to histology?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Okay. And 2000 to 2005 you indicate you're at Mount Sinai Hospital and you're key LIS operator, pathology. What does that mean?
MS. WEGRYNOWSKI:
A. In addition to taking on my general duties, I was also involved with the laboratory

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information system, so I was implementing that, as well.
CHAYTOR, Q.C.:
Q. Okay. So that would include report forms?
MS. WEGRYNOWSKI:
A. Synoptic reporting, including the stain codes, ensuring that the processing that workload units were being captured, yes.
CHAYTOR, Q.C.:
Q. So was this a new system brought in in Mount Sinai in this time period?
MS. WEGRYNOWSKI:
A. The system, yes, the system was brought in around 2000, that's correct.
CHAYTOR, Q.C.:
Q. Okay. And from 2000 to the present, of course, you're senior MLT, immunohistochemistry?
MS. WEGRYNOWSKI:
A. That is correct.
CHAYTOR, Q.C.:
Q. So you went back to, or you went to Mount Sinai from 1999 to 2000 as a general MLT?
MS. WEGRYNOWSKI:
A. Correct.

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CHAYTOR, Q.C.:
Q. And within a year became a senior MLT?
MS. WEGRYNOWSKI:
A. That's correct.
CHAYTOR, Q.C.:
Q. And what's the difference?
MS. WEGRYNOWSKI:
A. In the present scope I'm responsible for five different--responsible for five employees, I'm responsible for the immunohistochemistry laboratory and everything that comes out of there.
CHAYTOR, Q.C.:
Q. And you've been an invited lecturer?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. On a number of occasions?
MS. WEGRYNOWSKI:
A. I've--yes.
CHAYTOR, Q.C.:
Q. Perhaps you can take us through that?
MS. WEGRYNOWSKI:
A. Okay. I've had the opportunity to speak at the Michener Institute for Applied Science to

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the second, first or second year students, I can't recall. I gave a lecture with Dr. Aaron Pollett at the NSH convention when it was here in Toronto. I've done a number of in-service continuing education programs for immunohistochemistry at Mount Sinai, and when we do have rotating students from different sites, I also provide a lecture for them. Because I was involved with the laboratory information system at that time we were doing an integration between up--excuse me, between cytology and histology, one of the cytotechnologists and I did a lecture at that particular convention. And then recently I did several lectures at Lakeridge Health Corporation in Oshawa.

CHAYTOR, Q.C.:

Q. Okay. And then we have a number of publications you've been involved in?

MS. WEGRYNOWSKI:

A. Um-hm.

CHAYTOR, Q.C.:

Q. And courses attended include 1997, immunohistochemistry workshop?

MS. WEGRYNOWSKI:

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A. Yes. May I go back to 1983?

CHAYTOR, Q.C.:

Q. Sure, absolutely.

MS. WEGRYNOWSKI:

A. Okay. In 1983 it was very fledging in immunohistochemistry and there was no formal course out there to really find, and this particular course was offered that Michener Institute and it certainly provided me with the groundwork of what I've learned today. We learned about the proteins and the epitopes and words that were completely strange because they were certainly nothing that you take in your training.

CHAYTOR, Q.C.:

Q. It was nothing you had learned in your formal education?

MS. WEGRYNOWSKI:

A. Absolutely not.

CHAYTOR, Q.C.:

Q. Okay.

MS. WEGRYNOWSKI:

A. From there it was finding workshops from different suppliers. And at that time because it was such a fledgling area, many of the

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suppliers were bringing in guest lecturers and providing lectures, so I took one through Biogenex and then I took one through Miles, that one was held at Sick Children's. So that's basically where a lot of the knowledge came from. I attended the OHA convention, that was again QMPLS, understanding the accreditation proposal requirements. The one in 2001 actually was a very interesting workshop, it was a multi-functional lab station. I'll go in this later in my lecture, but I could just, if you'd like, just point on it. They were different pretreatments that we use in the laboratory, and at this particular workshop they presented a microwave that would assist us, so it was--it's having the ability to go to different workshops for the technologists that provides them with what is out there in the marketplace. And then, of course, Brian Hewlett's lecture on optimizing IHC techniques.

CHAYTOR, Q.C.:

Q. Okay.

MS. WEGRYNOWSKI:

A. I've gone to a number of workshops with the

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NSH, but this one in particular I highlighted because it was immunoenzymatic double staining methods. So a lot of the staining that is done in immunohistochemistry is just done as a single staining method, you're just looking for one particular epitope. When we get into the double staining methods, we were looking for more than one. I went to a workshop in DAKO, excuse me, in Santa Barbara, they were releasing a new autostainer. And then last year I had the privilege of going to the Biological Stain Commission meeting in Baltimore, Maryland.

CHAYTOR, Q.C.:

Q. Okay. And if we could just go back then to your employment. You started out at Women's College Hospital in Toronto. And that's where you were initiated in 1983 into IHC. Were you actually involved in the setting up of the IHC lab at that time?

MS. WEGRYNOWSKI:

A. Yes, I was. We didn't have anything at the time, it was a brand new area, and I had the opportunity to work with Dr. Wedad Hanna and Harriett Kaha, both of whom are at Sunnybrook

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and Wedad Hanna is also respected in the breast community as it Frances O'Malley. At that time they were beginning to look at different epitopes in tissue. We did not have the knowledge to be able to use formalin fixed paraffin-imbedded tissue, so at that time we were using frozen tissue, and one of the first antibodies that we began working with was estrogen and progesterone.

CHAYTOR, Q.C.:

Q. And that was back in 1983?

MS. WEGRYNOWSKI:

A. That is correct.

CHAYTOR, Q.C.:

Q. Okay. And what did you have to do at that point in time to validate your process?

MS. WEGRYNOWSKI:

A. Validating the process was very similar as to what we do today. It was--if I could just step back? Because we could not find the epitope in paraffin-imbedded tissue, we only used frozen material. At that time, I believe Frances spoke to you about this yesterday, there was a DCC, the charcoal method, and they were able to get a quantitative figure. So

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what we were looking at was determining whether or not the estrogen and progesterone that we were finding in frozen tissue was mirroring what they were finding in that particular method.

CHAYTOR, Q.C.:

Q. Okay. And had you been involved in the prior method?

MS. WEGRYNOWSKI:

A. No.

CHAYTOR, Q.C.:

Q. So were you comparing your results then in the new IHC method to what they had been using through the, I guess it was the bioassay method?

MS. WEGRYNOWSKI:

A. That's correct. So the word "validation" I'm not sure would apply in the same sense as it would today.

CHAYTOR, Q.C.:

Q. Yes. Because you're not comparing apples to apples, I guess?

MS. WEGRYNOWSKI:

A. That's correct.

CHAYTOR, Q.C.:

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Q. Okay. Perhaps you could tell the Commissioner then how IHC has evolved over time from the time that you first got involved in the early days in 1983?

MS. WEGRYNOWSKI:

A. Okay. Immunohistochemistry has evolved immensely because we now have the opportunity to find these epitopes in formalin fixed paraffin-imbedded tissue. It's quite amazing that these epitopes, if treated correctly from the very beginning, survive decades. And we have actually gone back and done work on family members that are 20 and 30 year old blocks. So we were not able to do this way back in the '80s. We were not able to do our lymphoma markers on paraffin-imbedded tissues. All this is available to us today.

CHAYTOR, Q.C.:

Q. Okay. And in terms of the number of antibodies that you would be using, how has that evolved over time?

MS. WEGRYNOWSKI:

A. When I first began, the bulk of the antibodies were fluorochrome so they were simply what we do now for kidney biopsies. But there were

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very few primary antibodies on the market, at least at the service end. I'm sure there was many more on the research end. And now in the laboratory I presently have, there's over 200 antibodies, at least.

CHAYTOR, Q.C.:

Q. Over 200?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. At Mount Sinai?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Now, Ms. Wegrynowski, we understand that you've been to St. John's before?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Welcome back.

MS. WEGRYNOWSKI:

A. Thank you.

CHAYTOR, Q.C.:

Q. And you were originally asked to come in September, 2005?

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MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. To carry out a review. So perhaps you could tell the Commissioner who approached you to do that review and what it was that you were asked to do?
MS. WEGRYNOWSKI:
A. Okay. Frances O'Malley originally came to speak to me, and from what I understand that Bev Carter had contacted her and that they were having some concerns with their estrogen and progesterone receptors and that they wanted someone to look at their processes. So she ran that through my technical director and I had, I believe there's some e-mails that actually lay out the land of how I got here.
CHAYTOR, Q.C.:
Q. So if we could look at 1743, please? And this is an e-mail communications from Dr. Carter to yourself on July 28th, 2005.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And it says, "RE: Immunohistochemistry, St.

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John's"?
MS. WEGRYNOWSKI:
A. Um-hm.
CHAYTOR, Q.C.:
Q. You say, "Hi Bev, Thank you very much for this opportunity and I look forward to reviewing your lab protocol. My employer has agreed to give me two or three days to visit your site." And you're talking about different dates.
MS. WEGRYNOWSKI:
A. Um-hm.
CHAYTOR, Q.C.:
Q. "I have some questions that perhaps you could help me with. Total number of antibodies, number of IHC tests run per month, charge technologist involvement, contact and name and number. Could I please contact them prior to my arrival? MLT dedicated and rotating staff type of equipment, date installation and implementation, would you like me to review your SOP's?" And we understand those are standards of procedure?
MS. WEGRYNOWSKI:
A. That's standard operating procedures.
CHAYTOR, Q.C.:

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Q. Standard operating procedures?
MS. WEGRYNOWSKI:
A. That's correct.
CHAYTOR, Q.C.:
Q. Thank you. "Re: validation process prior to the trip or would you like this done on site? Would it be of any value to your staff if I was to do a basic lecture on IHC?" Then it's talk about your charges and your travel. And "At the end I expect I will produce a written summary of findings and recommendations within a month of the visit." And then some logistics about coordinating timing. Ms. Wegrynowski why was this information important, the total number of antibodies, the number of IHC tests run per month, why did you need that type of information?
MS. WEGRYNOWSKI:
A. I wanted to get a sense of the size of laboratory and the complexity of the testing that they were doing, how did they compare to what I do, and it would also provide me with a little bit of a sense of how much time I would need to provide them with their needs.
CHAYTOR, Q.C.:

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Q. Okay. And you asked whether or not they wanted you to review their standard operating procedures?
MS. WEGRYNOWSKI:
A. Right.
CHAYTOR, Q.C.:
Q. And whether that would be prior to coming or after you've arrived. And did you receive an answer to that?
MS. WEGRYNOWSKI:
A. I never received any standard operating procedures or validation process before I arrived.
CHAYTOR, Q.C.:
Q. Okay. And did you understand, though, that that would be part of what you were coming to St. John's to do?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Okay. And the question of whether or not it would be value to the staff to do a basic lecture on IHC, did you receive a response to that?
MS. WEGRYNOWSKI:

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A. I did that and I did that all through Barry Dyer, not Bev Cook.
CHAYTOR, Q.C.:
Q. Okay. Bev Carter, I guess? And then if we can -
MS. WEGRYNOWSKI:
A. Yes, sorry.
CHAYTOR, Q.C.:
Q. That's okay. And if we could have, please, 1731? On July 29th, 2005 Dr. Cook writes to you and says, "Hi Patricia, As discussed, the best time period for your review would be September 20th to 25th" and you could contact Mr. Barry Dyer, divisional manager for anatomical pathology, and his numbers are given.
MS. WEGRYNOWSKI:
A. Um-hm.
CHAYTOR, Q.C.:
Q. His contact information. So I take it then you did contact Mr. Dyer?
MS. WEGRYNOWSKI:
A. To my recollection that's who I contacted, yes.
CHAYTOR, Q.C.:

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Q. Okay. And did you have discussions with Mr. Dyer to arrange coming here?
MS. WEGRYNOWSKI:
A. Yes, I do, and I believe there's an e-mail accordingly.
CHAYTOR, Q.C.:
Q. Okay. If we could look at 1746, please? And this is an e-mail exchange between yourself and Mr. Dyer. And I'll take you--I believe it's again is at the bottom and we'll work our way up.
MS. WEGRYNOWSKI:
A. All right.
CHAYTOR, Q.C.:
Q. And you're writing to Mr. Dyer on--is that the first one? Yes. Here it is.
MS. WEGRYNOWSKI:
A. Okay.
CHAYTOR, Q.C.:
Q. Writing to Mr. Dyer on August 19th, 2005 at 4:10 p.m. And you indicate that it was "nice to touch base with you to discuss my upcoming trip to your institution." And you sent along a copy of your e-mail to Dr. Carter.
MS. WEGRYNOWSKI:

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A. Um-hm.
CHAYTOR, Q.C.:
Q. You indicate, "Thank you for your candour. I have a better understanding of the situation. I will await your call on Monday after your meeting." What did Mr. Dyer explain to you in your initial discussions with him?
MS. WEGRYNOWSKI:
A. To the best of my memory we spoke about they were having issues with their ER/PR, that they had changed and that they had changed equipment.
CHAYTOR, Q.C.:
Q. And what equipment did you understand had been changed?
MS. WEGRYNOWSKI:
A. I understand that their autostainer had changed from the DAKO autostainer to the Ventana benchmark, I believe.
CHAYTOR, Q.C.:
Q. Okay. And what equipment were you using at Mount Sinai?
MS. WEGRYNOWSKI:
A. We use the DAKO autostainer.
CHAYTOR, Q.C.:

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Q. And you continue to use that?
MS. WEGRYNOWSKI:
A. To today.
CHAYTOR, Q.C.:
Q. Okay, and then it's just a copy of your e-mail here that you sent to Dr. Carter with the same questions, and I take it Mr. Dyer answered some of those questions for you in the telephone conversation?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And Mr. Dyer then responds to you on September 5th, 2005, and he says that he's "met with the program director and clinical chief on Friday. They inquired as to when you will be arriving. I explained to them we conversed on Friday and I have asked you to wait until Monday to decide," and then there's just some logistics there. Do you recall this as some period, because you had spoken to him, it appears, sometime prior to August 19th and he's indicating that he's spoken to you, it appears, another time, conversed on Friday. Do you know what discussions you had with Mr.

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Dyer in the interim, between August 19th -
MS. WEGRYNOWSKI:
A. No, no.
CHAYTOR, Q.C.:
Q. Okay. Was there any further information provided to you?
MS. WEGRYNOWSKI:
A. Not that I recall. This is something -
CHAYTOR, Q.C.:
Q. Sure.
MS. WEGRYNOWSKI:
A. No. Thank you.
CHAYTOR, Q.C.:
Q. Okay, and he's wondering about a conference room for a meeting with the appointed pathologists over immunos, and his techs will be available for all three days that you are here.
MS. WEGRYNOWSKI:
A. Um-hm.
CHAYTOR, Q.C.:
Q. And I take it those are people that you met with. You met with the technologists and the pathologist -
MS. WEGRYNOWSKI:

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A. I did.
CHAYTOR, Q.C.:
Q. - in charge of immunos?
MS. WEGRYNOWSKI:
A. I did.
CHAYTOR, Q.C.:
Q. Okay, and who was that person?
MS. WEGRYNOWSKI:
A. Dr. Ejeckam.
CHAYTOR, Q.C.:
Q. Dr. Ejeckam, okay, and then you're back to Mr. Dyer the next day confirming that you've booked to come from September 20th to the 22nd. "After our conversation, I think it is best if I concentrate on the role of validation in the IHC laboratory." What was it about your conversation with him that made you concentrate then on the role of validation?
MS. WEGRYNOWSKI:
A. Because they were having concerns with the estrogen and progesterone receptor and I felt that if they were having concerns, then we need to look at how it is that they were validating it or ensuring that the product--

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the marker was exhibiting the way it should.
CHAYTOR, Q.C.:
Q. Okay, and you go on to offer to do a lecture for the technologists?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Okay, and did you in fact do that?
MS. WEGRYNOWSKI:
A. Yes, I did one on basic immunohistochemistry and another one quality control or quality assurance in the laboratory.
CHAYTOR, Q.C.:
Q. And I understand that you're going to walk us through that today as well?
MS. WEGRYNOWSKI:
A. If you'd like, yes.
CHAYTOR, Q.C.:
Q. Okay, and those will be the same presentations that you gave to the technologists when you were in St. John's in 2005?
MS. WEGRYNOWSKI:
A. That is correct.
CHAYTOR, Q.C.:
Q. Then why did you choose to do basic IHC and

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quality control in the IHC lab?
MS. WEGRYNOWSKI:
A. Because they were rotating technologists, I felt that a basic lecture never hurts and it's a good way to learn from others as well, with their input, and quality control/quality assurance because immunohistochemistry, you must ensure that your pre-analytical, analytical and post-analytical. So it was more just a brush up. I wasn't sure where they were in the process. It was more, this is what we could look at, and if it fits your needs, that's terrific. If it doesn't, we can certainly skip by it.
CHAYTOR, Q.C.:
Q. Okay, and you went ahead with the lectures, so I take it you determined that it did fit their needs?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And you indicate, as for the meeting, "as for meeting the pathologists, I would like to do this at the beginning so as to understand the policies and procedures of the IHC

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department." So that was Dr. Ejeckam, I take it?

MS. WEGRYNOWSKI:

A. Correct.

CHAYTOR, Q.C.:

Q. And you met with him and you found -

MS. WEGRYNOWSKI:

A. I did.

CHAYTOR, Q.C.:

Q. - did you find that helpful?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And were you able to obtain information about the policies and procedures of the IHC department?

MS. WEGRYNOWSKI:

A. No, not really.

CHAYTOR, Q.C.:

Q. Okay, and why not?

MS. WEGRYNOWSKI:

A. I didn't find any.

CHAYTOR, Q.C.:

Q. And you indicate, you go on to say that "I do want to spend time with your technologists,

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understanding the processes that they are presently doing, so that I can assist you with making recommendations," and you ask for the name of the Ventana equipment and kit that you're using, and a list of equipment that they have in the IHC lab?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Was that information provided to you?

MS. WEGRYNOWSKI:

A. Yes, it was.

CHAYTOR, Q.C.:

Q. Okay, and you ask a few questions again about the number of tests and whether or not the staff are dedicated or rotating?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And your "goal is to assist you in evaluating your IHC procedures and to make recommendations from my observations, based on CAP," and again, you can just remind us, please, what CAP -

MS. WEGRYNOWSKI:

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A. College of American Pathologists.

CHAYTOR, Q.C.:

Q. Okay, and OLA standards?

MS. WEGRYNOWSKI:

A. Ontario Lab Accreditation.

CHAYTOR, Q.C.:

Q. Okay, and I take it in Ontario, at Mount Sinai, your lab is actually put through an evaluation or accreditation process? Is that right?

MS. WEGRYNOWSKI:

A. Every lab in Ontario is, that's correct, for QMPLS, that's correct.

CHAYTOR, Q.C.:

Q. And CAP is a form of external proficiency testing?

MS. WEGRYNOWSKI:

A. It is. It's an American organization that we also belong to.

CHAYTOR, Q.C.:

Q. And you voluntarily participate in that, I understand?

MS. WEGRYNOWSKI:

A. Correct.

CHAYTOR, Q.C.:

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Q. Okay, and you're going to divide your day or your time. "I will spend my first day orientating myself and determining a course of action for the rest of the visit. Day two would be ideal for the lectures, if desired, as at this point in time, I should know what to highlight in the talks. Day three could be used with a follow up day with your staff." And then Mr. Dyer is back to you on the 8th of September with some logistics about where you could stay and he gives you the information about the Ventana benchmark, the type of kits, and he indicates that they do about 80 immunos a day by three rotating staff.

MS. WEGRYNOWSKI:

A. Um-hm.

CHAYTOR, Q.C.:

Q. And how does that compare to at the time about what you were doing at Mount Sinai?

MS. WEGRYNOWSKI:

A. We do several hundred a day.

CHAYTOR, Q.C.:

Q. Several hundred a day?

MS. WEGRYNOWSKI:

A. Yes.

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CHAYTOR, Q.C.:
Q. And how many staff do you have?
MS. WEGRYNOWSKI:
A. We have five that report to me.
CHAYTOR, Q.C.:
Q. Okay, and are they rotating or dedicated?
MS. WEGRYNOWSKI:
A. Dedicated.
CHAYTOR, Q.C.:
Q. "Only two are scheduled daily on immunos. One staining and one cutting. The pathologists want three dedicated staff on immunos. This may be a good recommendation." When you read this, Ms. Wegrynowski, did you have any concern?
MS. WEGRYNOWSKI:
A. My concern was the rotating staff, but I wasn't sure what that rotation was, so it was just something I tucked at the back of my mind.
CHAYTOR, Q.C.:
Q. Okay, and then Mr. Dyer concludes with suggesting that you contact Mary Butler, who is the senior tech and the most knowledgeable with immunos, and I take it you met with Ms.

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Butler when you were in St. John's?
MS. WEGRYNOWSKI:
A. I did.
CHAYTOR, Q.C.:
Q. Okay. Other than the indication here that the pathologists want to have three dedicated staff, prior to coming to St. John's or during your time here in your evaluation and review, were there any other ideas suggested to you that Eastern Health thought or Eastern Health personnel thought might be a good idea or a good way forward?
MS. WEGRYNOWSKI:
A. They too wanted--the pathologists, yes, they wanted to have permanent staff in there as well, and I got a sense, once I came here, that the technologists welcomed that idea as well.
CHAYTOR, Q.C.:
Q. And were there any other things that they had in their mind that might be good ideas that they bounced off you and thought -
MS. WEGRYNOWSKI:
A. They were talking at that time about pathology assistants as well.

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CHAYTOR, Q.C.:
Q. Okay, and if we could look, please, at 1747? I take it there's nothing else that you recall about your discussions leading up to coming to St. John's?
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. And this is a fax transmittal to you then on September 14th, and you're scheduled to come, I believe, on September 20th, and this is from Dr. Cook, and he's sending to you terms of reference, external quality review of the immunohistochemistry service. It indicates that your "purpose would be to review the operation and make recommendations as to the processes involved in a service of laboratory medicine program." Did you understand were you to undertake any peer review of the technologists?
MS. WEGRYNOWSKI:
A. Of the process only.
CHAYTOR, Q.C.:
Q. The process only?
MS. WEGRYNOWSKI:

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A. Yes.
CHAYTOR, Q.C.:
Q. So a review of the process?
MS. WEGRYNOWSKI:
A. That is correct.
CHAYTOR, Q.C.:
Q. And "accountability, the external quality review consultant will take direction from and make recommendations to the leadership team of the laboratory medicine program." First of all, did you receive this fax prior to coming to St. John's?
MS. WEGRYNOWSKI:
A. Yes, very shortly before.
CHAYTOR, Q.C.:
Q. Okay, and did you know you would be receiving such a document?
MS. WEGRYNOWSKI:
A. I believe I did.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:
A. I believe I did, because they had--as it was set up as a peer review, it was to be maintained as confidential.

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CHAYTOR, Q.C.:
Q. Okay, and who had that discussion with you?
MS. WEGRYNOWSKI:
A. I believe it was Dr. Cook.
CHAYTOR, Q.C.:
Q. And even though it's set up as a peer review, you were not to undertake a peer review of the people?
MS. WEGRYNOWSKI:
A. No, it was the processes.
CHAYTOR, Q.C.:
Q. Okay, and who did you understand the leadership team of the laboratory medicine program to be?
MS. WEGRYNOWSKI:
A. I thought it was your chief pathologist, your chief technologist, your managers.
CHAYTOR, Q.C.:
Q. And the time frame, they're looking to have you review and report within three months.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And the responsibilities were: to review the current practices and procedures within the

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immunohistochemistry service of the laboratory medicine program.
MS. WEGRYNOWSKI:
A. Um-hm.
CHAYTOR, Q.C.:
Q. To interview individuals who may have relevant facts or current and background information of the service, with particular emphasis in ER/PR. So I take it you weren't limited to ER/PR, but they're asking you to concentrate on ER/PR?
MS. WEGRYNOWSKI:
A. To focus on that, yes.
CHAYTOR, Q.C.:
Q. Okay, and I take it that you did that, you interviewed people?
MS. WEGRYNOWSKI:
A. Yes, I did.
CHAYTOR, Q.C.:
Q. Okay, and who is it that you spoke with besides you've told us Dr. Ejeckam and Ms. Butler, Mr. Dyer. Who else did you speak with?
MS. WEGRYNOWSKI:
A. It would be Dr. Bev Carter, Dr. Don Cook and

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it would be Les and Ken and Mary.
CHAYTOR, Q.C.:
Q. Okay. So Les Simms, Ken Green, Mary Butler, the technologists?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Yes, okay.
MS. WEGRYNOWSKI:
A. And of course, Barry Dyer.
CHAYTOR, Q.C.:
Q. Okay, and then thirdly, to interview other issues of concern using a systems approach, which may have contributed to the situation being reviewed. So number one is to concentrate on the current practices. Number three, you're to identify other issues of concern using a systems approach which may have contributed to the situation they're experiencing, I take it with the ER/PR issue?
MS. WEGRYNOWSKI:
A. Correct.
CHAYTOR, Q.C.:
Q. Okay. What did you understand it to mean when it says using a systems approach?

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MS. WEGRYNOWSKI:
A. Immunohistochemistry is based on the product that you receive from the specimen when it is first excised from the OR. So to assess the quality of that particular block, one needed to go back to see where it had originally arrived or derived from. So that's what I took that to mean.
CHAYTOR, Q.C.:
Q. So to follow the tissue right from the time it's produced in the OR all the way through to a slide being -
MS. WEGRYNOWSKI:
A. Presented to the pathologist.
CHAYTOR, Q.C.:
Q. - presented to the pathologist.
MS. WEGRYNOWSKI:
A. That's correct.
CHAYTOR, Q.C.:
Q. Okay, and in looking at what may have contributed to the situation being reviewed, I take it if you could shed any light on what may have caused or contributed to the problem, they were asking you to do that?
MS. WEGRYNOWSKI:

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A. Yes.
CHAYTOR, Q.C.:
Q. And to provide recommendations as appropriate to deal with any issues identified during your review?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And you, in fact, did that?
MS. WEGRYNOWSKI:
A. I did that.
CHAYTOR, Q.C.:
Q. In your report.
MS. WEGRYNOWSKI:
A. Um-hm.
CHAYTOR, Q.C.:
Q. And to summarize the findings of the quality review in a confidential report.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And then you're given a case summary, which indicates "in 1997, a DAKO semi-automated manual system was installed for immunohistochemistry service and replaced the

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bioassay method for testing for ER/PR receptors, and this DAKO system was replaced in 2004 by an automated Ventana system with on board antigen retrieval." And then "a patient, in 2005, initially tested in 2002 with the DAKO system reported as ER/PR negative, had been retested with the Ventana and now indicated strong positivity." And then "four other patients initially tested as negative were also retested and all tested positive with the Ventana system. Retesting was expanded to include all samples initially tested as negative in 2002 on the DAKO system. Of the 57 retested on the Ventana, 38 showed positive results. This high conversion rate then placed the sensitivity of the Ventana system in question and as a result, all negative samples since 1997 had been sent to an external laboratory for testing" and we understand that was your laboratory, Mount Sinai.
MS. WEGRYNOWSKI:
A. It was at Mount Sinai, correct.
CHAYTOR, Q.C.:
Q. And were you provided any other information in

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terms of the background leading up to the issue?
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. This was it?
MS. WEGRYNOWSKI:
A. This was it.
CHAYTOR, Q.C.:
Q. Okay, and the idea of a conversion, a patient converting, going from negative to then being strongly positive, when you read this or were told this, was that of concern to you? Had you heard of this happening before?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. You had heard of this happening before?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Okay, and in what context had you heard of such a situation?
MS. WEGRYNOWSKI:
A. I should rephrase that. Maybe not this

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situation, but the first thing that cued off in my mind is I wanted to look at the processes and the validations and the procedure manuals to determine how this could have come about.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:
A. There had to be something that happened.
CHAYTOR, Q.C.:
Q. And the idea then that "there was a high conversion rate placing the sensitivity of the Ventana system in question," did you then look at that while you were at their lab?
MS. WEGRYNOWSKI:
A. Not the sensitivity of the Ventana system, no.
CHAYTOR, Q.C.:
Q. Did you think that that was an issue?
MS. WEGRYNOWSKI:
A. At that point, I can't comment to that. I don't recall that being one of the top priorities or top thoughts in my mind.
CHAYTOR, Q.C.:
Q. And when you were here, in terms of observing the system, were you able to rule out whether

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or not the Ventana system in fact was too sensitive?

MS. WEGRYNOWSKI:

A. The Ventana system as being too sensitive, I can't comment to that. I didn't really have a chance to review a lot of slides here, so I wouldn't be the best to comment.

CHAYTOR, Q.C.:

Q. And again, Mount Sinai uses the DAKO system?

MS. WEGRYNOWSKI:

A. We do.

CHAYTOR, Q.C.:

Q. And how does that differ from the Ventana?

MS. WEGRYNOWSKI:

A. To the best of my knowledge, the Ventana has on board retrieval, so it's--if I recall correctly, you put your slides in and the entire process is done in an automated fashion. Sometimes we call it the plug and play system. You just put it in, off it goes, everything is pre-dilute and if optimized correctly and validated in the laboratory, should give you reproducible results. The DAKO system is using a autostainer, which is taking nothing more than a pipette that's

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timed. It works on an XYZ axis, so it--you program it and you say what you would like dispensed for what period of time. The rest of the procedures are all done outside the autostainer.

CHAYTOR, Q.C.:

Q. Okay, and in terms of the DAKO system, we've heard reference to a 40-step process. Do you know what that refers to?

MS. WEGRYNOWSKI:

A. I believe what people are speaking to is the process of actually taking a section from the block and walking it through all the way until it completes its staining. Where in the Ventana system, there is much less hands on.

CHAYTOR, Q.C.:

Q. So if we were to count up from the time that the tissue is excised, all the way through to producing the slide, perhaps there could be 40 steps in the process?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Sort of like a recipe in carrying out.

MS. WEGRYNOWSKI:

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A. It's cumulative.

CHAYTOR, Q.C.:

Q. Yes, to get to your end product.

MS. WEGRYNOWSKI:

A. Correct.

CHAYTOR, Q.C.:

Q. Okay, and then in terms of your report, it says "the external quality review shall be in writing and include the team's recommendations. The recommendations shall be shared with involved staff members. The peer review, its conclusions and the final report are protected under the Evidence Act and as such, the final report will not be available to any third party and as well, the final report is protected from any subsequent legal proceedings." And Ms. Wegrynowski, it seems to differentiate between the external quality review and the peer review. What did you understand, which portion are you involved in here?

MS. WEGRYNOWSKI:

A. I felt that I was under the peer review, that the conclusions that I would derive in the final report were to be protected under the

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Evidence Act.

CHAYTOR, Q.C.:

Q. Okay, and that was told to you by whom?

MS. WEGRYNOWSKI:

A. I can't recall whose name to use to that, but.

CHAYTOR, Q.C.:

Q. Did you expect that your report would be shared with others in the lab?

MS. WEGRYNOWSKI:

A. I thought it would be curtailed to the laboratory to the chief pathologist and the laboratory manager, yes, I did.

CHAYTOR, Q.C.:

Q. Okay, and would such distribution in fact be something you would expect and be a positive move, to have your report shared with those who are carrying out the process?

MS. WEGRYNOWSKI:

A. Correct.

CHAYTOR, Q.C.:

Q. So who did you understand would not be able to see your report?

MS. WEGRYNOWSKI:

A. I never thought of it in an exclusionary way.

CHAYTOR, Q.C.:

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Q. Okay. So I take it September 20th comes, a few short days later, and you arrive in St. John's.
MS. WEGRYNOWSKI:
A. I did.
CHAYTOR, Q.C.:
Q. Okay, and tell us about that then. Take us forward from there. Where did you go upon arrival in St. John's?
MS. WEGRYNOWSKI:
A. I met with Barry Dyer on site at Eastern Health.
CHAYTOR, Q.C.:
Q. And I understand there were two sites, the St. Clare's Hospital and the Health Sciences. Did you, in fact, visit both sites?
MS. WEGRYNOWSKI:
A. I did.
CHAYTOR, Q.C.:
Q. Okay, and you started at the Health Science with Mr. Dyer?
MS. WEGRYNOWSKI:
A. I did.
CHAYTOR, Q.C.:
Q. Okay, and what did Mr. Dyer talk to you about

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then or what observations did you make?
MS. WEGRYNOWSKI:
A. Could we--is there--my notes, are they in here?
CHAYTOR, Q.C.:
Q. I think we do have your notes at P-1745, please. Are those your notes?
MS. WEGRYNOWSKI:
A. Yes, they are.
CHAYTOR, Q.C.:
Q. Okay. So if you could just walk us through that, please?
MS. WEGRYNOWSKI:
A. I had a conversation with Mr. Barry Dyer on Tuesday, September the 20th '05 and it was in his office, and he gave me an overview. I asked him to tell me a little bit about the organization and that's what he did for me. He explained to me that the organization was reorganized in 1996 and that fixation was site dependent. In 2002 -
CHAYTOR, Q.C.:
Q. What did that mean, sorry, fixation was site dependent?
MS. WEGRYNOWSKI:

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A. I take that to mean that each site was following their own processes and procedures.
CHAYTOR, Q.C.:
Q. So there wasn't a standard procedure between -
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. - St. Clare's and the Health Science?
MS. WEGRYNOWSKI:
A. That's correct.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:
A. And that in 2002, the organizations, all the immuno was done then at Eastern Health for the St. John's area. He explained to me that 60 to 70 percent of the breast cases are actually done at the St. Clare's site. He also told me that -
CHAYTOR, Q.C.:
Q. So meaning the surgeries? I'm sorry, I didn't mean to interrupt.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:

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Q. So most of the surgeries, but the actual IHC, the producing of the slides was taking place at the Health Science?
MS. WEGRYNOWSKI:
A. That was my understanding.
CHAYTOR, Q.C.:
Q. Okay, I'm sorry, go ahead.
MS. WEGRYNOWSKI:
A. And he spoke to me that 50 percent of the blocks from St. Clare's are needing to be reprocessed.
CHAYTOR, Q.C.:
Q. And what does that mean, in your world?
MS. WEGRYNOWSKI:
A. In my world, that means that the blocks were not handled correctly from the beginning. That there would be concerns whether or not the protein was actually stable and we could find it.
CHAYTOR, Q.C.:
Q. Perhaps you can explain to the Commissioner, and for those of us who, like myself, who were not in the know, tell us what reprocess is and why that is of concern.
MS. WEGRYNOWSKI:

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A. Okay. I'll start at fixation. The theory for fixation is to actually fix the protein and that's exactly what we're looking for in immunohistochemistry, and in doing so, it stops two things. One is autolysis which is the breakdown of a cell. When the cell breaks down, first you lose your cytoplasm, then you lose your cell surface membrane. It becomes very fuzzy, and then you start to lose your chromatin and your nucleus and that's where you start seeing hollow nuclear pattern and you won't be able to find a signal.

The other thing that fixation arrests is putrefaction, which simply means that when the body dies, all of the natural flora or any other bacteria that you have starts eating away at the body because it's hungry. Fixation also is able to harden the tissues, so that when we move through processing, we are able to produce a block. So from your original fixation in neutral buffered formalin, you then move into gradient alcohols, so you will start off with, like a 70 or 95 percent alcohol and this is also a form of post fixation. Once it reaches a

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hundred percent alcohol, it then moves into the cylene. From the cylenes it moves into the wax and that is how one is able to make those little blocks that I'm sure you have seen where the slides are created. When you start talking about reprocessing, it tells you something has gone awry within the system. It can either be that your alcohols were not clean, so that you were not able to remove the water. If you do not move the water, the water is not miscible in the cylene and so you're going to end up with this mush and you will not be able to get a section.

CHAYTOR, Q.C.:

Q. Okay, and so Mr. Dyer indicated to you about fifty percent of the blocks at St. Clare's were having this problem and were having to be reprocessed?

MS. WEGRYNOWSKI:

A. That's what he stated.

CHAYTOR, Q.C.:

Q. Okay, and if you could continue on then, please?

MS. WEGRYNOWSKI:

A. This has taught me to print neater next time.

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Health Sciences and St. Clare's, he spoke to me a little bit about where things were coming, biopsies would come in in formalin and larges would come from St. Clare's and there was a grossing and processing going on at St. Clare's. There was no weekend processing at the Health Sciences, but they were planning to bring this on.

CHAYTOR, Q.C.:

Q. So what did you understand then was happening with tissues over the weekend?

MS. WEGRYNOWSKI:

A. That they were hopefully sitting in formalin, is my understanding.

CHAYTOR, Q.C.:

Q. Yes, okay, sorry, go ahead.

MS. WEGRYNOWSKI:

A. Okay, and the embedding, cutting and staining was done at the Health Sciences and then the slides were being sent back to the original site for the pathologists to review. For fixation, he said to me that the policy at Health Sciences was to fix for 24 hours and St. Clare's site, he told me the fixation was up to the pathologist. But I did not get that

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confirmed by the pathologist and I have written here it was because there was a lack of policies and procedures and I'm not sure where that communication came from, but that's what I was told. He just spoke to me a little bit about the workload, that they were four days behind, that they were doing about 150 biopsies a day and three hundred large blocks and he stated to me that the protocols they used were very informal. He wanted to change the transport of tissue to come over to Health Sciences to be fresh and that he would also like to have a PA and they were beginning to-- they didn't have one, but they wanted to get one and that they were beginning to write their standard operating procedures.

CHAYTOR, Q.C.:

Q. And I take it then at this point in time when he's telling you the protocols are very informal and you've noted here a lack of policies and procedures, up to this point in time you were still expecting to see documentation with standard operating procedures?

MS. WEGRYNOWSKI:

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A. Absolutely, because this was just my first introduction to him and I just met him in his office, so I wasn't quite sure what I was going to find.

CHAYTOR, Q.C.:

Q. And were you surprised by that?

MS. WEGRYNOWSKI:

A. It was--I couldn't use that term, I was just there for information gathering.

CHAYTOR, Q.C.:

Q. Okay, continue on please?

MS. WEGRYNOWSKI:

A. As I said, he spoke that they didn't have any PA's and that they were beginning to write their SOP's and we just kind of went through, you know, what sort of specimens that they were looking at, the skin, the bowel, the breast, the thyroid and that St. Clare had the same mix as what Eastern Health did, and we're talking about specimens, but their focus was on breast, head and neck. And he told me that they did have someone over there, I'm--at this point, I couldn't tell you if it was a technologist or not, that they were doing the gross assists and they were doing about 20 per

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day. They took care of the frozens and two autopsies a month because all the biopsies came over to the Health Sciences. He just gave me a little bit about what their expectations were, that their urgent specimens or their stats had to be cut by 7 a.m.; the biopsies were to be cut by 9 and then the routine would follow. The cutting was done at St. Clare, they had four technologists there. Okay, I'll have to come back to that one, I'm not sure what that means. So then this would be back over at the other site, they had 9 technologists who assisted with the grossing. They did the cutting, the staining, the labelling, they were handling about a thousand slides a day. Their slide labels were not interfaced with the computer, but they did have a Meditech system. They also had two technicians that were included in the mix of the nine technologists, but these technicians had 30 years experience and according to Barry, that they were doing everything a technologist does.

CHAYTOR, Q.C.:

Q. So what does that mean, that they weren't

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dedicated to one particular specialty, I take it?

MS. WEGRYNOWSKI:

A. That meant to me that their scopes of practice were being used the same as a technologist, as opposed to being used as a technician. They had a data entry operator who entered all the specimens and sent out some receiving specimens. And their cut off was some 75 patient cases a day, but the biopsies were 35 and the routine get dropped if the biopsies get picked up. It's just what you would do if you have to get your work through. Prior to 2003, he advised me that the formalin was made up in house and then sent out to everyone and since then, they had purchased the--they were purchasing their ten percent neutral buffered formalin.

CHAYTOR, Q.C.:

Q. And did he tell you why they had made the change in January, 2003?

MS. WEGRYNOWSKI:

A. I don't recall what the reason was.

CHAYTOR, Q.C.:

Q. Okay.

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MS. WEGRYNOWSKI:

A. I just remember making myself a note saying that we needed to look at the pH's for that, the pathologist fill out requisitions--oh, this is when it goes to the immunohistochemistry group and so the surgical pathology number and the requisition would be filled out and cut by the immuno and so I as thinking, okay, let's start looking at our microtomes, our numbers, our documentation because in immunohistochemistry you're not the first person to touch the block, you want to ensure that you're not cutting through the block, you're not cutting through the tumor, so in my world it's called enfacing the block where the section, the block is taken up to the microtome knife so that the technologist would not lose any of the crucial tissue. So I wanted to see what they had in their protocols about that and how they were doing it. I just mentioned histocolometer there, that's a particular item that we use at Mount Sinai Hospital, but it depends on your microtome whether or not it can assist you. We talked about the number of pathologists a

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day, one does biopsy, the technologist does the gross and one pathologist routinely does gross and reads. Just talking about the routine and how it comes through and that the medical director is in charge of immunohistochemistry. And then this later on goes on to, this is a little bit later in the process.

CHAYTOR, Q.C.:

Q. So this is not your initial meeting on the next page with -

MS. WEGRYNOWSKI:

A. Yes, no, that's a little bit later on.

CHAYTOR, Q.C.:

Q. This comes later.

MS. WEGRYNOWSKI:

A. Yes, it does.

CHAYTOR, Q.C.:

Q. All right, and perhaps then we can continue on though, this is all information that you gathered during your visit?

MS. WEGRYNOWSKI:

A. Yes, it is.

CHAYTOR, Q.C.:

Q. What's written here on page 5?

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MS. WEGRYNOWSKI:

A. Okay, I asked him whether or not there were any competency programs in place for the technologists and there wasn't any.

CHAYTOR, Q.C.:

Q. And what's a competency program?

MS. WEGRYNOWSKI:

A. You want to ensure that the individuals performing the procedures are well within their scope of practice, that they have the knowledge, the skills and the tools to perform them.

CHAYTOR, Q.C.:

Q. Okay.

MS. WEGRYNOWSKI:

A. I asked him whether or not there was any external quality assurance programs and they did not have one.

CHAYTOR, Q.C.:

Q. Is it standard to be involved in a competency program?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Okay, and when you set up, for example, the

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lab at Women's College Hospital back in 1983, were the competency programs put in place then?

MS. WEGRYNOWSKI:

A. They were rather informal, I must say that as I was preparing for this, I found my procedure manual that I wrote around that time, so even in there these things were addressed and what the expectations of the technologists were, were in that -

CHAYTOR, Q.C.:

Q. Back in 1983?

MS. WEGRYNOWSKI:

A. Yeah, I think it was a little bit later than that, but yes, within that timeframe.

CHAYTOR, Q.C.:

Q. Okay, and EQA, don't have one, what's EQA?

MS. WEGRYNOWSKI:

A. External Quality Assurance programs, he wanted to ensure that what you're testing is--how it compares to those in the external, so what you're getting is correct.

CHAYTOR, Q.C.:

Q. And again, is that standard to have an external quality assurance or be involved in -

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MS. WEGRYNOWSKI:

A. You know, to speak to that, I couldn't say back in the '80's--it was more interlaboratory comparison at that point, if you, you know, pathologists would look at some slides and they would both -

CHAYTOR, Q.C.:

Q. And by 2005, is it standard?

MS. WEGRYNOWSKI:

A. Oh absolutely.

CHAYTOR, Q.C.:

Q. And I take it it became standard sometime before that? Would you be able to say around when, being involved in external quality assurance program would have become standard in the industry?

MS. WEGRYNOWSKI:

A. From my personal--when I became involved with Mount Sinai Hospital in 1999, they were already presently enrolled in the CAP.

CHAYTOR, Q.C.:

Q. And if you could continue on please? And I'll get you to explain a little more about external quality assurance when you do your presentation.

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MS. WEGRYNOWSKI:
A. All right. So I did ask about new antibodies, how they were brought into the laboratory setting and it was explained to me that the pathologist would provide the controls and they would check the slides and it was up to them whether or not the product would go forward. They would only use positive controls when they were doing their validation, not negative in a composite block. I asked whether or not there was any technical input, were the technologists abreast of why this antibody was coming into the laboratory? Were they given articles to read and they were not. I was looking for whether there was documentation, a formal documentation about the validation process. I did not find that. I asked about what validation--do you want me to explain a little bit about what validation is?
CHAYTOR, Q.C.:
Q. Sure. That was not occurring, validation.
MS. WEGRYNOWSKI:
A. Was not occurring, is not what I found. If you were to purchase a product and you run

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through the product, you are going to order a new one and it may not be of the same lot, it may come from a different lot, so you want to assure that that lot is working in the exact same manner as the previous lot because things can change between lot to lot, there's definitely lot to lot variation. So I asked about that in regards to the antibodies, the detection system that they were using and any ancillary products that they were using. They were not running negative controls.
CHAYTOR, Q.C.:
Q. And what's the importance of negative controls?
MS. WEGRYNOWSKI:
A. The sensitivity of your testing, you want to ensure that you are not getting any non-specific staining.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:
A. This is much later, this is probably when I was getting together to write the report, these are all the questions that I was asking when I was there for those three days. I

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asked about the water. Water has a huge impact in IHC, the calibre of the water, we use deionized water and I can tell you for a fact that at Mount Sinai when we have our chambers changed, we do not use the water for at least 24 hours because of the amount of oxygen that gets into the system, so we actually stock pile our water and then we go forward with that. We need to ensure that our buffers are maintained.
CHAYTOR, Q.C.:
Q. And what were they doing in St. John's?
MS. WEGRYNOWSKI:
A. I wasn't able to find any information out on that. They didn't know what type of water they were using, there is different types of water depending on the amount of, I believe it's bacteria in the water, so the purer the water, the more you have to just be cognizant of this, but I never got a sense of what type they were using.
CHAYTOR, Q.C.:
Q. And does this say "water on tap"?
MS. WEGRYNOWSKI:
A. Yeah, it's not tap water.

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CHAYTOR, Q.C.:
Q. I was hoping -
MS. WEGRYNOWSKI:
A. Sorry, my deionized water is tapped into the laboratory, so we have a central still and it comes through all the labs and we have little indicator lights on them and whatnot. No, I wasn't using tap water, sea water.
CHAYTOR, Q.C.:
Q. And then that's the next bullet then, micro -
MS. WEGRYNOWSKI:
A. Oh, and I was asking because what does your micro and chemistry department use because you're going to use that for your autoanalyzers, so I would assume -
CHAYTOR, Q.C.:
Q. Yes, okay. I think it says "calibration records, type unknown".
MS. WEGRYNOWSKI:
A. Yes, and that would be in regards to the water, what type of water it was. I asked whether or not their pipettes were being calibrated and no, they were not.
CHAYTOR, Q.C.:
Q. And perhaps you could just tell us what does

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that mean? What is a pipette and why would you need to calibrate it?

MS. WEGRYNOWSKI:

A. Okay. The pipette in immunohistochemistry, when you are using concentrated antibodies is the most important tool that you would use on a daily forum. You will have predetermined what all your dilutions are and from those pipettes, you are going to make that dilution. If your pipettes are not calibrated and maintained, then you never have the assurance that the dilution is made correctly. In addition to that, you must always assure that your pipette tips are sterilized because you do not want to include any contaminants into the antibody.

CHAYTOR, Q.C.:

Q. And if you could continue on, please?

MS. WEGRYNOWSKI:

A. I asked to see, well at this point I guess one of the thought processes would have been service records. You need to ensure that all of your equipment is working, your PMs, preventative maintenance, on annually, daily, weekly, monthly, quarterly, whatever they

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should be for whatever instrument, they need to be done and there needs to be documentation for that. And I've written something in the corner, but I can't read that. Oh, Sakura from Ventana apparently from Montreal, perhaps, had looked at their Ventana but that, I think that's just going by memory, that's perhaps why I scrawled that. They were doing manual staining for the HER2/neu and using the DAKO kit and for their immunofluorescence, they were doing that manually and they were doing kidney biopsies. Their medical laboratory technologists are--they write the same national exams as all other technologists in the country; however there is no provincial college, but they do have a medical laboratory group and I was told that they are supportive of continuing education.

CHAYTOR, Q.C.:

Q. I'm sorry, who was supportive of continuing education?

MS. WEGRYNOWSKI:

A. This would be from Barry.

CHAYTOR, Q.C.:

Q. The medical technical group was supportive of

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continuing education?

MS. WEGRYNOWSKI:

A. Yes, so that there was--I know that he had stated that he was going to--he had gone to the National Society of Histotechnology and I believe that was in one of the e-mails that we had corresponded with. So we were talking just about on rotating technologists and the grossing, that they were doing grossing and immuno and just the different techniques that were being done and who was doing what, but nothing in--they were sending their FISH to Sunnybrook and did you want to know what FISH was?

CHAYTOR, Q.C.:

Q. Sure, it might mean something different in Newfoundland.

MS. WEGRYNOWSKI:

A. It's fluorochrome in situ hybridization. It's your goal standard when you start doing the HER2/neu testing. HER2/neu testing leads to breast cancer patients receiving Herceptin. So a patient which would be equivocal would be, their case would be reflux to FISH.

CHAYTOR, Q.C.:

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Q. Okay.

MS. WEGRYNOWSKI:

A. Re-embedding, I needed to look at their temperatures, I wasn't quite sure where they were at and they did have a fridge in the immunohistochemistry on an alarm, which is very important because there's thousands of dollars worth of antibodies in that particular fridge.

THE COMMISSIONER:

Q. I'm sorry, they had it on a?

MS. WEGRYNOWSKI:

A. They did have it on an alarm.

THE COMMISSIONER:

Q. Oh, an alarm.

MS. WEGRYNOWSKI:

A. Yes, because some of these antibodies can cost several thousand dollars for one ml., so that's quite critical. They weren't using NIST traceable thermometers.

CHAYTOR, Q.C.:

Q. And what's that?

MS. WEGRYNOWSKI:

A. NIST just is a acronym for the National Institute of Standardized Testing, so all the

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equipment that I use at Mount Sinai Hospital is traceable, all the timers, thermometers, that kind of stuff. They had stated that they had changed equipment from a DAKO to the Ventana, so I wanted to know what the protocol for purchasing the new equipment was.

CHAYTOR, Q.C.:

Q. And what were you told about that?

MS. WEGRYNOWSKI:

A. You know, I don't have a sense of a response for you right now.

CHAYTOR, Q.C.:

Q. So do you recall whether you were given a response or was anybody able to tell you what happened through that change over?

MS. WEGRYNOWSKI:

A. I really can't recall that.

CHAYTOR, Q.C.:

Q. Okay.

MS. WEGRYNOWSKI:

A. They had three technologists, they were training a MLT, this was with a go-ahead plans that they were wanting to have three technologists that were to be permanent, train MLT, I was surprised they were doing their--

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immunofluorescence was being done in the light and there was no controls being run. The specimen storage and that we can speak to, if we may, when I have my notes up from my report.

CHAYTOR, Q.C.:

Q. Sure.

MS. WEGRYNOWSKI:

A. They had fume hood that was stuck in the corner of the immunohistochemistry laboratory and they had different chemicals on top and they should be put away, that's just for safety. Oh, it was, their last comment here, "does our institution support your Ventana computer?" If I recall correctly the technologists were telling me or explaining to me, rather, when they were doing some of their maintenance, it wouldn't be the daily, I believe it would be either quarterly or semi-annually that that was done, actually with inside of the computer and that they didn't have the ability to get that, so I had suggested that perhaps they look at their computer people to assist them with that.

CHAYTOR, Q.C.:

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Q. And does this say "Have IF SOP.

MS. WEGRYNOWSKI:

A. I must have left them a standard operating procedure for IF, could very well be.

CHAYTOR, Q.C.:

Q. Did they have their own?

MS. WEGRYNOWSKI:

A. For me to leave that note, I'm suggesting I did not see a formal one.

CHAYTOR, Q.C.:

Q. And on the top of the next page says "ER/PR 300 to 400 cases". Is that what you understood they were doing per year?

MS. WEGRYNOWSKI:

A. Could be, yes.

CHAYTOR, Q.C.:

Q. And what does this say?

MS. WEGRYNOWSKI:

A. The notes on the express, I made notation in my actual report about the express, it's a different processing and different fixations.

CHAYTOR, Q.C.:

Q. And I'll ask you to take us through that and explain it when we get to your report.

MS. WEGRYNOWSKI:

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A. I'd be happy to do that.

CHAYTOR, Q.C.:

Q. Kohler illumination?

MS. WEGRYNOWSKI:

A. They were not doing that.

CHAYTOR, Q.C.:

Q. Calibration (unintelligible - 113) no, and what's this say?

MS. WEGRYNOWSKI:

A. Again, as I spoke to Madam Commissioner about the sterilizing of the tips so that you ensure that you're not contaminating any of your antibodies, the answer to that was no. They were using a product from Ventana called Easyprep for the depariffinization and I asked whether or not they were pHing it to ensure that it was doing what--it was living up to what the commercial standards of what the company had said and they weren't doing that. The reaction--I can't read that, oh again, that would be again with their buffers, their DABs, they're presently not neutralizing them. I asked about gloves and goggles, negative patients were not done.

CHAYTOR, Q.C.:

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Q. And I take it again that's a safety concern?
MS. WEGRYNOWSKI:
A. That's a safety concern because some diaminobenzidine is carcinogenic, negative patient controls were not being done and dates on the slides were missing.
CHAYTOR, Q.C.:
Q. Dates on the slides -
MS. WEGRYNOWSKI:
A. I'm going to take that--oh, there's Ken's e-mail address he left for me. So this would be Wednesday, September 21st, we're just talking about the quality of tissue.
CHAYTOR, Q.C.:
Q. And do you recall who you had that discussion with the next day?
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. And then on the next page, I believe is notes of, a brief note of your discussion with Dr. Ejeckam, so I take it Dr. Ejeckam, you said was able to assist you in understanding the current state of affairs. Did he also give you any indication as to what may have been

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the state of affairs over the last couple of years? Anything that had happened in the past?
MS. WEGRYNOWSKI:
A. No, not to me, but I recall meeting with Dr. Ejeckam and we sat and we spoke about immunohistochemistry. He felt that they should not be rotating in there, he would have liked the technologist to start reading the controls. He had begun to teach the technologists about the theory, I got the sense that he was rather frustrated because they were rotating, it was very difficult to get all three or how many people that you were using in that area at the same time. He was also rather frustrated because he felt that he, and I wrote this, this was the only comment that I wrote from that entire meeting, that they had to get clearance from Barry. So he did not feel as being the director of that particular subsection that he was able to go and speak with the technologists directly, but rather he was to speak to the manager and the manager was then to speak to the technologist.
CHAYTOR, Q.C.:

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Q. What were you able to conclude from your discussion with Dr. Ejeckam as to his own understanding and level of knowledge of IHC?
MS. WEGRYNOWSKI:
A. I felt that Dr. Ejeckam had a very good thorough knowledge of immunohistochemistry. He certainly understood the procedures and we talked about some of the textbooks that are out there and we both agreed that they were very strong textbooks, that certainly would assist one in the troubleshooting area of immunohistochemistry. It's the troubleshooting of immunohistochemistry that is truly where your knowledge needs to lay.
CHAYTOR, Q.C.:
Q. Okay, and I believe there is actually reference to the Dabbs text here.
MS. WEGRYNOWSKI:
A. Yes, we both kind of agreed on that one and I like Delias, so there you go.
CHAYTOR, Q.C.:
Q. So this was in trying to recommend a textbook for the laboratory?
MS. WEGRYNOWSKI:
A. They had nothing, yes.

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CHAYTOR, Q.C.:
Q. They had nothing. And this was a text that you and Dr. Ejeckam agreed would be useful?
MS. WEGRYNOWSKI:
A. That would be correct.
CHAYTOR, Q.C.:
Q. Did Dr. Ejeckam or anybody else, while you were in St. John's, indicate to you that he had detected some issues in 2003 and in fact had shut down the laboratory for a period of weeks at that point in time?
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. That wasn't told to you?
MS. WEGRYNOWSKI:
A. No.
MR. SIMMONS:
Q. Not the whole lab.
CHAYTOR, Q.C.:
Q. Not the whole lab, I'm sorry, not the whole lab, they had stopped doing certain stains, including the ER/PR. I think there were eight stains altogether.
MS. WEGRYNOWSKI:

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A. No.
CHAYTOR, Q.C.:
Q. That wasn't made known to you.
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. Okay, and in terms of 2005 and the concerns that he raised, there was the issue obviously of the communications or the reporting channel to Mr. Dyer.
MS. WEGRYNOWSKI:
A. Correct.
CHAYTOR, Q.C.:
Q. The fact that the staff were rotating. Did he identify any other concerns? Did he identify anything in terms of consistency or standards that were being maintained, anything along those lines?
MS. WEGRYNOWSKI:
A. I can't recall. It was more that there was this frustration and trying to be able to teach the technologists about immunohistochemistry.
CHAYTOR, Q.C.:
Q. About the theory.

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MS. WEGRYNOWSKI:
A. Yeah, and to be fair because at this point I didn't know what the cycle was of people rotating, I got the sense after meeting with the technologists, if I may interject at this point, they too felt this sense because if you received a phone call from someone that this didn't work, you have to recall they are not reading any of their controls and you did not do that work that day, you really don't have a sense of where you can start as well, so there was a lot of frustration, I would say on both sides.
CHAYTOR, Q.C.:
Q. So were the technologists reading the controls?
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. And did Dr. Ejeckam indicate to you whether or not these were issues that, you said he's frustrated, you sense a frustration.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:

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Q. So was it that these were issues that he had identified and brought forward but was having difficulty getting his concerns addressed? Was that the nature of the frustration?
MS. WEGRYNOWSKI:
A. That was my sense, he had asked me what we were doing at Mount Sinai Hospital and I told him what we were doing there. So that's basically what our conversation was about.
CHAYTOR, Q.C.:
Q. And the reference to the textbook and you indicated they didn't have any textbooks, was the internet available to the technologists?
MS. WEGRYNOWSKI:
A. They had nothing.
CHAYTOR, Q.C.:
Q. What was your sense of the role that the technologists were being asked to play at Eastern Health as compared to your role as a technologist at Mount Sinai?
MS. WEGRYNOWSKI:
A. As a technologist at Mount Sinai Hospital, I am expected to produce a product at the end of the day that is reproducible, as is my staff. We do not sign out anything out of the

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laboratory if it does not pass our control, so if our positive control does not pass, none of the slides go out that day until that problem is rectified. At Eastern Health the technologists were simply doing what stain was being ordered and they would put it through and then it would end up in the pathologist's desk and then they would be working backwards.
CHAYTOR, Q.C.:
Q. And I believe Ms. Wegrynowski in your interview you made a statement that it was your sense that they were to come and do, not to think, is that a fair statement?
MS. WEGRYNOWSKI:
A. I think that's a rather fair statement. It's not meant to be critical, but if you don't have the tools, you can't think to troubleshoot. If I may, there's a difference between working in a regular histology laboratory and doing special stains, as compared to immunohistochemistry when you're actually doing, it's an enzymatic process, so it's very different and you need to know this.
CHAYTOR, Q.C.:
Q. And so you need to have a high level of

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knowledge to be able to do your job?
MS. WEGRYNOWSKI:
A. Yes, and simply because of all the issues that can occur.
THE COMMISSIONER:
Q. Can you elaborate a little on that and that is the requirement that the technologist understand more than what it is you physically have to do in the context of this kind of testing.
MS. WEGRYNOWSKI:
A. Okay, I guess this would then go back to the academic setting because when you're in school, you're not learning about immunohistochemistry, so a lot of what goes on in immunohistochemistry one learns on the job. If everything thing is working perfectly, then you are going to have a positive result. So if I may just use something so simple as a hepatitis surface antibody, so you have an infected liver and we have a hepatitis surface antigen and we find the antibody and it's stained. If we were to do that same test the next day and it was to be negative, that would tell us that something has gone askew. In so

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doing, it would be then our role to determine what that was prior to sending that work out, so you would go back and look at the processes. So it's a very analytical departmental learning. Does that answer your -
THE COMMISSIONER:
Q. I think it does in part, so what you're saying is that you really need people who are not just going through the motions, they are acutely aware of what they have to do and do they also need the knowledge of what can go wrong if they do not do things in a particular way?
MS. WEGRYNOWSKI:
A. They need to understand the why they are doing things.
THE COMMISSIONER:
Q. Okay, but it makes sense to me, but it seems to me that if you do not understand the importance of doing things correctly, then you just don't have that mindset which says it matters.
MS. WEGRYNOWSKI:
A. I agree.

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THE COMMISSIONER:
Q. When small measurements are slightly off or whatever.
MS. WEGRYNOWSKI:
A. I agree and that also brings into the different types of testing that occurs in immunohistochemistry. The majority of the testing that we do are class 1, but there are some class 2 testing which affects definitely the patient's therapies and this would be one of them.
THE COMMISSIONER:
Q. And class 1 verses class 2 means?
MS. WEGRYNOWSKI:
A. It's just a level of testing, so class 2 testing would have a direct impact on the patient, so in that category we have things like HER2/neu and our ER/PR's.
THE COMMISSIONER:
Q. Okay.
CHAYTOR, Q.C.:
Q. And I take it that during your visit when you went to St. Clare's, you met with Dr. Cook, I take it, at St. Clare's?
MS. WEGRYNOWSKI:

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A. Yes, I did and Bev Carter.
CHAYTOR, Q.C.:
Q. And Bev Carter.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Just based on what you've told us so far and through review of your notes, at any point in time did your expectations as to what you were coming to St. John's to do, did those expectations change and if so, why?
MS. WEGRYNOWSKI:
A. I thought I was coming to St. John's, and as I detailed in the original e-mail, to look at their standard operating procedures and I wanted--they had an issue, so I thought, all right, if I can look at your standard operating procedures before I arrive, it will save me a lot of time, so if perhaps there's something in them that we can focus on and I wanted to see them not just for the immunohistochemistry laboratory, but right from when they are excised from the patient. So when I didn't receive them, I wasn't quite sure--I thought, okay, well maybe they're--

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there's not going to be a lot to read, I really didn't have a sense of what was going to unfold.

CHAYTOR, Q.C.:

Q. So then at some point in time your expectations, I take it, did change as to what you were here to do?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And what you could do?

MS. WEGRYNOWSKI:

A. That would be correct.

CHAYTOR, Q.C.:

Q. Were you aware that the lab, the IHC lab had been running the ER/PR testing since 1997?

MS. WEGRYNOWSKI:

A. I believe that was set out for me in the initial e-mail, yes.

CHAYTOR, Q.C.:

Q. Okay, and the state of affairs that you observed then in St. John's in 2005, given that the lab had been running since 1997 with respect to those two stains, were you--did you have any concerns as to the state of affairs

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that you found?

MS. WEGRYNOWSKI:

A. I was surprised.

CHAYTOR, Q.C.:

Q. And what is it--why were you surprised?

MS. WEGRYNOWSKI:

A. That there were not standard operating procedures, that documentation was completely remiss.

CHAYTOR, Q.C.:

Q. At St. Clare's did you also visit the lab there and the operating room?

MS. WEGRYNOWSKI:

A. I went to the lab. The reason I wanted to look at the lab and the main reason that I went there, in my mind, was because of the reprocessing rate that had been highlighted by Barry Dyer. I wanted to look at their processors, I wanted to see their documentation. I wanted to see their standard operating procedures for how they were handling the breast tissue. There has to be a reason for that number to be so high.

CHAYTOR, Q.C.:

Q. And what did you observe then at St. Clare's?

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MS. WEGRYNOWSKI:

A. I didn't find any documentation.

CHAYTOR, Q.C.:

Q. And in terms of your visit to the operating room?

MS. WEGRYNOWSKI:

A. I looked in the operating room, I wanted to ensure that there are refrigerators in the operating room. There are times when porters can't obviously pick up every second, every specimen as soon as it is excised, so you need to put your specimens into the refrigerator to maintain them at 4 degrees, because you want to stop autolysis and putrefaction, you need to arrest that and they had no refrigerators, things were left on bench.

CHAYTOR, Q.C.:

Q. Perhaps we could look then at P-1736 please? And these aren't your notes, these are -

MS. WEGRYNOWSKI:

A. No, I've never seen this.

CHAYTOR, Q.C.:

Q. No, these are notes of Dr. Cook, it just indicates in his notes here, he's doing a bit of a chronology, we understand, and he

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indicates here that "On September 21st, 2005, Dr. Cook, Dr. Carter interviewed Trish" and we understand that's just his notation of your meeting.

MS. WEGRYNOWSKI:

A. Uh-hm.

CHAYTOR, Q.C.:

Q. The actual "at St. Clare's" and you met there with Dr. Cook and Dr. Carter, your review of the lab itself, the lab process itself, did you actually observe what was happening in the lab, how the tissue was being processed?

MS. WEGRYNOWSKI:

A. No, I did not. The technologists--I don't recall anybody doing any of those, there were technologists there and that's how I asked to see the standard operating procedures. I wanted to see their temperature checks and they were telling me that they were just looking at the temperatures by the digital read out and I explained to them that the digital read out on a piece of equipment is not acceptable because there can be differences between that particular read out and the actual read out, especially for the

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paraffin wax. The reason I mention that is that some of the antibodies are heatedly laible so you can lose them at high temperatures.

CHAYTOR, Q.C.:

Q. And is ER/PR though in that category?

MS. WEGRYNOWSKI:

A. I can't comment. Off the top of my head, I wouldn't comment.

CHAYTOR, Q.C.:

Q. Okay, if we could look at P-1738 please?

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. And this again is not your notes, these are notes that Dr. Cook took, "Spoke to Trish, Dr. Bev Carter September 21st, 2005 at 11 a.m.", he's indicating and these are some notes that he took, apparently, from your meeting.

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. And it looks like he's saying "Start with CAP proficiency testing, techs are overwhelmed." Are these things that you would have indicated

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to Dr. Cook and Dr. Carter?

MS. WEGRYNOWSKI:

A. I did have a conversation with them while we were at St. Clare's. I had not met with the technologists, I don't believe at that point. I believe I met with Barry Dyer and then went straight to St. Clare's.

CHAYTOR, Q.C.:

Q. And so was this something that was related to you by Barry Dyer or perhaps this is, do you recall Dr. Cook and Dr. Carter telling you this?

MS. WEGRYNOWSKI:

A. You know, I have not seen this before, I couldn't comment.

CHAYTOR, Q.C.:

Q. Okay, well tell us what you recall about your meeting with Dr. Carter and Dr. Cook.

MS. WEGRYNOWSKI:

A. Okay, I recall Dr. Cook just knowing that there was something wrong, he didn't know what it was, but he definitely just wanted it fixed and Bev Carter had trained in Ontario and I didn't know it at that point and she had told me this, and she had explained to me that she

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knew something was wrong, she just knew that this would never have happened and she told me the name of the technologist that she had worked with. She said this just would not have happened and his name was Brian Hewlett and he's also a colleague of mine and we were talking about, like what is happening when something goes wrong, what is it that you do if you get these slides and you recognize that they're not working, the controls are not working. And this is when I found that they were then calling over to the immunohistochemistry laboratory at Eastern Health and telling them, you know, this didn't work and then the technologist at the other end of the phone--it gets back to this issue if you're rotating and you didn't do it, so there was this sense of frustration. There was no continuity of service.

CHAYTOR, Q.C.:

Q. So did you understand that Dr. Carter and Dr. Cook both had recognized there was a problem, that their controls weren't working and were trying to communicate with the technologists to figure out what--why that would be? What

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was going wrong in the process?

MS. WEGRYNOWSKI:

A. That was my sense.

CHAYTOR, Q.C.:

Q. Okay, and how long did you understand that they were aware there was problems with the staining?

MS. WEGRYNOWSKI:

A. I didn't get a timeline from them.

CHAYTOR, Q.C.:

Q. Okay, was that something that had just come up in 2005 or -

MS. WEGRYNOWSKI:

A. Ms. Chaytor, I really don't know that.

CHAYTOR, Q.C.:

Q. Okay, do you recall anything else then from your meeting with them?

MS. WEGRYNOWSKI:

A. Not much more. A lot of it would have just been, you know, the validation processes, policy manuals, I mean -

CHAYTOR, Q.C.:

Q. These are things that he's noted here.

MS. WEGRYNOWSKI:

A. Yeah, that would have been very basic things

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that I would have been looking for, so I mean, they could have asked me what it was that I was looking for. They did put their controls, they have--they do put their positive controls on slides and so, I mean, that is terrific. We don't have that opportunity at Mount Sinai Hospital, we batch ours. We just don't have that same amount of control tissue. So they're really at a terrific advantage out here. We spoke--I did speak to them about fixation and that stemmed again from the reprocessing. You definitely need standardized fixation, you need the same processing at both sites. This is just basically all the things that I would have gone through.

CHAYTOR, Q.C.:

Q. Okay, and it indicates here that "need to be trained how to read controls", I take it that's the technologists?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And you indicate "keep the control tissue on the slides" -

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MS. WEGRYNOWSKI:

A. Uh-hm

CHAYTOR, Q.C.:

Q. You saw that as a good thing.

MS. WEGRYNOWSKI:

A. Uh-hm, absolutely.

CHAYTOR, Q.C.:

Q. And the fixation issue again, and I can't confess to be able to read all of his writing, but this appears, what you're saying to be a list of things that you would have discussed with them.

MS. WEGRYNOWSKI:

A. You know, it's almost as if this was--yes, yes.

CHAYTOR, Q.C.:

Q. And the issue of validation, need to validate the lots, which you've already mentioned.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Need to document pH.

MS. WEGRYNOWSKI:

A. This would have been the big picture of what I would have looked for.

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CHAYTOR, Q.C.:

Q. Okay, and I think it's written over here "DAKO system worked fine", would that be something you discussed with them?

MS. WEGRYNOWSKI:

A. Yes, I do because Bev, I believe, had had experience with the DAKO and I gather there were those that thought it was the DAKO itself that had led to this, so there was some discussion with that, as well.

CHAYTOR, Q.C.:

Q. I take it though the DAKO system they had been using was no longer at Eastern Health -

MS. WEGRYNOWSKI:

A. I never saw it.

CHAYTOR, Q.C.:

Q. You never saw it.

MS. WEGRYNOWSKI:

A. I never saw it, no.

CHAYTOR, Q.C.:

Q. And did you understand that it was no longer there?

MS. WEGRYNOWSKI:

A. Yeah.

CHAYTOR, Q.C.:

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Q. And this says, does it say "no calibration of the DAKO system" or -

MS. WEGRYNOWSKI:

A. It says that, but I mean there was nothing for me to review. It wasn't as if I could go back and say could you please show me how you calibrated that DAKO to begin with, what you used and how you changed to this. I did not find that.

CHAYTOR, Q.C.:

Q. And you would have expected to have that information where?

MS. WEGRYNOWSKI:

A. In your service manuals.

CHAYTOR, Q.C.:

Q. And this also says "no assurance things can be done the same day, no standardization of DAKO system, no validation of system, don't know where to look to troubleshoot" and again, that's referring to the technologists, I take it?

MS. WEGRYNOWSKI:

A. Yeah.

CHAYTOR, Q.C.:

Q. "Need Ken or Mary to be in charge of

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immunoservice?"
MS. WEGRYNOWSKI:
A. You need somebody to be responsible and there.
CHAYTOR, Q.C.:
Q. Okay. And over on this column then, continuing on down it says, "need protocol" and I believe we spoke about that one, "for fixation".
MS. WEGRYNOWSKI:
A. Uh-hm.
CHAYTOR, Q.C.:
Q. "Things are being done but no documents, need to be trained to troubleshoot. Need to get involved with the CAP program. Techs don't have the skills, need training at"--looks like "computer" and "need PA's"--that's the pathology assistants.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. "Need spec sheets"?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And what are the spec sheets?

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MS. WEGRYNOWSKI:
A. The spec sheets are the sheets that come in--they're documentation that come in with the antibodies, they tell you about the clone, the lot, the expiry date, the approaching concentration, what the subclass is of the antibody, so it's all the vital information that you need to use to be able to work with the product.
CHAYTOR, Q.C.:
Q. And "Need external QA program" and we'll talk about that some more in your presentation.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. "Need to send to well organized, well run lab" and I take it you're referring to getting the techs further training?
MS. WEGRYNOWSKI:
A. Yes, I think that was something that Dr. Cook suggested, not myself.
CHAYTOR, Q.C.:
Q. Okay, other than Dr. Ejeckam, Dr. Carter and Dr. Cook, did you meet with any of the other pathologists?

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MS. WEGRYNOWSKI:
A. Not that I recall.
CHAYTOR, Q.C.:
Q. And the technologists then, you believe your meeting with the technologists came after your meeting with the doctors?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And what do recall about your meeting with Mary Butler, Les Simms and Ken Green?
MS. WEGRYNOWSKI:
A. If I could just back this up just a bit.
CHAYTOR, Q.C.:
Q. Sure, absolutely.
MS. WEGRYNOWSKI:
A. So on the first day I met with Barry, Bev, Don and Dr. Ejeckam, so that was what--so when I completed that day, I needed to go on a forward basis and because I had not found any documentation or supporting evidence for what I was looking for, I took it upon myself to use the rest of the visit as simply a learning took because there was not any documentation to review.

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CHAYTOR, Q.C.:
Q. So to teach the technologists?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Okay, and your discussions then with them, did they voice any concerns to you?
MS. WEGRYNOWSKI:
A. No. Any concerns other than they too were very frustrated, they felt that they were being pulled in many different directions, you know, they were trying to do their very best, they certainly were doing so, but they couldn't because they were being pulled in far too many directions.
CHAYTOR, Q.C.:
Q. And did they feel they had the tools to be able to do their job properly?
MS. WEGRYNOWSKI:
A. I would think they would have preferred had they had more.
CHAYTOR, Q.C.:
Q. And did they express to you that they felt things were inconsistent or that there was a lack of continuity in the lab?

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MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. When was it that you met with the technologists?
MS. WEGRYNOWSKI:
A. I met with them on my second day.
CHAYTOR, Q.C.:
Q. So that was on the second day?
MS. WEGRYNOWSKI:
A. Correct.
CHAYTOR, Q.C.:
Q. And then you decided to use this as a teaching time for them?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Did you feel that they had the information that they required for them to understand the theory of IHC sufficient for them to be able to actually put it into practice?
MS. WEGRYNOWSKI:
A. You know, I never evaluated that, I couldn't comment.
CHAYTOR, Q.C.:

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Q. Okay. Did you meet at any point with Mr. Gulliver?
MS. WEGRYNOWSKI:
A. Very briefly.
CHAYTOR, Q.C.:
Q. And at what stage of your visit would that have happened?
MS. WEGRYNOWSKI:
A. It was more, hello, how are you, glad to see you here, but nothing -
CHAYTOR, Q.C.:
Q. Nothing substantial?
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. And no discussion around the issue or issues of concern?
MS. WEGRYNOWSKI:
A. Not that I recall.
CHAYTOR, Q.C.:
Q. Okay. Well then perhaps what we can do is move into looking at your PowerPoint that you would have taken the technologists through. Did anyone else attend the presentations?
MS. WEGRYNOWSKI:

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A. No, I would have liked it, I would have hoped that we could have set it up in a conference room of some sort, at least for the quality assurance portion, but that did not occur.
THE COMMISSIONER:
Q. Ms. Chaytor, I just wanted to remind you it's about 11:00 and ask you your preference in terms of the morning break, would you prefer to take it before this or -
CHAYTOR, Q.C.:
Q. Sure, we're going to go through two or three PowerPoint presentations then, so perhaps this is a good time.
THE COMMISSIONER:
Q. All right. Why don't we take the morning break now then.
(RECESS)
THE COMMISSIONER:
Q. Please be seated. Ms. Chaytor.
CHAYTOR, Q.C.:
Q. Thank you, Commissioner. I believe when we broke, Ms. Wegrynowski, we were about to go into the presentations that you gave to the technologists during your visit in September of 2005.

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MS. WEGRYNOWSKI:
A. Okay.
CHAYTOR, Q.C.:
Q. When you met with the technologists on the second day that you were here, where did you meet with them?
MS. WEGRYNOWSKI:
A. I met with them in Barry Dyer's office and I gave the three of them the lecture on his computer, so they sat behind me.
CHAYTOR, Q.C.:
Q. Okay, so it was done in Mr. Dyer's office with them standing behind you looking at the computer screen?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Okay, and the first presentation that we have is P-1765 and I understand this is basically a summary of what you do in Toronto or basically what your lab has in Toronto. So if you just want to take us through from there, you can go ahead.
MS. WEGRYNOWSKI:
A. I just started the PowerPoint off just a

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little bit about Toronto. I always find that when I ever go places, people always like to know where you're from, so--anyway, that's just a picture of our waterfront, not nearly as beautiful as yours, of course, but it is ours. Okay, this is just--this is the legislature for the Province of Ontario and this is Mount Sinai Hospital. Mount Sinai Hospital is located on University Avenue in Toronto, affectionately known as "hospital world" so we have Hospital of Sick Children across the street from us and we're flanked on either side then of the UHN and then we have the Queen Elizabeth Rehab Centre to the other side of us. This was just a little bit about the amount of work that we do at, what was in the year of 2004, and Mount Sinai Hospital just to provide--it's a comparative. People like to know how you compare with them in size and structure and whatnot. So at that point we were doing 21,000 surgical specimens, 7000 outside consultations, 203 autopsies of which 177 were perinatal. Mount Sinai has one of the largest NICUs in the Province of Ontario, so that explains that number.

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CHAYTOR, Q.C.:

Q. So the 7000 outside consultations were coming from hospitals?

MS. WEGRYNOWSKI:

A. Everywhere.

CHAYTOR, Q.C.:

Q. Everywhere?

MS. WEGRYNOWSKI:

A. Yeah. Presently for my work in immunohistochemistry over half my work is global consults. This was just to give a little breakdown of the number of people that are on site, that we has 14 pathologists, 3 pathology assistants, 35 MLTs, 4 technicians and we have secretarial and transcriptional pool of 16.

The pathology sections that we have at Mount Sinai Hospital is basic histology, we also have sarcoma because that's one of the leading programs at Mount Sinai. We have electron microscopy department, immunopathology, we have special histology which does morphology and plastics, which then evolved into doing immunohistochemistry and many other things. We have autopsy services

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and then the research services also went into special histology since that time. Woops.

In 2004 we were doing 40,000 tests annually. At that point our consultation group was 35 percent. We were doing about 200 slides a day, 150 antibodies and the kind of immunohistochemistry, we were working on paraffin sections, cytospins, which would come out of the Department of Cytology and frozen material. This is mainly for IF. And again, we do over 200 IF cases a year, and we had four MLTs at that time.

CHAYTOR, Q.C.:

Q. Okay. And since then, since 2005 your number of antibodies are now over 200?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And you now have five MLTs?

MS. WEGRYNOWSKI:

A. Yes. And our global consultations are over 50 percent, yes. Okay. So this is the--at that particular time this was the equipment that we had in the immunohistochemistry laboratory at Mount Sinai Hospital. We had two DAKO

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autostainers, we had a microMED T/T Mega, a microscope, the Accumet pH meter, the mettler balance, two incubators, slide drier, pipettes, fridges, freezer, microtomes, water bath, cover, slippers. The main portion of the screen is just to let you know that there's documentation for all these products and their maintenance schedules and whatnot.

Again, we had at that time four detection systems, we were doing single and double labelling for cocktails, we used different chromogens and--chromogens are just the colour, so whether you get the brown or the red, that's all that is.

CHAYTOR, Q.C.:

Q. And what's a detection system?

MS. WEGRYNOWSKI:

A. A detection system is how you--what you use to be able to find the antibody. So if you have an antibody, it's the detection system that you use, then you apply a chromogen to be able to identify it.

CHAYTOR, Q.C.:

Q. And the chromogen is the colour?

MS. WEGRYNOWSKI:

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A. The colour. It's the -
CHAYTOR, Q.C.:
Q. So in the ER/PR it's the brown colour that shows up when it's positive?
MS. WEGRYNOWSKI:
A. Right. That's correct. It's an enzymatic reaction. So it's not a dye, it's not a stain, okay.
CHAYTOR, Q.C.:
Q. And what's, I'm sorry, FITC?
MS. WEGRYNOWSKI:
A. FITC, it's the green fluorochrome that you would use when you're using your kidney biopsies. It's very common. The EQA that we were presently using at that point was the CAP and OLA and there was some external quality assurance going on with involvement with the immuno users group. Whoops. That's the end of that one. That was just my little introduction.
CHAYTOR, Q.C.:
Q. Okay. And if we could have then, please, 1764? So that was basically, I take it, to give the technologists some idea of the equipment that you have in your lab?

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MS. WEGRYNOWSKI:
A. Yeah.
CHAYTOR, Q.C.:
Q. The size of your lab and how it would compare to what they have?
MS. WEGRYNOWSKI:
A. That's right, who I am, what we have and what we do, that's correct. Okay, so you want to do this one next. Okay, immunohistochemistry, this was a PowerPoint, it's basic IHC, talking about what we do in immunohistochemistry, a little bit about the theory. Immunohistochemistry is an accurate localization of tissue or cellular constituents within antibodies.

So there are three major functional roles of our antibodies. We identify the tissue of origin in a metastatic tumor; we can provide data for therapy; and we can measure tumor antigen levels.

When we talk about tumor of origin, again, this is just very basic, it's not meant in any way, shape or form to be, you know, this is the only way. When we're looking for carcinoids, they would use, a pathologist

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would select a panKeratin; for sarcoma specimens they would be looking at Vimentin and Desmin; neuorendocrine tumors stain with Synaptophysin and lymphomas would stain with CD45, which is just your BNT cells. Okay.

When we talk about therapies, and this is where it comes in for hormone receptors, and this again, Madam Commissioner was talking about with the Class 2 because it has a direct impact on patient. We are talking about estrogen and progesterone because the results of that will result in the patient either receiving or not receiving Tamoxifen therapy. The same can be said of Her2/neu and the patient receiving or not receiving Herceptin. And with gastrointestinal stromal tumors it's the positivity of a CD117, which would provide the clinician with the knowledge to provide the patient with Glivex.

Tumor antigen levels are almost ancillary. No one would take a biopsy out just to look at a tumor antigen levels because these can all be found during a serum, so you would just use blood work. But occasionally we use this, if the tumor has been taken out,

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this would be just part of the product. So CA125 will mark your ovarian cancer; CA will mark, excuse me, your colon and gastric cancer; and the prostate, specific antibodies for your prostate cancer. But we have, we use other things now for our prostate cancers. Okay.

So what is an antigen? An antigen is a substance that can induce an detectable immune response and in that antigen we are looking for the epitope, and that's your structural part of the antigen. So it's that epitope that is sitting on the cell surface that you're actually looking for.

And antibodies are immunoglobulins that are produced as a result of the introduction of an antigen. So, for example, you would go to your doctor, you would get an immunization and your body would get the antibody, your body produces the antigen.

This is a brief, just a schematic of the IGG molecules, got your two heavy and two light chains. You've got your interdisulfide bonds, and that's just something in my world that we have to look at.

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This is the Fc portion that carries the specific antigen determinant which is raised towards that particular IGG. So if you're looking at the structure of the IGG molecule you know at which end the antigen will bind and which end the antibody will bind.

And I have a schematic and I use that a lot for teaching, because I think it provides people--if it's all going on in a test tube and it's clear, it's very difficult to visualize. So what you have is your primary antibody. Your primary binds to the antigen in the tissue, your epitope, and then it becomes, it acts as an antigen for the second antibody and then the second antibody binds to the antigenic sites of the Fc portion of the primary antibody molecule. The best way I could describe it, it makes a sandwich. And there it is. So if we use the cell surface as being the green portion and your little white portions, that would be your little epitopes and you can see that your primary antibody is bound. You can see that the shape is correct, that it absolutely fits right in. So, it wouldn't non-specifically bind had that been

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like perhaps a cube or a triangle, it could be washed away. So, you would not have that binding and that's why you have that specificity. If you were to imagine those great big floaty balloons out there with the little IG molecule floating down was to bind to the primary, they would just absolutely slip right in, almost like docking when you think of a jet or something.

In my world we use three kinds of antibodies. I think in this lecture we just talked about two, but they're monoclonal and polyclonal. For ER and PR I think we can concentrate on the monoclonal because that's what they are, they have particular clones. Polyclonals don't have clones. So polyclonals are just immunochemically dissimilar, they react to various epitopes. So you inject the antigen, the titre gets measured of the rabbit and once it's high enough, it's bled and then the serum has to be purified.

Monoclonal antibodies, that's exactly what you may be talking about. They have identical clones of plasma and they're directed towards one epitope. So in the

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estrogen you're talking about the clone 6F11 and the progesterone that we were using at Mount Sinai, we used the 1294. And so this is just a little tiny bit of how it's created. The--it's injected into the antigen. They sacrifice the mouse and their B-lymphs are fused with the myeloma cell and their hybrid myeloma cell. And what ends up actually happening is that they're harvested at the manufacture level, so then that's what they sell to the public or to the laboratories. So that's at that point that they can--that's where your protein concentration, how much of that antibody is actually in that particular natant (phonetic).

Okay, so we talked a little bit about antibody characteristics, the affinity and the avidity.

CHAYTOR, Q.C.:

Q. And maybe you could tell us what those are?

MS. WEGRYNOWSKI:

A. I did, it's definitions of all. The affinity is the binding sites, they fit well with the antigen sites on a specific antigen because you don't want any other antibody to bind. So

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it's almost like this affinity is like an attraction, it is so strong that it is the only way that those two sites will bind. And I think that the first schematic showed that very well.

The avidity is the binding strength and stickiness. And it depends on the number of fitting sites between the antigen and the antibody. And that occurs so what when you wash your sections off, that the antibody does not come dislodged from the epitope.

The staining methods that we reviewed that day were the direct method, two step indirect method, a PAP method and an Avidin Biotin Method. There are other methods out in the market, but that's what we discussed that particular day.

So we're going to look right now at the requirements for immunohistochemistry. You must have the preservation of the antigen in the tissue and this goes right back to the cornerstone of formalin fixation and how the specimens are procured. We talk about specific an sensitive staining. And then we speak about the efficient labelling and

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detection.

Preservation is achieved by fixation. You want the antigen made insoluble but available for detection. So by using formalin you get the cross linking of the formaldehyde fixation and it is there to preserve the protein. The longer piece of tissue is left in formalin, the greater degree of cross linkages.

So in the direct method, the most basic example I could give you would be if you had a kidney biopsy taken, the kidney biopsy would be brought to the laboratory. We use a transport media called Michelles, but the reason you do this is that the work will be done on the tissue in a frozen state. All you need to do is then the technologist would cut the sections and apply the correctly diluted primary antibody which is already conjugated to the fluorochrome and if the deposit is there, then it stains, and if it's not there, it's not. So that's a very, very simplistic, not the most sensitive but very simplistic.

The two step indirect technique is very rarely used in immunohistochemistry. It's used

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more in hematology. But it's the same premise where you have in green the primary antibody attached to the epitope, which is the blue, and in the yellow you have your conjugated enzyme being labelled so that you'd be able to visualize it.

The PAP method is one of the first methodsthat were used in immunohistochemistry. It's peroxidase, antiperoxidase where the secondary antibody was against the specific animal. So if the primary was a monoclonal, then we would have a secondary against that mouse and then our tertiary, the last one that goes on is the peroxidase, antiperoxidase. And if you notice at the top of the scheme, there are two fushcia coloured dots. So this is binding sites of the chromogen. So for every one epitope that you can detect there are two chromogen binding sites. The reason why I bring this to your attention is that with sensitivity and with different methods your amount of chromogen that you have is greater so that actually you can use less antibody. So in using concentrates this is more, when

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you're using pre-dilutes it isn't as much a big deal because manufacturers have taken care of this for you.

This is the Avidin and Biotine method we presently use just in immunohistochemistry and this is actually what is used on our estrogen and progesterone. So if I can walk you through that. If we have the epitope at the bottom, say that's a SF11 and we're applying--okay, so we have an epitope at the base, which is our nucleus, and then we apply our 6F11 which is a teal green, to that we're going to put our secondary antibody which is a Biotinylated horse anti-mouse, so it's going to be against that primary antibody. The Strip Avidin, there's such a strong affinity between Strip Avidin and Biotin that they cannot be taken apart, and that's why this method, just basic science, works as well as it does. You can see the number of chromogen binding sites are greatly increased than we have seen in any of the previous slides, which has provided us with a very sensitive method.

So then we talked about the controls that are necessary in the immunohistochemistry

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laboratory. So as far as reagent controls, we were talking about the primary antibodies, controls to do with the reagent and the detection system, and again to do with the chromogens. With tissue you need to ensure that there's positive controls and negative controls and internal controls but only when they are present. Internal controls are not present in every single tissue, only in some. For example, if you were looking for--how about if we go back to the hepatitis surface. You would have no internal control there because that would mean the patient had the virus. But on a piece of skin, and were to do a panKeratin, that would cover all the different parts of skin, you would expect it to stain. Does that provide some clarity? Okay.

So then I went on to speak a little bit about what these reagent controls and all the different controls mean. For reagent controls, when we're looking at positive and negative, we are doing this to validate the staining technique. We can do an assessment of the handling and fixation of the tissue.

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We can standardize our methods and results among laboratories and we also use it for education and for performance and interpretation. We want to ensure that every single reagent that we use in any procedure is working optimally.

The one thing that you must know about your tissue controls is that they must always be processed identically to the specimen. And I recognize that this becomes an issue because for fixation there are no national standards, there's no one that says every single piece of tissue is going to be fixed at the exact same rate. But what you need to do is ensure that in your particular facility that they are processed identically to the specimen and that is why your standard operating procedures must be in place describing this. So with our positive control we ensure that, again, it's processed identically and that it contains your target protein because if it didn't, it wouldn't be positive. And the negative, again, the same thing is true, so that it does not contain that tumor marker or that tissue marker. And internal controls are built-in

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controls. And again, they're not always present in every tissue, they can't possibly be. But when they are, they're a really great backup.

CHAYTOR, Q.C.:

Q. And in doing ER/PR testing, would they normally be an internal control would be present in doing ER/PR testing?

MS. WEGRYNOWSKI:

A. I can speak to this, although this is outside of my scope of practice because I would not be selecting the section that would be used for the testing. You know, you certainly are at liberty to ask a pathologist this if you don't -

CHAYTOR, Q.C.:

Q. Yes. And I take it the--you can go ahead, though.

MS. WEGRYNOWSKI:

A. Oh.

CHAYTOR, Q.C.:

Q. Your knowledge of it, as well.

MS. WEGRYNOWSKI:

A. Okay. The knowledge is that if you use normal tissue, the ducts will stain, so you would try

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and pick a piece that is not just totally tumor.

CHAYTOR, Q.C.:

Q. Okay. So usually what you see and what block would be presented to you would include both normal epithelium and tumor?

MS. WEGRYNOWSKI:

A. I wouldn't be looking at that, but that would be the pathologist's call, that is in their scope of practice.

CHAYTOR, Q.C.:

Q. Yes. But when you see it and what gets put onto then eventually the slide, it would normally include the normal tissues as well as the tumor in doing ER/PR testing?

MS. WEGRYNOWSKI:

A. I wouldn't be reviewing the HNEs to determine that.

CHAYTOR, Q.C.:

Q. Okay.

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. Fair enough.

MS. WEGRYNOWSKI:

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A. Yeah, okay. Oh, my goodness, I think I've done something, it's not going forward.

CHAYTOR, Q.C.:

Q. If we can get rid of that? Okay. Here we go, we're good.

MS. WEGRYNOWSKI:

A. Okay, thank you. I think I pushed one too many buttons. So then we discussed pretreatments. So there are several pretreatments that we use in immunohistochemistry presently. The first being and the gentlest would be proteolytic digestion. The next would be heat-induced epitope retrieval. For proteolytic digestion, the two proteins that we use are Pepsin and proteinase K, proteinase K being the gentlest of the two. So by scientific theory we recognize that formalin fixes by forming cross linking methylene bridges. And this goes back, even back to the '80s when I first described in immunohistochemistry how we were only able to work on formalin, excuse me, on fresh tissue, that we were not able to work on formalin. It wasn't until the advent of heat retrieval on digestive natures that we were

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able to expose the epitopes, so this is a little bit down the line. So what it does is the digestion compensates for the impermeable nature of the fixative, which is formalin and it's very gentle and the enzyme enters the tissue allowing the epitope to be exposed.

So this is just a photomicrograph of panKeratin without Pepsin, so it's a piece of skin and it's supposed to show all the epithelium, so your squamous epithelium. And so once you do your digestion, I'm sure you can see that it's quite noticeably different. So by doing so, by doing that one pretreatment, you have now been able to expose the epitope and you are able to visualize it.

Heat retrieval, heat retrieval, it was described by SHE (phonetic), it's almost, I don't really understand myself how it all works and I--there are many pieces in literature, but really no one understands the direct mechanism because we speak about maintaining temperatures and not having your waxes, but yet, here we are taking things up to 115 degrees celsius, 120 degrees celsius under pressure. It's quite a phenomenal

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science. Heating provides the energy to rupture the hydroxyl bonds and releases the tissue bound calcium ions which breaks a fixative bond, permanently exposing epitope. Efficiency of heat induced epitope retrieval is a function of time, temperature, pH and the chemical composition of the buffer. And those are definitely the cornerstones of heat induced epitope retrieval.

This is just an example of particular antibody PGP, and what we're looking for here are nerve cells, that's all you need to know. So this would be a very basic validation process. You can say, okay, so how do we get to expose this epitope, what is it going to take to be view it? I can tell you that in this particular example, I've kept everything the same in relationship to the dilution and the detection system. The only thing that was changed was the pretreatment.

So this one we used Pepsin under 37 degrees, ten minutes, and there's obviously no staining because all you see are blue dots. So then when we took it into heat retrieval and we use something called a Citrate buffer

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at pH 6 for three minutes at 115 degrees celsius, you start seeing that some of the nerve cells are starting to come up. But maybe it didn't photograph very well, but rest assured that when we took that pH up to 9 for the same time at temperature, the end product was intensified and the high signal low noise would then be done and then this is where you would go on with the process. So at this point you would say--because there was a question raised to me, I know that you raised it to me in Toronto, maybe I could -

CHAYTOR, Q.C.:

Q. Sure.

MS. WEGRYNOWSKI:

A. If I could take the liberty now? Someone asked me, and I don't recall which of you, does a pathologist see all the slides when you validate? And I'll tell the answer is no. If I had showed the pathologist the first slide and there was absolutely no staining, he would probably, or she would look at me and say, why are you bothering showing me this. So it is the judgment of the technologist at that point to say, okay, there's no signal here, where do

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we go from here. So at this point if we had moved through this process and recognized that this was already giving us the best signal to noise, then from there we would start working on our dilutions.

COMMISSIONER:

Q. Signal to noise?

MS. WEGRYNOWSKI:

A. Signal to noise. Signal being the brown or the chromogen, what we're seeing, and the noise being the background is very clear.

COMMISSIONER:

Q. Okay.

MS. WEGRYNOWSKI:

A. Because you want to ensure that the signal that you're seeing, it's much, it's much easier to interpret than if you have this noise in the background, which would also indicate that the validation process wasn't correct.

COMMISSIONER:

Q. Okay.

MS. WEGRYNOWSKI:

A. Okay. Is that okay?

CHAYTOR, Q.C.:

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Q. Yes, thank you.
MS. WEGRYNOWSKI:
A. Okay. So section quality. Section quality, there is no part of immunohistochemistry laboratory that is not important. There is no role in there that is not important. The section quality taken by the microtomist is imperative. The reason for this in this example, and if I was to explain it to you, it was like the ridge going down the centre is a fold, so the tissue is absolutely, it's overlapping on itself. So you can see that this is, you know, this is using the DAKO, I don't have the Ventana so I don't know what it would look like on the particular equipment that you have here, but on the DAKO autostainer, it applies by drop, so it drops in two or three different sections. So the antibody will spread but if it hits this wall, it will not be able to spread any further because we are only using 100 microlitre drops, which are very small volumes. And as you can see, that if you provide a section like that, there is no staining if you look at the right side of the screen, so this would be

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an invalid test.
The score works in the exact same manner, except obviously in reverse. The score then acts as a well. And as you can see, the antibody will--the detection systems and chromogens will only be able to spread a certain amount and then they can't go any further and I think there's a higher power of that, so this is a cycling--it's a self--it's a CD marker, cluster differentiating marker, so that you can see that even in the sections that are underneath in the well, the antibodies start getting up underneath it because it lives, so these are invalid tests.
CHAYTOR, Q.C.:
Q. So and what was it that made these tests invalid?
MS. WEGRYNOWSKI:
A. Because the staining is not complete.
CHAYTOR, Q.C.:
Q. Okay. And why would that be, does it have anything to do with how the tissue was sliced, the size of the tissue?
MS. WEGRYNOWSKI:
A. No. This has all got to do--geez, I've done

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it again, sorry. This has all got to do with the microtomist.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:
A. Okay. So at sections, taking the sections in the laboratory so the pathologist is provided, the technologist says this is the test I want done, so they are now preparing the slides. And these are some TMAs, I think Frances provided me with for this lecture. And you can start seeing even they start to lift. And that's an issue that you can find with TMAs, tissue microrays. How they're created very simply is that a pathologist would look at a slide, they would mark the area of tumor and it's like a very fancy hole punch, and they just pop up that piece and then they get put into blocks, and that's basically what tissue microrays are.
CHAYTOR, Q.C.:
Q. So in your laboratory if you were--would you look at those slides and determine yourself that this had not worked effectively and it would never go to the pathologist?

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MS. WEGRYNOWSKI:
A. Shouldn't go out. And I can speak to this is that, well, I shouldn't say never, things do happen, if it was an antibody that you were expecting to stain the entire slide, let's say, and it was a lymphoma and you were doing a marker that should show every B and T cell on that and you noticed yourself that there was only half a staining, you would stop and take a look yourself, of course.
CHAYTOR, Q.C.:
Q. Okay. And you would have a microscope in your lab and -
MS. WEGRYNOWSKI:
A. We have a microscope in our laboratory, yes.
CHAYTOR, Q.C.:
Q. And when you arrived in St. John's in 2005, did the technologists have a microscope in their lab?
MS. WEGRYNOWSKI:
A. I don't believe they did, no. I should say I don't recall, I don't--they certainly didn't have a multi-header, that's for sure. Okay, so then we're talking a little bit about background staining. There are two reasons

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that you can get background staining. The first is the endogenous peroxidase activity and the second one is endogenous Avidin Biotin activity but this is completely dependent upon the detection system that one employs. Okay. So this is endogenous peroxidase. This is just a section of spleen. Okay, bottom line here is the dark dots, that's the proliferating cell marker. The little brown dots, that's red blood cells, which should not be staining, and it's simply because they haven't, we haven't--it's endogenous peroxidase that we have to remove prior to testing. And you do this, and again, once I show you the next photograph, then you'll see that it's gone. Again, that's that noise to signal. So it's just part of the procedure, just part of the regular, every immunohistochemistry test.

Endogenous Avidin binding activity is a little bit different. This goes back to that photo that I showed you, the photo of--schematic, I should say, of the Avidin and Biotin procedure. Because Biotin is a vitamin coenzyme, it binds specifically with Avidin or

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Strepavidin. That's just science. So non-specific staining resembles a very diffuse cytoplasmic pattern and you can very easily eliminate this with Avidin and Biotin blocking. We, if we use the Avidin Biotin technology, we always block for it, we do not select by specimen, it's automatically gets done. And I have a photograph I should be able to show you. This is a section of gut and you can see in the mucosa--okay. See at the top of the screen how it's beige, kind of, like they have little round holes and they kind of like beigy, okay, that's non-specific staining, that's absolutely it. But when we block for it, you end up with this clear, again, it's that low noise. Just part of the technology. There are certain tissues that exhibit endogenous biotin at very great amounts and I don't know if it's in this lecture, but if you didn't block for it, you would never know what you were marking because it's laden with it.

Then we moved into the chromogens and there are two types of chromogens that we use in immunohistochemistry, the first being for

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immunofluorescence and the second being for immunoenzymatic. Immunofluorescence are the most common fluorochrome that is used is FITC and that is presently used at St. John's, as it is for Mount Sinai for the kidney biopsies. TEXAS RED is another color and DAPI is just the name of--it's a nuclear marker. So this is just an example of what you would expect to see with a positive kidney biopsy. So that's a glumeruli, all lit up with the fluorochrome. So, you can see that great apple green.

So, when the fluorochrome is left in light, the actual amplification diminishes, as does the staining intensity, so that you'll get a much less bright signal. So when the pathologist reads it, you never--you don't work in light with these. You don't cover slip in light with these and even when the work is being read by the pathologist, it's not--the slides are left in the fridge and they're not repeatedly looked at, because once the light hits it, it excites it and the emissions reduce. This is just an example of a project I did with just--anyway, it just shows the three different chromogens, that

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it's doable.

CHAYTOR, Q.C.:

Q. And was the lighting an issue here in St. John's?

MS. WEGRYNOWSKI:

A. In St. John's, they were not--I did it again. In St. John's, they were not doing their fluorescence in the dark and they weren't using controls and they weren't storing their tissues. So yes, it was an issue.

Immunoenzymatic staining, this is more to do with the regular everyday paraffin stuff. It allows the visualization of a cell component, and if I could just reiterate again, it's so different than doing a special stain because with the special stain, you're going to put on your hematoxylin and all your nuclei are going to stain, and then you're going to put in your eosin and everything else is going to stain. Immunohistochemistry does not work like that. It's that enzyme substrate reaction that allows you to visualize that colored end product. It's a very different theory. So the end precipitates and at best site and the antigen

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is localized and the reaction is insoluble upon oxidization. So in other words, once it's there, it's not going anywhere.

We don't use Nova Red any more, but we can go through that. DAB, which is, I think most people use, gives you that brown end product. I'm sure you've all seen pictures with the brown, and it is extremely insoluble in alcohol and other organic solvents and so that once it's bound to the site, it is not coming out. The red products, well, they can. They can be insoluble in alcohol and other organic solvents, so if you're not careful, you can use them, but that was not an issue here at St. John's.

This is just an example of what the different colors look like. We use HMB45 as a melanoma marker. We use Nova Red with all our melanoma markers, only because of the amount of melon--melanin is a natural pigment and it's brown. So if you stain stuff with brown, it can be more difficult to see. So if you put red in, it's just very easy for the pathologist to see it. It's just quicker and easier. It does not change the sensitivity,

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nor the specificity. Again, might want to--there you go.

Okay, so then, we worked on the kinds of specimens that we work with at Mount Sinai Hospital. We worked at with formalin fix, paraffin embedded tissues and then we walked a little bit through the pre-treatments that we used to expose the epitopes. Frozen specimens, acetone fixed, and again, this would be just like St. John's with working on your kidney biopsies. We occasionally do blood smears, not very often, but we do, and we also work on some cytospins, which are alcohol fixed.

Factors that affect the quality of immunostaining. Okay, all these factors are used not only for validation, but these are the same critical components that you would look at when you were doing your troubleshooting. So you would look at your antibody titre. During the validation process that antibody titre would be used--would you like me to go both ways with it?

CHAYTOR, Q.C.:

Q. Yes, and you can explain to us what exactly

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that means.

MS. WEGRYNOWSKI:

A. Okay. So antibody titre is the amount of antibody compared to diluent that you need to use to give you this end product. So, for example, you may try different titres to get that final end product, whether it be 1 in 50, 1 in 100, it doesn't really matter. At the other end of it, this is one of the components that you need to look at if you have negative staining or if your staining is not correct. The question then arises did you make that dilution correctly. To make that dilution, you need to ensure that your pipettes were calibrated. You needed to ensure that your diluting buffer was pHed and that your lot had lot to lot comparison.

So everything that we go forward with, we go back with. It's a circular--maybe I'm not coming up with the right words, but -

THE COMMISSIONER:

Q. Wait now, let's make sure I understand this. You are saying that in the process--if we go back to getting in a new batch.

MS. WEGRYNOWSKI:

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A. Okay.

THE COMMISSIONER:

Q. When you get your new batch in, you do a series of what I shall loosely call tests, which you then compare with your old batch to make sure that you have that continuity? Is that correct?

MS. WEGRYNOWSKI:

A. Yes, that would be if it was an antibody already in service, that is correct.

THE COMMISSIONER:

Q. Okay. Assuming that, for the moment, then you get your new batch, which I, a knitter, like to think of as dye lots, but that's just my way -

MS. WEGRYNOWSKI:

A. Okay, no, that's good.

THE COMMISSIONER:

Q. You get your new batch and if somewhere along the way, using your new batch, the product that you are getting, you begin to doubt, you will go back through those -

MS. WEGRYNOWSKI:

A. I would.

THE COMMISSIONER:

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Q. - same kinds of things you did to make sure that, in fact, you did it the proper way in the sense of the percentages used were what you had determined should have been used?
MS. WEGRYNOWSKI:
A. Correct. So that's why when we go back and we look at those spec sheets from the antibody when they first come in, that's where we're looking at the protein concentration. Is that protein concentration the same for this lot as it was for the last lot?
THE COMMISSIONER:
Q. Okay.
MS. WEGRYNOWSKI:
A. That is correct.
THE COMMISSIONER:
Q. All right. So it's a matter of determining what you do with this new batch and then having determined that, if something goes wrong, you check every single one of those little steps -
MS. WEGRYNOWSKI:
A. Absolutely.
THE COMMISSIONER:
Q. - along the way to determine what caused it to

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go wrong?
MS. WEGRYNOWSKI:
A. That's correct.
THE COMMISSIONER:
Q. Was the mix correct? Was the -
MS. WEGRYNOWSKI:
A. That's correct. It gets a little bit more -
THE COMMISSIONER:
Q. - sterilization done, etcetera, etcetera.
MS. WEGRYNOWSKI:
A. That's right. It gets a little bit more multi-layered and I can walk you through that as well, if you want, but that's the basic premise.
THE COMMISSIONER:
Q. Okay.
MS. WEGRYNOWSKI:
A. Okay. So then antibody titre and then you have your antibody dilution which you've seen, and I'm not sure why I wrote that twice, but I did. So we start talking about the incubation time, inconsistencies, but once your stainers are set up, regardless of what you're using, that is all going to be logged in in the set up. So that is not as critical an issue.

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Incubation temperature most certainly can be. Most people in the service environment, you are going to be doing it at room temperature. It becomes an issue in the summer time with humidity and whatnot and we end up getting specific background staining, but that we can't do much about.
Pre-treatments. Ah, yes, pre-treatments. When you are doing pre-treatments, regardless if you're making them in house or you're buying them commercially, you must ensure that there is no lot to lot variability. So before any product goes into service, you must ensure that you do a side by side comparative with the previous to ensure it's giving you the same results. If you are making it up, it's the same thing. You want to make sure, if you're making that buffer, that your scales have all had the preventative maintenance on it, so that they are actually weighing the correct way, that your water is correct, so that the whole process is correct, and of course, your pH is correct at the end. Again, we did that with pre-treatments and then buffers, again it's the same thing. So your

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buffers that you're using, just in the laboratory, that they're all pHed.
If I may, there are times when something can go wrong with a test, but everything else worked, and so you have to go back through the process as well there and look at how that was handled. Was the detection system the same? And you start going through the row. Okay, the detection was the same. Then it can't be that. And you just start working through it at a bigger picture to start coming down to what you think the issue could be, because as you can appreciate, there are a number of steps and as you don't visualize anything along the way, it can be rather labour intensive and sometimes frustrating.
THE COMMISSIONER:
Q. Tell me, is there a recording process along the way that goes with this as well, in terms of when you are starting to work with a new batch, for example -
MS. WEGRYNOWSKI:
A. Yes.
THE COMMISSIONER:
Q. - and going through the process of ensuring

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that the results are consistent with your old batch, is there a recording process for recording each of the steps you took or is that sort of something that everybody does so automatically that you don't record these things?

MS. WEGRYNOWSKI:

A. I do record them. So what ends up happening, if I may, depending on what the uses is going to be, if I am going to purchase a product that I'm going to be using every single day and I have a pretty good idea of what the volumes are going to be, then I will contact the supplier and we will sequester lots, so that I have that lot for an extended period of time.

THE COMMISSIONER:

Q. Okay.

MS. WEGRYNOWSKI:

A. Those lot numbers are then recorded in a book. We have the date that we receive them. We have all that information there. We do the same thing, not only for our buffers, but we do the same thing for antibodies. For example, we do--we are one of the two

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laboratories in the province of Ontario that do HER2/neu testing. So I will sequester lots that will last us almost--well, I hope to last us a year, so that we don't have issues with reproducibility.

THE COMMISSIONER:

Q. Okay.

MS. WEGRYNOWSKI:

A. But that is all--we have all that documented, yes.

THE COMMISSIONER:

Q. Okay, thank you.

CHAYTOR, Q.C.:

Q. If you could continue on then, please.

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. Is there anything else on this slide that you -

MS. WEGRYNOWSKI:

A. I don't think so. I think that's it.

CHAYTOR, Q.C.:

Q. Okay.

MS. WEGRYNOWSKI:

A. Oh, yes, okay. So antigen loss upon storage,

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I'm sure everyone in the room is familiar that we have blocks and from the blocks, we create slides, and one of the wonderful things about immunohistochemistry is that you learn something all the time, and this actually came from a researcher, and they had given us some slides and we had run the work for the day, and this was a control slide and I'm telling you, it was a failure, and I couldn't figure it out. I thought I know we did everything right. There were tests that we had run that day in that batch and they had worked. So what had happened to the control? So one of the things I looked at was when the date of that control was cut, and the antigenicity of this particular epitope does not survive storage, long-term storage. So when we re-cut it, there you go, there's your staining. So we are of a habit of always writing on our slides, our control slides, the date we cut them, and even in our control slides, they all have their own, some like to be frozen, some are kept at room temp--well, they're never kept at room temperature, but they're always covered and kept at four degrees. So even

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with that, it's--and you don't find that information on spec sheets.

THE COMMISSIONER:

Q. Okay.

CHAYTOR, Q.C.:

Q. Okay, thank you.

MS. WEGRYNOWSKI:

A. And that's it.

CHAYTOR, Q.C.:

Q. Ms. Wegrynowski, how was your--how was this presentation received by the technologists?

MS. WEGRYNOWSKI:

A. Very well.

CHAYTOR, Q.C.:

Q. And I take it they found it informative?

MS. WEGRYNOWSKI:

A. I believe so.

CHAYTOR, Q.C.:

Q. And they asked questions of you?

MS. WEGRYNOWSKI:

A. Yeah, we walked through it. I mean, there was things obviously they knew and you know, they were just--it was a very collegial environment. It was--I was certainly there only to teach. If they had wanted me to skip

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through slides, I would certainly have done that for them, but this was up to them what they chose to do with it.

CHAYTOR, Q.C.:

Q. And they seemed to learn from what you were doing and what you were telling them?

MS. WEGRYNOWSKI:

A. They seemed to be very accepting of the--I think the schematics helped them the most, if I recall. Again, as I say, because everything is colorless, it's very difficult sometimes to grasp in your mind the schematic and they're very helpful, I think.

CHAYTOR, Q.C.:

Q. And Ms. Wegrynowski, where did you, yourself, learn all this information, such that you're now able to teach it to others?

MS. WEGRYNOWSKI:

A. As again, it started with the base course in 1983, all the different courses along the way, a tremendous amount of reading and most of it is just troubleshooting in the laboratory. It's being in there every single day and seeing what can go wrong and recognizing that it's okay to say it didn't work and go

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backwards. But if I can add to that?

CHAYTOR, Q.C.:

Q. Sure.

MS. WEGRYNOWSKI:

A. I also have the support of the pathologists that when I say it's not working, that's where it stops.

CHAYTOR, Q.C.:

Q. Okay, and if we could look then, please, the third presentation you did, I believe was called quality processes. That's 1761, please, and perhaps you could walk us through this presentation as well.

MS. WEGRYNOWSKI:

A. Okay. Okay, so the quality processes, all right, so we're talking about--we're following a specimen from the physician order to the reported results. So we're going to be talking about the pre-analytical, the analytical and the post-analytical parts of immunohistochemistry. They all vary a little bit, depending on where your start-off point is, but I'll explain that, flow charts, how important they are, and of course, documentation.

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All right, the processes that we're going to discuss in this lecture are the pre-analytical, the analytical, the post-analytical, equipment and reagents, quality assurance. So just to break it down a little bit, so we talk about specimen collection. How is the specimen collected? You need to always be cognizant of specimen transport, both internally and externally. Receipt of the specimen in the lab, that they must be labelled correctly, that all viable patient information is there, and then it gets successioned into the laboratory information system. There should be the responsibility that if any of these things are not correct, there should be a mechanism in place to send the specimen back to the originator.

For example, if there's--just for example, if something came without a patient name and birth date, you would automatically send that back from whence it came, because upon receipt, you could never validate or verify that that's whose specimen it was. So in a hospital setting, all your specimens are going to come--I don't know what they're

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called, bradma plates, you know, those little sticker things that they go on the specimen. So you want to ensure that the name on the requisition would match that of the specimen container.

These are really helpful if you start doing audits, because if you find that a particular area has not been able to do that, maybe they need an in-service to understand what the mechanisms are. It gets hard with nursing sometimes, because they're working 24/7, to be able to track people down. So it's something you'd like to stop at the front door.

CHAYTOR, Q.C.:

Q. And if we could, just the specimen transport, I take it that would include the actual getting the specimen from the OR, in the case of breast surgery, getting the specimen from the OR down to the lab?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And there would be protocols then in place for that to take place?

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MS. WEGRYNOWSKI:
A. There has to be protocols for everything in place to take place.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:
A. So that you know what to expect.
CHAYTOR, Q.C.:
Q. And internal being within your hospital and external being samples that you receive or specimens you receive from outside Mount Sinai?
MS. WEGRYNOWSKI:
A. Anywhere outside, yes.
CHAYTOR, Q.C.:
Q. Okay, and in terms of if we just think internal for a moment, what is appropriate transport to have the sample come down from the OR down to the lab?
MS. WEGRYNOWSKI:
A. Okay. So you're talking about porters being able to pick up specimens out of the refrigerator up in the OR, so in the OR, we would have a sticker in our OR book that would match the sticker on the requisition and on

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the specimen, so that when the porter would pick it up, they would look at the two pieces and sign off in time that they've actually removed them from the refrigerator and then brought them down. Is that what you're looking for?
CHAYTOR, Q.C.:
Q. Yes, that's--yes, and in terms of, I take it, that the protocol would say how often the porter would -
MS. WEGRYNOWSKI:
A. Yes, the times and things.
CHAYTOR, Q.C.:
Q. The times and -
MS. WEGRYNOWSKI:
A. I'm not familiar with that part of it.
CHAYTOR, Q.C.:
Q. - and you would document then--is there documentation then which would indicate when it arrived in the lab as well?
MS. WEGRYNOWSKI:
A. Yes, they do.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:

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A. So then requisitions, what is the expectation of your requisitions, what information needs to be on that requisition for you to accept it from the patient. Okay.
All right, so I deal, in my world, with the analytical stage. Pre-analytical, for me, is everything that kind of shows up at my front door. So for me, I'm looking at my methodology. I want to maintain it that it's current, that it's based on published practice guidelines or in-house procedures validated by the laboratory and that they're approved by my laboratory director or designate, and I actually have a medical director that I report to who will sign off on this. All my procedures are documented. All the procedures are available at the workstation for the end user, and all the documents are reviewed annually.
CHAYTOR, Q.C.:
Q. And who is the medical director that you would report to in Mount Sinai?
MS. WEGRYNOWSKI:
A. My medical director is Dr. Bob Riddell.
CHAYTOR, Q.C.:

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Q. Okay, and is this, I take it, when you say "I" this is for all technologists who work within your group?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And they would stay current on published practice guidelines as well as your in-house procedures?
MS. WEGRYNOWSKI:
A. Absolutely, yes. It's--I don't know whether you want to go into this now, but when you talk about procedures, Bob Riddell will sign off on my procedure manual, but when we're talking about different antibodies and whatnot, his specialty is gut, so he doesn't necessarily--no, let me rephrase that. When it comes to the breast work, so we're talking about a HER2/neu and our ER and PR, when I do my validation slides, they go to the pathologist of interest with those particular antibodies, because those are the pathologists that read those slides. The ER/PR are not read by every pathologist, neither are HER2/neu. The lines get very blurred. A lot

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of bosses at times.

So as I say, all our procedures are documented. Everything must be available at the workstation for the end user. It is not unusual for pathologists to come in and take a look at our procedure manuals, our antibodies data sheets. Everything is--it's transparent and they're all there for everyone to use, and we do use them.

Okay, annual review. For us, it's a little--our documentation is all done online. We have a--I don't know what--the company is called Paradigm, so all our procedure manuals are up online so that everybody can see.

CHAYTOR, Q.C.:

Q. Can we just go back, please, for a moment?

MS. WEGRYNOWSKI:

A. Sure.

CHAYTOR, Q.C.:

Q. The annual review, is that annual review of your methods? Annual review of your technologists? What is that referencing?

MS. WEGRYNOWSKI:

A. It can be both.

CHAYTOR, Q.C.:

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Q. So what gets reviewed annually?

MS. WEGRYNOWSKI:

A. Our procedure manual would get reviewed. There'd be updates on it. You would--I mean, manuals are living, breathing documents. They are never stagnant. So if you were to bring in a new antibody, it would get introduced to that manual long before the annual review would come up. If you are changing detection systems, that would be included in your procedure. So, they're not stagnant. It's not like once a year, okay, you read this and you do it. They're always being worked on.

CHAYTOR, Q.C.:

Q. So, it's reviewed more frequently as required, but at least annually the entire -

MS. WEGRYNOWSKI:

A. Yes, documents -

CHAYTOR, Q.C.:

Q. - manual is reviewed.

MS. WEGRYNOWSKI:

A. Yes, it's signed off then.

CHAYTOR, Q.C.:

Q. Thank you, that's fine, go ahead.

MS. WEGRYNOWSKI:

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A. Okay. So, postanalytical, so most of this--well, the reporting is not done by the technologist. That is done by the pathologist and there's certain parts that they need to do. The turnaround times and stats, that falls more into the technologist side. We want to ensure that things are done in a very timely manner and record retention, I don't throw anything out.

CHAYTOR, Q.C.:

Q. So, turnaround times and what's STAT?

MS. WEGRYNOWSKI:

A. Stats, we get something if somebody comes in and says that we need this now. How quickly -

CHAYTOR, Q.C.:

Q. So, urgent -

MS. WEGRYNOWSKI:

A. Yes, how quickly we can move it through the process and then record retentions. All our records are maintained, so that we can certainly go back to them.

CHAYTOR, Q.C.:

Q. And are they maintained electronically or hard copy?

MS. WEGRYNOWSKI:

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A. Prior to Paradigm for our documents, for our procedure manuals it was hard copy, but I have, like, the hard copies on old antibodies that go back a decade or whatever. They're all maintained.

CHAYTOR, Q.C.:

Q. Okay. Anything else?

MS. WEGRYNOWSKI:

A. I don't think so. Okay, equipment and reagents. Okay, so, for example, if I could speak to what we just done at Mount Sinai. We have two autostainers and we've purchased more. So, we're looking at some new equipment. You can't just go and plug in the equipment and say, we're ready to go. You must ensure that the equipment is working and it is reproducible and consistent and the results are comparable to what you're presently using. So, I think the next slide may explain that. Okay.

So, when we're looking at new equipment, we will do a market evaluation and that's what we did when we purchased these new stainers. There are certain criteria that will fit to your institution, whether you want an open

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system, closed system or whatever it is that your institution is looking for. You want to look at who's got what out there. But more importantly you must ensure that the manufacturers performance claims must be validated prior to use. People can tell you a whole lot of stuff, it doesn't necessarily work like that or may not work for you in your settings. So, you have to ensure that.

THE COMMISSIONER:

Q. Tell me more about validating, that kind of thing.

MS. WEGRYNOWSKI:

A. Would you like me to walk you through that?

THE COMMISSIONER:

Q. Yes, I'm curious actually.

MS. WEGRYNOWSKI:

A. Okay.

THE COMMISSIONER:

Q. I'm not sure in the end it means anything, but -

MS. WEGRYNOWSKI:

A. Okay, all right. So, we just bought on a couple of months ago, have all the slides and the documentation for this. So, one of the

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first things we did was we took, this is for ease of expense as well, we took a marker that was relatively inexpensive on a tissue that I could get lots of. So, I used CD31, it's an endothelium marker. So, what we did is that we set up two runs. Our machines hold 48 slides, so we had 48 slides set up for our regular machine and 48 slides set up for the next machine. Everything was handled the same way. The tissues were cut the same time, everything was the same. They were taken down and pretreated until they got to the stainers, the dilution. So, what we did, when we knew how much of the reagents we required, we made up double of everything so that we could ensure that the dilutions were exactly the same. So, we just loaded up the machines and let them run. So, what you want to see at the end was that the staining that was exhibited from the machine that you're presently using is showing up in the second machine. When it passed that and it did, then I knew that I was going to be using this particular piece of equipment for my estrogen and progesterone and my HER2/neu testing.

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So then what I needed to do is I went through that process again, but using those specific antibodies because we wanted to make that I'm getting the stain--well, the real reason was that I was using antibodies that stained the three different cell sites. So, I was staining the cell surface, the cell cytoplasm and the nucleus. So then once that was done and we were satisfied that that was done, we brought that product--then we started using that machine to process.

THE COMMISSIONER:

Q. Okay, thank you.

MS. WEGRYNOWSKI:

A. And then we have all our records, of course, that we keep.

CHAYTOR, Q.C.:

Q. Okay. If you could continue on then please.

MS. WEGRYNOWSKI:

A. Okay. So, existing equipment, you must always maintain your service records. It doesn't matter what you product is, your autostainers, your microscopes. There should always be a routine PM done. Service problems and documentation. If for whatever reason--I

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could give you an example that we've had on our autostainer. It'll tell us that there's not enough antibody or whatever in there and it's partially picking up. But you will visually see that there's enough product in there. You have to have the wherewithal to stop the process and call in the manufacturer and say, we've got a problem; this is what the problem is; describe the problem to them and expect that a technical person will show up to make sure that that machinery is working properly. You have the documentation that you've made the call which corrective action is followed by the documentation given to you by the technical person from the company.

CHAYTOR, Q.C.:

Q. Ms. Wegrynowski, when you were in St. John's was there any discussion or did you make any inquiries of the people that you met with as to what they did in bringing in the new Ventana machines?

MS. WEGRYNOWSKI:

A. I don't -

CHAYTOR, Q.C.:

Q. Did you ask whether or not there had been any

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comparisons done?
MS. WEGRYNOWSKI:
A. No, and I didn't find any document, like there was not binders there for me review. So, -
CHAYTOR, Q.C.:
Q. So, there was no documentation -
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. - which would have give you the answers.
MS. WEGRYNOWSKI:
A. No, and to be fair, I was only there for three days. So, if you can understand, the first day, I was meeting people. The second day I was doing my lectures and trying to look through the laboratory and try to glean as much information as I could. And on the third day, it was a shortened day, I needed to get to the airport and I was also meeting with the VP. So, you know, I did the best I could with what I had.
CHAYTOR, Q.C.:
Q. Yes, absolutely. Now, in terms then of the existing equipment and service records, maintenance records, were you able to observe

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any documentation regarding that?
MS. WEGRYNOWSKI:
A. No. They told me that they were cleaning the machines and so I suggested then maybe they'd like to start printing that up on a daily basis.
CHAYTOR, Q.C.:
Q. So, was it that you just didn't have time to review it or it didn't exist?
MS. WEGRYNOWSKI:
A. I'd say both.
CHAYTOR, Q.C.:
Q. Do you know whether or not they had any records, service records and maintenance records?
MS. WEGRYNOWSKI:
A. I didn't see them.
CHAYTOR, Q.C.:
Q. Okay. And did you ask to see it?
MS. WEGRYNOWSKI:
A. I don't recall.
CHAYTOR, Q.C.:
Q. You can continue on please.
MS. WEGRYNOWSKI:
A. Okay. So, the service records though, I mean,

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we're talking for absolutely every single piece of equipment. I mean, we know there wasn't any for the pipettes. So, I mean, we could -
CHAYTOR, Q.C.:
Q. I'm sorry, you know that -
MS. WEGRYNOWSKI:
A. I know there wasn't any for the pipettes because I asked if they had ever been calibrated. So, if we want to answer the service one for the pipettes, we could say definitely no to that one.
When we're talking about maintenance records, we talking about replacement of scheduled parts, pH standardization and that was definitely not being done.
CHAYTOR, Q.C.:
Q. So, the pH standardization was not being done?
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. Okay. So, there wouldn't be, I take it, any record of it if it's not being done.
MS. WEGRYNOWSKI:
A. That is correct.

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CHAYTOR, Q.C.:
Q. Okay. And the pipettes again, you said had never been calibrated?
MS. WEGRYNOWSKI:
A. Not to my knowledge.
CHAYTOR, Q.C.:
Q. And you asked that specifically?
MS. WEGRYNOWSKI:
A. I certainly did.
CHAYTOR, Q.C.:
Q. And so we won't expect to see any documentation if it's not being done.
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:
A. And again, your scales as well.
CHAYTOR, Q.C.:
Q. And what's the importance of the pH standardization? What's the importance of that?
MS. WEGRYNOWSKI:
A. Okay. They were using--Eastern Health uses the Ventana system, it's a closed system. So,

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they purchase all their products from Ventana. And even on the literature that comes with the product, they will tell you themselves to pH it. Regardless if that information is there or not, you should know that you have to assure that the pH is as written by the manufacturer. So that you can ensure that you're getting a final end product. And one of the issues that I bring this up for was because of the demographics of this Island, I don't imagine you have the same store houses that I would have in Toronto. So, certain pieces or certain reagents are not going to do well if left to the elements, so you have to ensure that there's no freezing and you have different climate than I do, so you want to ensure that that is all in place and that that pH is as expecting.

CHAYTOR, Q.C.:

Q. And what can be the effect if that's not done to -

MS. WEGRYNOWSKI:

A. Your staining would be completely off. You could have incorrect staining.

CHAYTOR, Q.C.:

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Q. And could you have no staining?

MS. WEGRYNOWSKI:

A. I couldn't speak directly to that, but your staining procedure would be off because you're not following your standard operating procedure. Because you're not following your standard operating procedure, then the consistency between the work that you were doing yesterday would not be the same as that of today.

CHAYTOR, Q.C.:

Q. And it take it that could run the full gambit?

MS. WEGRYNOWSKI:

A. Absolutely. And that was as well again is with your formalin because you find, formalin is bought in great big carboys and it can start dissipating up to formic acid.

CHAYTOR, Q.C.:

Q. Sorry, if you'd like to continue on?

MS. WEGRYNOWSKI:

A. Yes, okay. Temperature dependant, you must daily check the temperature on day of use of any product that you're using. It is not acceptable to use the digital read outs on equipment, they have been known--well they can

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fail and -

CHAYTOR, Q.C.:

Q. Is that what was happening here in St. John's?

MS. WEGRYNOWSKI:

A. Yes, yes. And that one needs to use internal thermometers that must be NIST certified and I spoke to that, that is a standardized thermometer. Even those standardized thermometers have due dates on them, so they're only good for so long as well. Monitoring devices with alarms, we have, at Mount Sinai, I can only speak to Mount Sinai, all my fridges and freezers are on a resystem so that if there's any change in the temperature that we can get in there and either we have to relocate them or make sure that the manufacturer is aware so that we can get those serviced, but as I stated, yours was on an alarm here.

CHAYTOR, Q.C.:

Q. Yes, the fridge that you observed here was on an alarm -

MS. WEGRYNOWSKI:

A. Absolutely, because you must maintain those temperatures, they must be stringent.

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CHAYTOR, Q.C.:

Q. Sorry, go ahead.

MS. WEGRYNOWSKI:

A. Okay, you want to use up-to-date instructions. Again, they must be on the workbench and they must be approved, so standard operating procedures, instructions, whatever term you would like to use.

CHAYTOR, Q.C.:

Q. So that's the same thing, instructions are your standard operating procedures?

MS. WEGRYNOWSKI:

A. Yeah, I mean, even if you have equipment, I mean, your equipment comes with instructions, you must be using it the correct way as well, correct, so you would have all your manuals for that as well.

CHAYTOR, Q.C.:

Q. Thank you, continue on.

MS. WEGRYNOWSKI:

A. Okay, so I think I've spoken a little bit about this, so when we're talking about equipment and reagents, you want to ensure that your storage requirements are maintained. Manufacturers will tell you on their data

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sheets what the storage requirements are, so it's not unusual for them to say 48 degrees celsius and then the list would just go into your refrigerator or they'll say freeze aliquots or whatever, and then we have like the freezers for that. They'll tell you whether they have to be at room temperature. It's all on your data sheets what you need to do with it and it's your job to ensure that that occurs. Inspection and I think we've highlighted this a little bit. We have the ability to accept and reject different kits that come in if they don't perform as expected and it does occur. You can bring something in and for whatever reason, that lot does not work, we've had it happen with our slides, we've had slides come in and we used a particular kind of slide so that the section doesn't come off the slide and they have not done what they're supposed to do and so it is my job to stop the process and say, we call the manufacturer, we look for all the lot, we pull the lot, it doesn't go forward and they need to come and explain to us what has occurred and we do do this and it is

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documented.

CHAYTOR, Q.C.:

Q. And that's part of your job of troubleshooting, as you say?

MS. WEGRYNOWSKI:

A. That's part of my job, yes, it is. We must always verify performance prior to use. Again, whether it's a pH or a result comparison, you have to ensure that reproducibility is there on a day-to-day basis.

CHAYTOR, Q.C.:

Q. And in this situation what was happening in St. John's if they were running, they ran out of a reagent and a new batch was brought in, what was their procedure?

MS. WEGRYNOWSKI:

A. My understanding was they took another (phonetic) fridge and used it.

CHAYTOR, Q.C.:

Q. And the prior slide where you were talking about the importance of the daily check on the temperatures, what's the possible effect if your temperature is off?

MS. WEGRYNOWSKI:

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A. Heat laiblity of the protein so that the protein would break down so that you would not be able to find it, so, it would be gone.

CHAYTOR, Q.C.:

Q. And it wouldn't stain.

MS. WEGRYNOWSKI:

A. It wouldn't stain. But again, as I showed you in that previous lecture, just that slide storage at room temperature could do the same thing as well.

CHAYTOR, Q.C.:

Q. Could do the same -

MS. WEGRYNOWSKI:

A. Absolutely.

CHAYTOR, Q.C.:

Q. Sorry, I don't know if there's anything else on this slide that you wanted to point out to us.

MS. WEGRYNOWSKI:

A. I don't think so. Okay, so then we start talking about our reagents and so this is where a lot of the binders come from, all the documentation that goes through bringing the product into the marketplace or into the laboratory using it. Inventory control is a

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very huge component of immunohistochemistry. Every single thing that comes into the lab is logged in, so all the lots are maintained. Even with our antibody data sheets, so for a particular antibody, even with that, I will have a historical data sheet that sits on top if it, so I can tell you how quickly I went through it, what the expiry dates were, if there was a change in how that produce was used. It's just a--I use it like historical data just in case there's changes in the--in how it's used or how it's performing, so we keep all of that. We have, as I say, we have our lot numbers, our expiry dates, our date in service and all the conditions that go with it. We have a workplace--we don't--I don't keep that information in the immunohistochemistry laboratory, we keep that information in the pathology laboratory which is right across the hall from me, and in there all our manufacturer's safety data sheets are kept. We use very few chemicals in the immunohistochemistry laboratory, so -

CHAYTOR, Q.C.:

Q. And that's for Workplace Health Management

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Informational Systems.
MS. WEGRYNOWSKI:
A. Yeah.
CHAYTOR, Q.C.:
Q. The record keeping, the idea of keeping track of the lot number expiry dates, keeping track of reagents that in fact had expired, was that happening here in St. John's?
MS. WEGRYNOWSKI:
A. On my first visit? No.
CHAYTOR, Q.C.:
Q. And how were the antibodies stored?
MS. WEGRYNOWSKI:
A. Well the data sheets were kept in a box in the cupboard and the antibodies were simply put in the fridge.
CHAYTOR, Q.C.:
Q. And what would you have expected to see?
MS. WEGRYNOWSKI:
A. At the very least I would have expected the antibody data sheets to be at the very least in alphabetical order. I would have expected them, to each of them have their own instructions for use. What was the lot? What was the expiry date? What was the

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dilution? What was the pre-treatment? Everything, I would have expected to see there, so that it would have been a very easy reference for all the technologists to use. I thought that was critical, especially because they were rotating, and one of the issues then is when you have people that are not in a place every single day, communication, in my opinion, then becomes even more important, as does transparency.
CHAYTOR, Q.C.:
Q. And the antibodies being stored in the fridge, as you say, what would you have expected to see there?
MS. WEGRYNOWSKI:
A. Nice orderly fashion, alphabetical.
CHAYTOR, Q.C.:
Q. So I take it there were quite a number of these antibodies in the fridge?
MS. WEGRYNOWSKI:
A. They were all stored correctly, yes.
CHAYTOR, Q.C.:
Q. Yes, they were stored in the fridge where they should be?
MS. WEGRYNOWSKI:

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A. Yes, they were.
CHAYTOR, Q.C.:
Q. But it was just that in terms of being able to access them -
MS. WEGRYNOWSKI:
A. Ease of use.
CHAYTOR, Q.C.:
Q. Okay, so that was the issue, and were there any issues though in terms of, I guess, what's the consequence or possible consequence if an antibody is expired?
MS. WEGRYNOWSKI:
A. You have to guard against expiration dates in ensuring that the efficacy of the antibody is still in use. Now they were using the Ventana system. The Ventana system, to the best of my knowledge, will not allow you to use a product post expiry. So it's a done deal with them, the bar coding. The issue, however, becomes if you are loading your own cylinders--I'm not familiar with the term that they use--with a concentrated antibody that you yourself dilute, because you can--I'm not sure, I don't know enough about that equipment. I don't want to say something out of turn, but if I

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could, in layman's terms, say override the system and apply that antibody onto it, if that antibody was expired and the efficacy had not been ensured, then you would not know that.
CHAYTOR, Q.C.:
Q. Now we understand that from 1997 to April 2004, they were using the DAKO system.
MS. WEGRYNOWSKI:
A. Okay.
CHAYTOR, Q.C.:
Q. And does the DAKO system also not allow you to use an expired antibody?
MS. WEGRYNOWSKI:
A. The DAKO system is a completely open system so that it's the technologist who creates absolutely everything on the system. So you would employ your own detection system, your own primary antibodies, whatever dilutions that you saw fit. Yes, you could go ahead and use an expired antibody.
CHAYTOR, Q.C.:
Q. Okay, and the system wouldn't alarm you to that?
MS. WEGRYNOWSKI:

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A. It's not--no.
CHAYTOR, Q.C.:
Q. Or alert you to that?
MS. WEGRYNOWSKI:
A. No, that's not it's -
CHAYTOR, Q.C.:
Q. Okay. Sorry, if you could continue on then with this slide. Water, I think you're up to the bullet about water.
MS. WEGRYNOWSKI:
A. Okay, and I believe I have spoken about water earlier this morning, that you--it doesn't--I can't give you the numbers off the top of my head, but there are certain different kinds of water or the criteria that is used to select the appropriate water is very, very important.
Okay, so then we spoke a little bit about quality assurance, and I've broken it down into three areas. We have internal quality control, interlaboratory comparatives, and external quality assessment. Okay. So under internal quality control, I have five bullets. You must ensure that there's a policy and protocol established.
CHAYTOR, Q.C.:

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Q. And I take it based on what we've already discussed today, that wasn't--that hadn't happened?
MS. WEGRYNOWSKI:
A. No, it had not.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:
A. You must assess with an each user defined run.
CHAYTOR, Q.C.:
Q. What does that mean?
MS. WEGRYNOWSKI:
A. Well, it's going to mean something different for Eastern Health than what it does for me at Mount Sinai, and I've spoken of this as well. Because we run a control with a batch, we want to ensure that everything run in that batch, so let's say we have five of antibody A and four of antibody B, we would only have one control for antibody A and antibody B, although every single patient would have their own negative control. So we want to ensure that within that run, everything is working as compliant. In the Ventana system, it would be the same way, and you would be checking that

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within that run, your controls were always working, whether they be positive or negative.
CHAYTOR, Q.C.:
Q. So if you run two different runs, you're going to ensure that your results are reproducible?
MS. WEGRYNOWSKI:
A. Absolutely.
CHAYTOR, Q.C.:
Q. By doing it the same way each and every time?
MS. WEGRYNOWSKI:
A. Each and every time, absolutely. The stringencies that are required to do reproducible immunohistochemistry are paramount. Okay. Controls must be treated in the same manner as the patient sample. There was--in the notes that Barry had--no, when Barry had spoken to me the first day, I believe I made an exert in my note about the tissue express, the Sakura express.
CHAYTOR, Q.C.:
Q. Yes.
MS. WEGRYNOWSKI:
A. That particular piece of equipment does not--the fundamentals of that equipment are not the same as what the historical processing are.

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It's based on alcohol fixatives.
CHAYTOR, Q.C.:
Q. So what you're saying is that the control tissues that they would have had in their system would have been processed a different way?
MS. WEGRYNOWSKI:
A. Yes. If you are going to use a completely different system, then you must ensure that the controls that you are using are the same as what your tests are. You must compare apples to apples and oranges to oranges, right.
CHAYTOR, Q.C.:
Q. And so the Sakura Express, how is that different?
MS. WEGRYNOWSKI:
A. It's based on alcohol based fixatives, as opposed to formalin.
CHAYTOR, Q.C.:
Q. Okay, and so -
MS. WEGRYNOWSKI:
A. I don't know whether they were using it or not. It was something that was stated to me, so I put it in my report and it was just to

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guard against that. It's not that they were doing it.

CHAYTOR, Q.C.:

Q. So if they were to in the future use this Sakura Express, they would have to build up a control bank or a bank of controls based on the alcohol-based system.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. The Sakura Express, as opposed to what they had used, the formalin based controls which they would have in their bank?

MS. WEGRYNOWSKI:

A. Yes, so that you can ensure that the testing that you're doing is accurate for the way the tissue was handled.

CHAYTOR, Q.C.:

Q. So then your patient tissue, if it's tested on the Sakura Express, is treated in the same manner as what the control slide is?

MS. WEGRYNOWSKI:

A. That is correct.

CHAYTOR, Q.C.:

Q. Yes, okay.

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MS. WEGRYNOWSKI:

A. And again, this is exactly what this speaks to. New reagent lots are checked prior to use against prior reagent lots for reference material, and it's the same thing, just reiterated yet again that to get that consistency, you must ensure that every single piece of the puzzle is done in the exact same way and that each piece is behaving or reproducible.

CHAYTOR, Q.C.:

Q. Okay, thank you.

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. And then interlaboratory comparisons.

MS. WEGRYNOWSKI:

A. I think I went too far again. I'm sorry, Sandy.

CHAYTOR, Q.C.:

Q. That's all right.

MS. WEGRYNOWSKI:

A. Okay. It's optimal that an evaluation of performance and/or laboratory competence in the testing of defined samples by two or more

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laboratories. Interlaboratory comparison can also be done within your own site with case and control review and histological and cytological correlation, and this is much more focused at what the pathologist would do than what a technologist would do. But it's a way for the pathologist to ensure that the technical accuracy of their work is right.

CHAYTOR, Q.C.:

Q. And can you give us an example of how does Mount Sinai do interlaboratory comparison?

MS. WEGRYNOWSKI:

A. Okay. Well, we belong to QA testing with OLA, so our work is--we send our work to them and then our work is compared to those of our peers.

CHAYTOR, Q.C.:

Q. Okay, so external quality assessment then can be something different than -

MS. WEGRYNOWSKI:

A. Than internal. If you have--okay, so if you have more than one laboratory in your area or whatever, you could certainly, if you don't want to go into a big external, the two laboratories can even just ensure that both

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sides are producing--okay. If you are producing blocks at St. Clare's and you are producing blocks at Eastern Health.

CHAYTOR, Q.C.:

Q. At the Health Sciences.

MS. WEGRYNOWSKI:

A. Yes, yes, I'm sorry, at Health Sciences, and I'm just talking something very basic as HNE section. You want to ensure that the quality of the staining is the same regardless of where the blocks are taken from. So you can take it out of the--take it out of IHC and just take it down to the very rudimentary values. It doesn't matter where the work is done. This is the same organization. You want to ensure that both sites are providing you with the same quality work.

CHAYTOR, Q.C.:

Q. Okay.

MS. WEGRYNOWSKI:

A. Does that make sense?

CHAYTOR, Q.C.:

Q. Yes, and was that the -

THE COMMISSIONER:

Q. Really no different than treating the other

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site as a separate institution?
MS. WEGRYNOWSKI:
A. Absolutely, and that's example of two or more laboratories because you're both doing the same work and you want to ensure the consistency and reproducibility both sites.
CHAYTOR, Q.C.:
Q. And with standard operating procedures, you would ensure that that was taking place? If they were implemented and being followed.
MS. WEGRYNOWSKI:
A. Absolutely. Do you see how they all start-- it's very interwoven. Okay, so then again, you have your case control review and this is, you know, the pathologists. I have no idea. I didn't ask for any documentation on this, but an example of this would be you have your frozen sections, how do they compare with what your paraffin sections look like? You know, if you're calling it malignant at frozen, what are we seeing post, and what are we seeing-- what's our correlation between our histology and our fine needle aspirates of cytology? Those are just examples.
CHAYTOR, Q.C.:

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Q. And in terms of your internal quality control within Mount Sinai, I take it you keep records of that?
MS. WEGRYNOWSKI:
A. Yes, and our records are kept for years.
CHAYTOR, Q.C.:
Q. And who--I realize within the laboratory that you are the senior person there, but who ensures that you're doing your job, from a quality assurance point of view?
MS. WEGRYNOWSKI:
A. That's where we belong to external quality assurance programs.
CHAYTOR, Q.C.:
Q. That's the external. Is there anyone else within Mount Sinai, in terms of the quality department, is there anyone else who ensures that "well, Ms. Wegrynowski has her SOPs. The SOPs are in place."
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. That they are doing the maintenance checks. How does that happen at Mount Sinai?
MS. WEGRYNOWSKI:

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A. Well, we have a quality manager by the name of Gaman Modi. We belong to CAP. We belong to OLA. They send us their checklists. So we must ensure that what we do is that we have everything that they're stating on the checklist. So we do this again in this document control and we will put it in there and then hyperlink it to our manual, so we can answer our questions that way. But prior to having that, it would be the same way. We would still get your checklist, no different than anything I came to Eastern Health with, and just start looking through it. It's a really great just basic building block, what is here, and what you need to do your work.
CHAYTOR, Q.C.:
Q. Yes, okay, and so I see how when you send your product out and it comes back and you do your comparison to make sure that it's satisfactory and I'm just thinking, what checks and balances are in the system though to make sure that when it came back and it wasn't okay, that then the follow up takes place?
MS. WEGRYNOWSKI:
A. Can you reiterate the last comment?

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CHAYTOR, Q.C.:
Q. Yes, I'm just thinking, within Mount Sinai itself, what roles does the quality department play to ensure that, yes, the external quality assurance is taking place. We have the programs we're registered with and that that is taking place, that the product coming back is fine and I understand that you personally take responsibility for that, but who makes sure that it's done?
MS. WEGRYNOWSKI:
A. Okay.
CHAYTOR, Q.C.:
Q. What if there wasn't a Trish? Who makes sure that it happens?
MS. WEGRYNOWSKI:
A. Okay. What ends up happening is the documentation goes to Gaman Modi. He signs off on it and then sends it to me.
CHAYTOR, Q.C.:
Q. Okay. So it goes to the quality -
MS. WEGRYNOWSKI:
A. So there's somebody above me.
CHAYTOR, Q.C.:
Q. - someone above you.

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MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. It goes to your quality department. They have to sign off?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. All right, thank you.
MS. WEGRYNOWSKI:
A. You're welcome.
CHAYTOR, Q.C.:
Q. Okay. I'm sorry, continue on then. I think we're on to the next slide then.
MS. WEGRYNOWSKI:
A. Okay. So this is when we discuss the external quality assessment. So we're talking about the proficiency testing and we spoke about the interlaboratory testing. So again, if there's no formal EQA program, they need some mechanism to compare results with other sites and I mean, I just went through the very loose one of just doing the HNEs, if you have two sites and the same thing. It's still the same thing. It's between laboratories. A peer

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review assessment, you can certainly sent it to your peers and it doesn't have to be the most rigid of it. You know, this is what we see. This is what you see, and you want to ensure that. But what you want to do is that you want to ensure that the quality of the lab's results, to determine accuracy and reliability of the procedure. That's the whole goal of this.
CHAYTOR, Q.C.:
Q. And at the time you were here in September 2005, your first visit, Eastern Health was not enrolled in any external quality program?
MS. WEGRYNOWSKI:
A. No, not at that point in time, no.
CHAYTOR, Q.C.:
Q. And do you know whether or not there was anything happened then through interlaboratory to compare the results?
MS. WEGRYNOWSKI:
A. Not that I can recall.
CHAYTOR, Q.C.:
Q. Okay. Is there anything else on this particular issue?
MS. WEGRYNOWSKI:

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A. I don't think so.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:
A. Okay, so then I walked them through the validation process. Okay, so this is the definition of validation. Validation refers to establishing documented evidence that a process or system, when operated within established parameters, can perform effectively and reproducibly to produce pre-determined specifications and quality attributes. So if you keep your parameters the same every single time and you perform effectively, you are going to get that pre-determined end result, and that's what you're looking for.
CHAYTOR, Q.C.:
Q. And why is that important?
MS. WEGRYNOWSKI:
A. Consistency and reproducibility.
CHAYTOR, Q.C.:
Q. And in terms of the issue that you mentioned earlier, I believe you said about manufacturers can change the protein

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concentration?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. What do you do then? If you've noted that and the protein concentration has been changed, what do you do to guard against that?
MS. WEGRYNOWSKI:
A. Okay. So if I was to receive a new lot of antibody and the protein concentration was, just for simplicity, half as much as what I originally had, I would never start the dilution as where I was before. I would cut that in half as well, probably go down below a half and then a little bit above. So it's like a sliding scale. You need to have that information so that you can produce that best end product.
CHAYTOR, Q.C.:
Q. Okay, and you still dilute your own antibodies?
MS. WEGRYNOWSKI:
A. Yes, we do.
CHAYTOR, Q.C.:
Q. And I believe that procedure changed in St.

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John's. That's not--that they use the manufacturers antibodies.

MS. WEGRYNOWSKI:

A. When I was here, they were still using some concentrated antibodies from DAKO. I don't know what they're using now, but they certainly had switched a fair amount over to the Ventana, which were all pre-dilutes.

CHAYTOR, Q.C.:

Q. And do you know what they were using for ER/PR?

MS. WEGRYNOWSKI:

A. You know, I don't want to hazard a guess.

CHAYTOR, Q.C.:

Q. That's fine.

MS. WEGRYNOWSKI:

A. I thought they were the DAKOs, but I don't want to hazard a guess.

CHAYTOR, Q.C.:

Q. And even if it is though a pre-diluted antibody, do you still have to do these checks and balances?

MS. WEGRYNOWSKI:

A. Absolutely.

CHAYTOR, Q.C.:

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Q. Okay.

MS. WEGRYNOWSKI:

A. Absolutely. If an antibody is only supposed to stain the nucleus and you're getting test results back that more than nucleus, so you're getting the cytoplasm and the nucleus is coming up, then I suggest perhaps you may want to go back and look at that protocol, because if the antibody only resides in the nucleus, then how come you're getting this cytoplasmic staining? So the validation process needs to be looked at, whether or not it's incubations that are correct, pre-treatments that are too strong. There's something--that result is telling you something and you need to guard against it.

CHAYTOR, Q.C.:

Q. Okay. Thank you.

MS. WEGRYNOWSKI:

A. You're welcome.

CHAYTOR, Q.C.:

Q. And if you can continue on then.

MS. WEGRYNOWSKI:

A. Okay. So what are we going to validate? Well, antibodies, detection systems,

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chromogens, pre-treatments, buffers ancillary products and I'm sure, as you can tell, the lectures how they all start melding together and it's to drive home the same point of the importance of the same things and how they all inter-react and interrelate together. So I've gone through this, I think.

CHAYTOR, Q.C.:

Q. Maybe just the buffers. We haven't spent much time on that. Maybe you could explain what that is and how you validate your buffers?

MS. WEGRYNOWSKI:

A. Okay. Well, there's a number of buffers that we use at Mount Sinai. I can tell you that we use two particular buffers for pre-treatments. We use a citrate buffer pH6 and we use a Tris pH9, that you need to know that part. But what we need to guard against is that when we make up these buffers that they're made up in the exact same manner every single time. So what we'll do is we'll take them and then we'll pH them and send them through to process. The same thing comes true with our washing buffers as well. Everything has to be pHed.

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At Eastern Health, they're receiving in buffers and they too need to be pHed. Whether it's for washing, diluting, it doesn't matter. They have to live up to what the manufacturers.

CHAYTOR, Q.C.:

Q. And was that happening?

MS. WEGRYNOWSKI:

A. Not to my knowledge. There was no pH meter, so no.

CHAYTOR, Q.C.:

Q. Sorry?

MS. WEGRYNOWSKI:

A. I did not find the pH meter in the immunohistochemistry laboratory, so I'm going to say no, and I found no documentation.

CHAYTOR, Q.C.:

Q. Okay, and what are you referring to by ancillary products?

MS. WEGRYNOWSKI:

A. Oh, all your extras, your haematoxylin, your cover slips, your glass slides, anything else that you would use in the course of staining that does not fit into these particular categories.

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CHAYTOR, Q.C.:
Q. So it all has to be validated?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Okay. Next slide then, please.
MS. WEGRYNOWSKI:
A. Okay. So this was a little bit--and when we were talking about, Ms. Chaytor, when you asked me about the antibodies and you were asking me about the spec sheets and you asked what had I expected.
CHAYTOR, Q.C.:
Q. Yes.
MS. WEGRYNOWSKI:
A. This is probably the list that I would have expected, and it mirrors very much what we do at Mount Sinai. You want to know the clone, because there are so many different clones out on the market for different antibodies and the clone that is selected is always selected by the pathologist. The storage conditions and temperature, and I know we've spoken to that. The supplier manufacturer information, we've spoken to that, the lot number, the expiry

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date. The iso-type and concentration, we've spoken to that. The antibody dilution, what I would put on my sheet was I would put in all my testing criteria and then what we came up with at the end. Incubation temperature, pre-treatments, detection system. The control tissue that is used and then the signatures, and signatures just mean who did the work, who signed off on it, and where do we go from here.
CHAYTOR, Q.C.:
Q. And you would expect all that to be documented?
MS. WEGRYNOWSKI:
A. Well, I would expect at least some of this information. I mean, this is just something that I came up with at Mount Sinai. This is certainly not an all-inclusive list. I mean, you can even add things to it where you would expect the staining to be seen. There is no standardized form that needs to be filled.
CHAYTOR, Q.C.:
Q. But at least this?
MS. WEGRYNOWSKI:
A. Yes, some of this, at least.

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CHAYTOR, Q.C.:
Q. I'm sorry, then go ahead.
MS. WEGRYNOWSKI:
A. All right, can I got to the next screen?
CHAYTOR, Q.C.:
Q. Absolutely.
MS. WEGRYNOWSKI:
A. So this is just an example. It's taken from my standard operating procedure that I brought to Eastern Health. I didn't know--I mean, I didn't know what they did have or did not in place, but I always find that no matter where you go, you can always learn something. So I thought it would be an opportunity I could see what you had and we could come up with a better end product. So this is just from my SOPs, which I left here at Eastern Health, and so it's just everything that was on those previous pages, just put up -
CHAYTOR, Q.C.:
Q. So you gave them your SOPs?
MS. WEGRYNOWSKI:
A. I gave them this particular template from my SOPs, I did.
CHAYTOR, Q.C.:

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Q. Okay, and the purpose being so they could develop their own?
MS. WEGRYNOWSKI:
A. Absolutely. I mean, if you had one, then maybe there would be something here of value that you would wish to add to yours, or in this case, they didn't have one, so perhaps you would like to use this as a starting point to create your own.

Again, this is part of my antibody record, when I spoke to you about some--just historical data. So in my book, I have--they're all sitting on top of each other, so I know the dates when products received, who manufactures it. Just--you probably may not know, but in my world, manufacturers stop carrying things because they don't sell. So then you have to go looking around, if that antibody is still required, you need to go find different manufacturers and then what ends up happening is you have to start the validation process again. But it's that information that would be kept in here in the antibody record that you can see where it is, when that antibody would change from one

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manufacturer to the other.
The flip side of that is that the actual documentation from that previous manufacturer then goes into another binder which is called the old expired antibody. It's not a very fancy name, but it all stays there, and then there's always a reason given why we've done that. So it's a really great concise history.
We have our expiry dates, iso-types, concentrations, dating service, dilution, pre-treatment systems and MLTs and that's our signature, of course, that goes on the far right-hand corner.

CHAYTOR, Q.C.:
Q. Okay, thank you.

MS. WEGRYNOWSKI:
A. You're welcome. And again, this was another template from my SOP that I left here at Eastern Health.

CHAYTOR, Q.C.:
Q. Okay.

MS. WEGRYNOWSKI:
A. So now we're moving into detection systems. You must always inspect your products when they come in. You want to make sure that if

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they're supposed to come in on ice, they come in on ice. I mean, HER2/neu, those detection systems are thousands and thousands of dollars and they have to be received on dry ice. If they are not, we are not going to accept them and we have no problems calling manufacturers and telling them so. Again, there's documentation, lot number, expiry dates. I think I've said it enough. Then again, the acceptance, once you test the validation, the ability of someone in that laboratory to be able to say "this isn't working, this old batch, new batch comparison," and just stop it before it goes into the actual service. It's much easier to stop it than once it gets in. It's insidious.
Okay, chromogens, again, it's the same thing for everything. Whether or not you purchase it or you make it up, you have to follow these same guidelines. I don't think there's any need for me to reiterate that. Pre-treatments, it's the exact same thing again, regardless of whether you're making it or you're purchasing it. These are standards that one must do.

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Again, when you prepare things in-house, it's a little bit different because you don't have all the lot numbers and whatnot. I'll give you an example. We prepare our own Mayer's hematoxylin in-house. We've tried different commercial ones, but we like ours the best, so we make it. And so when we make it up, we have to ensure that it is working correctly, the same thing with our pepsin. So we will always test a lot before we go live with it and we'll sign off on that as well.

CHAYTOR, Q.C.:
Q. So why does Mount Sinai choose to do that, as opposed to purchasing the pre-diluted? I hear what you're saying, you like your own better, but is there any other reason? Is there--do you have more control over the process by making your own?

MS. WEGRYNOWSKI:
A. I'm sorry, in what--to what are you speaking to?

CHAYTOR, Q.C.:
Q. No, I'm sorry, I'm just thinking--I'm thinking back to the conversation we had about the antibodies.

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MS. WEGRYNOWSKI:
A. Oh, antibodies.

CHAYTOR, Q.C.:
Q. And why is it that you choose to do your own?

MS. WEGRYNOWSKI:
A. Well, we have an open--we use an open system at Mount Sinai. I would assume some of it's historical. Some of it is cost analysis. We're very--you can get a tremendous dilution out of some different products. We have found that different antibodies work with different detection systems and different pre-treatments, but that is what we have found at our house. When you are using a closed system, the manufacturer is selling you products that are all supposed to work together. It's a very different--it's just a different way of doing work.

CHAYTOR, Q.C.:
Q. Okay. So in terms of an advantage at the end of the day, in terms of producing a higher end product, do you feel you have any more control over that by doing it your way?

MS. WEGRYNOWSKI:
A. I don't know if the word is control as of the

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amount or perhaps the amount of antibodies that we would carry could be greater, because if you're into a closed system, you are locked into only what that manufacturer can produce for you. So if I could, we don't purchase our antibodies just from one source. We go to a multitude of sources. So if we were to tie it into one particular company, then we would not be able to offer that product and we would like to be able to do that.

CHAYTOR, Q.C.:

Q. So it gives you more flexibility?

MS. WEGRYNOWSKI:

A. Absolutely.

CHAYTOR, Q.C.:

Q. Okay, thank you.

MS. WEGRYNOWSKI:

A. You're welcome.

CHAYTOR, Q.C.:

Q. And are we finished with this slide then?

MS. WEGRYNOWSKI:

A. I think so.

CHAYTOR, Q.C.:

Q. Okay, and we can go to the next one.

MS. WEGRYNOWSKI:

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A. Buffers, again, it's--you're going to hear the same thing. I feel like a record.

CHAYTOR, Q.C.:

Q. And we just discussed that.

MS. WEGRYNOWSKI:

A. Yeah, so I think we can go on from there.

CHAYTOR, Q.C.:

Q. Sorry.

MS. WEGRYNOWSKI:

A. Again, it's the same thing, whether they're purchased or prepared in-house. Again, it's the same thing again, all your ancillary products, they all have to be maintained the same way.

CHAYTOR, Q.C.:

Q. Okay, and if you'd like to go to the next slide then. Okay, and we're about to then go into the ER and PR evaluation, so perhaps this would be a good point then for us to take the lunch break, please.

THE COMMISSIONER:

Q. We'll adjourn to ten after two.

CHAYTOR, Q.C.:

Q. Thank you.

(LUNCH BREAK)

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THE COMMISSIONER:

Q. Please be seated. Ms. Chaytor.

CHAYTOR, Q.C.:

Q. Good afternoon, Commissioner. Good afternoon, Ms. Wegrynowski. We're about to turn to the ER and PR evaluation part of you presentation. Before we do that, is there any peculiar about ER/PR antibodies as opposed to other IHC stains?

MS. WEGRYNOWSKI:

A. I wouldn't use the peculiar other than these are one of the fewer antibodies that live in the nucleus, the proteins are available in the nucleus.

CHAYTOR, Q.C.:

Q. Okay. And what's the import of that?

MS. WEGRYNOWSKI:

A. The importance of it?

CHAYTOR, Q.C.:

Q. Yes, what's the importance of -

MS. WEGRYNOWSKI:

A. Fixation, localization, so that when you're interpreting the slides and you're going through the validation process you know exactly where you're to find the epitope.

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That is only in the nucleus and nowhere else.

CHAYTOR, Q.C.:

Q. Okay. And if we can then, if you wouldn't mind, please, take us through the next slides.

MS. WEGRYNOWSKI:

A. Okay. I did a portion of these slides with Dr. Frances O'Malley when I was getting ready to come here because we knew that it was about ER/PR. So, we thought we'd give a little bit of an overview. So, when we talked about the clinical validation, we wanted to ensure that the test identified sub-sets of patients with significantly different risks of recurrence to survival. So, we had to ensure that the test is sensitive, specific, reproducible and interpreted in a uniform manner from lab to lab.

CHAYTOR, Q.C.:

Q. Thank you.

MS. WEGRYNOWSKI:

A. On the technical portion, we must ensure that the test is sensitive, specific, reproducible and interpreted again in a uniform manner from lab to lab. So, with ER validation we have a technical validation. So, we have several

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antibodies against the estrogen. We have several antibodies to ensure its specificity. So, we tried out several and then this is how they've come up with what they wanted to use. It's reproducible. We've tried different IHC methods and that's what we did during validation. So that we made sure that the one that we were using was what we needed to do. And this is part of the pathologist portion where arbitrary cutoffs and methods of scoring have been determined. So, in IHC the signals can be difficult to quantify and the reasons are the results can be affected by tissue handling; fixation; processing; the specificity and sensitivity of the primary antibody; the detection systems; antigen retrieval and of course, your methods of scoring. A little bit of the history, so you had the DCC charcoal method and then you had the IHC all or none method. So, if--present or not. And then they're presently using the Allred scoring method. And some places are also beginning with image analysis.

For ER and PR you must ensure there's quality control and quality assurance as you

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are with every antibody.

CHAYTOR, Q.C.:

Q. Okay. Maybe you could just tell us what the difference between those two, quality control and quality assurance.

MS. WEGRYNOWSKI:

A. Okay. With quality control, you want to make sure that you've batched batch reproducibility, that your controls are all working with quality assurance, that it's working within the bigger picture.

CHAYTOR, Q.C.:

Q. Okay, thank you. You can go ahead.

MS. WEGRYNOWSKI:

A. Okay. So, this is--and I'm sure Frances used this same slide yesterday. So, you've got a tumor that is negative, but then we're looking at the internal controls of the ductal epithelium which are positives, so that you would know this was a true negative stain. Okay.

CHAYTOR, Q.C.:

Q. Yes, I think that is the same -

MS. WEGRYNOWSKI:

A. This is the same slide -

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CHAYTOR, Q.C.:

Q. - that she showed us yesterday, yes.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Okay.

MS. WEGRYNOWSKI:

A. So, we just went over a little bit of what we were using. So, we fix in 10 percent neutral buffered formalin for eight to ten hours followed slicing to allow adequate fixation. They use the--Frances has followed the Baylor's methodology. The antibody clone that use for estrogen is 6F11 and for the PGR we're using the 1294. And they use the Allred scoring system.

CHAYTOR, Q.C.:

Q. And in terms of the impact of fixation, and in particular the impact of under fixation, can you speak to that?

MS. WEGRYNOWSKI:

A. With under fixation you can have an opportunity to lose the staining and end up with hollow nuclei in your nucleus because it had never been fixed. So that--actually the

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cell begins to degrade.

CHAYTOR, Q.C.:

Q. So your protein is deteriorated.

MS. WEGRYNOWSKI:

A. That's right. You wouldn't see your chromatin pattern in your nucleus.

CHAYTOR, Q.C.:

Q. And that's of particular importance, I take it, then to ER/PR in particular?

MS. WEGRYNOWSKI:

A. Yes, because that's where the protein should be exhibited.

CHAYTOR, Q.C.:

Q. What could cause under fixation?

MS. WEGRYNOWSKI:

A. Not leaving the sections--well, either not--under fixation? Not keeping it in a fixative long enough, not slicing it properly, so that even if you took a thick section and the fixative would not be able to penetrate the section in an adequate amount of time.

CHAYTOR, Q.C.:

Q. And we've heard about -

MS. WEGRYNOWSKI:

A. Bread loafing.

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CHAYTOR, Q.C.:
Q. - bread loafing, yes. Could you just explain that to us?
MS. WEGRYNOWSKI:
A. So that if you had a piece of tissue you would want to cut it, serely (phonetic) section like a bread loaf. Oftentimes paper towels will be inserted to increase the absorbency so that the formalin will be able to absorb into and starting fixing the tissue.
CHAYTOR, Q.C.:
Q. Okay. Sorry, if you could continue on then.
MS. WEGRYNOWSKI:
A. Okay. So, this was a the scoring that they're presently using at Mount Sinai. So, and I know you did this yesterday, so zero percent would be negative, low positive is one to nine and then the positive is ten to hundred. The technologist doesn't do the scoring. That's all done by the pathologist, but this was more to show this to the technologists so that they understood how the tests were going to be used and what interpretation was going to be given to it. Next?
CHAYTOR, Q.C.:

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Q. Yes, thank you.
MS. WEGRYNOWSKI:
A. Once again this is from Frances', so again, estrogen receptor protein, you'd want it greater than 90 percent of your cells, antibody use was 6F11 and the progesterone receptor protein was approximately 60 percent. So, the positive and negative laboratory controls stained appropriately and so they came up with their threshold. So, I can't speak exactly to the numbers here. As I say, I've taken this from Frances just to provide the technologist with some information.
CHAYTOR, Q.C.:
Q. Okay.
MS. WEGRYNOWSKI:
A. So this is just a piece of tissue. It's a core biopsy. No, excuse me, it's a TMA and it's positive for ER and it's just an example so that you can see it. It's on the nuclear detail. Again, it's the same thing. So, you can see, it's just your nuclei. You don't have any of the cytoplasma around it. It's just--they look like dots. So, it's just the nuclear--and then a TMA and this is just some

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HER2/neu work that we were showing. And then you can just see that cell membrane. That's a completely different marker, but just how it exhibits a pattern. And that's my joke, my levity.
CHAYTOR, Q.C.:
Q. It's good. So, thank you and thank you for taking us through that.
MS. WEGRYNOWSKI:
A. You're welcome.
CHAYTOR, Q.C.:
Q. The issue of fixation and the importance of fixation, when, in your career, did you become aware of that as being important, particularly with respect to nuclear staining.
MS. WEGRYNOWSKI:
A. Fixation is a cornerstone of pathology. So, this would have been taken--I would have learned about this when I was in first year school, med lab tech.
CHAYTOR, Q.C.:
Q. And as a technologist, you're dealing with the analytical stages you told us, are you able to tell when there are problems with fixation?
MS. WEGRYNOWSKI:

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A. It would be represented in the quality of a block that we would receive. We can tell that there problems with processing, a block will become concave or if there's a lot fat in it and it's never fixed, you can actually feel, on the block, the oily surface. Other issue occur when the microtomist is trying to obtain a section and they're not able to get a full section. And they end up getting holes actually in the section.
CHAYTOR, Q.C.:
Q. Okay. So, you took the technologists through those three presentations while you were here in St. John's in September 2005.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. What happened next in your visit?
MS. WEGRYNOWSKI:
A. That took up most of the day. So, we just did a little bit of a walk around through the laboratory and that was about it for the day. I can't remember anything else highlighted.
CHAYTOR, Q.C.:
Q. Okay. And then on your third day, how did you

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spend your time?
MS. WEGRYNOWSKI:
A. On my third day it was--I needed to determine how I was going to write my report. And so that was what most of the day was spent on, just checking through the laboratory, but nothing there was highlighted, I don't recall.
CHAYTOR, Q.C.:
Q. And did you have what we call an exit interview?
MS. WEGRYNOWSKI:
A. Yes, I did.
CHAYTOR, Q.C.:
Q. Who met with you?
MS. WEGRYNOWSKI:
A. Dr. Bob Williams.
CHAYTOR, Q.C.:
Q. Anybody else?
MS. WEGRYNOWSKI:
A. There were other people at the table. I don't recall their names.
CHAYTOR, Q.C.:
Q. Okay. And if we could look please at P-1737 and these again, I believe, Ms. Wegrynowski, these are notes from Dr. Cook.

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MS. WEGRYNOWSKI:
A. Okay.
CHAYTOR, Q.C.:
Q. And September 22, 2005 "meeting with Trish, Dr. Williams, Kara Laing, Terry Gulliver and Heather Predham", would that seem about right? Dr. Ejeckam's name is mentioned there as a point man, did he attend your exit interview?
MS. WEGRYNOWSKI:
A. No, I don't think so.
CHAYTOR, Q.C.:
Q. Okay. And do you recall if Dr. Laing, Mr. Gulliver and Ms. Predham attended?
MS. WEGRYNOWSKI:
A. I couldn't put a face to the names, so, I couldn't comment.
CHAYTOR, Q.C.:
Q. So there were a number of people -
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Okay. And what then did you discuss with Dr. Williams and the others before you left St. John's?
MS. WEGRYNOWSKI:

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A. I had determined before I had left St. John's basically what I was going to outline to them as far as the recommendations. So, I looked at the large picture and just started at the very beginning and thought I would speak to each point and from there I derived my report.
CHAYTOR, Q.C.:
Q. Okay. And did you take--what observations did you find or conclusions did you find to be most important to speak to them about and alert them to?
MS. WEGRYNOWSKI:
A. That there was absolutely no standard operating procedures and that documentation was not being done.
CHAYTOR, Q.C.:
Q. So, I take it basically this interview you outlined any concerns that you were able identify.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. What was their reaction? What was Dr. Williams' reaction?
MS. WEGRYNOWSKI:

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A. I think they were rather surprised. I mean, they were--I don't think they had an understanding of the depth of the concerns or the issues that I brought to them. They were certainly very interested in them.
CHAYTOR, Q.C.:
Q. Okay, but somewhat surprised as to the extent of the issues?
MS. WEGRYNOWSKI:
A. I think so.
CHAYTOR, Q.C.:
Q. Okay. And I take it based on your CV that we looked at earlier, you have done similar reviews for other laboratories?
MS. WEGRYNOWSKI:
A. Correct.
CHAYTOR, Q.C.:
Q. Okay. And how did what you observed here compare to what you have observed in other laboratories that you've assessed?
MS. WEGRYNOWSKI:
A. It was very different, but the other laboratories that I had gone to had already been through a CAT process. So, they were a little bit more aware of what one might be

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coming to look for. Where, in this laboratory, it was walking in and I was on the understanding I was here to do a peer review, to look at their processes and procedures and that's what I had asked for in my initial e-mail. So, for me I was surprised that I didn't find what I thought I was coming to see and that prior to coming here, no one had explained to me that those weren't in place. So, it was very different.

CHAYTOR, Q.C.:

Q. So, I take it, in saying that, that the basic or the basic cornerstones of what you were expecting to see weren't there.

MS. WEGRYNOWSKI:

A. Correct.

CHAYTOR, Q.C.:

Q. Okay. And if we could just look then, please at those notes to see if there's anything there. Now, perhaps this will all come out in your report. There's certainly the recommendation for external CAP, need this, you indicate. So, that's for the external q u a l i t y a s s u r a n c e a n d " h a v e frustrated/overwhelmed individuals" and we've

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spoken about that.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Both sides, I take it, the technology side and on the pathology side?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Not sure what that one says. "Don't understand the theory, work as a group" and that would have been something you would have pointed out in your meeting?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. "Need to have good lines of communications" and you've discussed that with us. "Need one technical director" I think that says. "Need better fixation", so that was something that you were able to determine while you were here in St. John's? "Standardization, fixation need pathology assistance" and we've talked about that. "Outside labs, need outside labs to advise if there's fixation". And the

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outside labs being the labs outside of Eastern Health that were sending their samples in, was that an issue that you raised?

MS. WEGRYNOWSKI:

A. One of the thoughts that I had was that if you were going to be doing the work for a number of other institutions in the area, that it would be wise to have you look at their protocols to ensure that they were doing the same thing as you.

CHAYTOR, Q.C.:

Q. Okay. And it says, "Health Care Corporation, St. John's, needs to advise other hospitals of fixation. So, they would need to advise of the fixation issue". Is that what you were indicating?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And "consider disclaimer on reports for referral labs", is that something that you brought up in the meeting?

MS. WEGRYNOWSKI:

A. I might have done that. I know we certainly do that.

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CHAYTOR, Q.C.:

Q. Okay. And controls "need to run positive and negative controls".

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And we've spoke about that. And negative controls for specificity, is that right.

MS. WEGRYNOWSKI:

A. Correct, ensure they're negative.

CHAYTOR, Q.C.:

Q. And validation then and we spoke, I think, about most of those points on validation.

MS. WEGRYNOWSKI:

A. Correct.

CHAYTOR, Q.C.:

Q. And I'm sure we can cover it off in what's written, in any event, in your report. So, we can see here that Dr. Cook wrote two fairly detailed pages of notes from your exit interview. How long was that meeting?

MS. WEGRYNOWSKI:

A. I don't recall.

CHAYTOR, Q.C.:

Q. But I take it, it was fairly thorough in terms

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of what you had discovered and you relayed to them most of what ultimately ends in your report.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And if we could look then at P-0596. And again, this is not a document, this is not your document.

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. And you've probably not seen it before, but it's another couple pages of hand written notes. And we understand this is Dr. Williams' notes and you'll see it's two and bit pages.

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. And this again is September 22nd, 2005 and it appears to be Dr. Cook, Dr. Laing, Ms. Predham, Mr. Gulliver, Dr. Williams and the Mount Sinai technical person. So this appears to be his notes then of your exit interview as

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well and we'll see the state of affairs, frustrated and overwhelmed comments, again referring to the staff. "Causes, communication issues, do not understand the theory." So similar items being mentioned here, the fixation being mentioned, the controls being mentioned and validation and it goes on from there. So this is, we understand Dr. Williams' notes of your exit interview and again, I just show that to you and I take it that's in keeping with the types of issues that you covered off with the senior management of Eastern Health before you left St. John's?

MS. WEGRYNOWSKI:

A. Correct.

CHAYTOR, Q.C.:

Q. If we could have, please, P-0047? Actually before we go there, if we could look at P-1755? And perhaps you can just explain what this document is?

MS. WEGRYNOWSKI:

A. Okay, this was the CAP guidelines for the year 2004, I believe, isn't it up there?

CHAYTOR, Q.C.:

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Q. Yes, it's there in the corner.

MS. WEGRYNOWSKI:

A. So it would have been in keeping in what we would have been looking at for accreditation in that year to do with anatomic pathology.

CHAYTOR, Q.C.:

Q. So is this what you used as a reference in carrying out your assessment?

MS. WEGRYNOWSKI:

A. I did use this as, yes, as some of the reference points, yes, I did.

CHAYTOR, Q.C.:

Q. Okay. And if we could have then, please, P-0047?

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. Ms. Wegrynowski, this is your first report which is dated November 9th, 2005 and it appears that we're looking at copy seven of eight, May 23rd, '07. And it's a fairly detailed report. I believe it goes on for some twenty pages--twenty-one pages. Does this report contain everything that you observed or could have written about?

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MS. WEGRYNOWSKI:

A. I could have written more, there's no doubt about it, but at some point I had to take stalk and look at the large picture and went after the very large components. It could have been much more detailed, but I wasn't sure that's what they wanted.

CHAYTOR, Q.C.:

Q. Okay, and what were you trying to then achieve or convey in writing this report?

MS. WEGRYNOWSKI:

A. I wanted to provide them with a blueprint of what their deficiencies were and provide them with a set of recommendations that I thought would assist them in correcting the issue that they had at hand.

CHAYTOR, Q.C.:

Q. Okay, and we have your table of contents and then we have "Introduction, Executive Summary" and here you write, "The inconsistencies associated with formalin fixation, tissue processing and pretreatment methodology, compounded by a rotating staff directly affected the repeatability of the immunohistochemistry results." What are you--

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what is it exactly that you are saying there, Ms. Wegrynowski?

MS. WEGRYNOWSKI:

A. Well the formalin fixation, there was no standard operating procedures as we've already discussed, so the amount of--well just inconsistency between case to case. The tissue processing, we knew that the processors, there was no documentation to ensure that they were being changed and corrected. We also knew that the reprocessing rate was very, very high. The pretreatment methodology of choice, I believe at that time what they were using was a steam pretreatment on the bench. I never found that they had any thermometers in there again, as we speak to NIST traceable, there were no NIST traceable timers around, so I would venture to say that I'm not sure that even the pretreatment was done in a very consistent manner, so you would also have lot-to-lot variation or day-to-day variation with that as well.

CHAYTOR, Q.C.:

Q. And you go on to say, "Also the lack of standard operating procedures across the

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pathology lab contributed to the problem. The high degree of proficiency required to perform, evaluate, and interpret immunohistochemistry testing must be assessed, as both the technical and professional staff are accountable for the final outcome." And what are you trying to emphasize in that statement?

MS. WEGRYNOWSKI:

A. Well they had no standard operating procedures either for the technical side or for the professional side or pathologist's side. So to ensure that there's proficiency, you need to have that. You want to ensure that the evaluation and the interpretation of the immunohistochemistry tests are done collectively. I never found any correlation between even the pathologists if they had ever taken a set of slides and looked at them and come up with their own results, so I--that's what that was meant to mean.

CHAYTOR, Q.C.:

Q. And under "Background", you indicate that from 1997 until 2004 the semi-automated DAKOCytomation, is that correct?

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MS. WEGRYNOWSKI:

A. Yes, those two companies were linked at that time.

CHAYTOR, Q.C.:

Q. Okay, auto-stainer was used for IHC staining.

A heat induced epitope retrieval pretreatment using steam was performed manually outside of the auto-stainer and concentrated primary antibodies were diluted at the discretion of the laboratory." Can you just explain that to us laypeople? What is a heat induced epitope retrieval pretreatment?

MS. WEGRYNOWSKI:

A. So the sections are taken and the slides, you have to remove the wax, the tissue sections on the slide and to do that, you go through solvents, you go from cylene through alcohols, gradient alcohols to get down to water. Once they're into water, then you can remove the indogenous peroxidase, as I showed you in my PowerPoint this morning with hydrogen peroxide and water. Some antibodies to be able to see them, you need to use a pretreatment and for the particular one that they were using, they were using steam, and that is performed prior

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to the slides being put on the auto-stainer. It was what they chose to use at Eastern Health to break the aldehyde bonds to expose the epitope.

CHAYTOR, Q.C.:

Q. They use steam.

MS. WEGRYNOWSKI:

A. They use steam, yes.

CHAYTOR, Q.C.:

Q. And then you go on to talk about in 2004 how that was replaced with the automated Ventana benchmark.

MS. WEGRYNOWSKI:

A. Uh-hm.

CHAYTOR, Q.C.:

Q. "Prior to January 2003, the formalin fixative was prepared in-house at the Health Science Centre and distributed; however, no documentation concerning the pH of this fixative was found. It has since been replaced with commercially prepared ten percent neutral buffered fixative." And again the importance of having documentation or what would you expect to have in the way of documentation around the pH of the fixative?

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MS. WEGRYNOWSKI:
A. That they would ensure that it was the same, regardless of whether it was being made in-house or just commercially prepared.
CHAYTOR, Q.C.:
Q. So that you can compare it then and reproduce the same results?
MS. WEGRYNOWSKI:
A. Absolutely, absolutely.
CHAYTOR, Q.C.:
Q. And then you outline the objectives of your review which were to "review the current practices and procedures within the IHC service, identify issues of concern which may have contributed to the present situation and provide recommendations pertaining to issues identified." And you indicate in your scope that you visited both sites, that you focused on the immunohistochemistry service and the procurement and processing from both sites, because tissue fixation and processing provide the basis for accountable immunohistochemical staining."
MS. WEGRYNOWSKI:
A. Yes.

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CHAYTOR, Q.C.:
Q. And your methodology we've talked about, you met with the technologists, the pathology manager, laboratory director, chief pathologist and pathologist.
MS. WEGRYNOWSKI:
A. Uh-hm.
CHAYTOR, Q.C.:
Q. And at the histology lab, again you speak about the lack of consistency with fixation and the issue of reprocessing, both had had a direct effect on the staining outcome with IHC. And perhaps you could just spend, I know we've talked about that, but perhaps you could just once again explain why you think that had an effect on the staining outcome?
MS. WEGRYNOWSKI:
A. Because if you never fix the protein at the beginning and stopped it and kept it in as true a form as it was as when it left the body, the results that you would get would not be indicative of that particular time.
THE COMMISSIONER:
Q. Is there a guideline, as I understand it it's in formalin hopefully from the time it's taken

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out of the body, it's placed in formalin and then it goes through the process of getting from the OR to the lab.
MS. WEGRYNOWSKI:
A. Yes.
THE COMMISSIONER:
Q. And then at some point there's the cutting done.
MS. WEGRYNOWSKI:
A. Yes.
THE COMMISSIONER:
Q. And then it goes back into formalin.
MS. WEGRYNOWSKI:
A. Yes.
THE COMMISSIONER:
Q. The smaller pieces.
MS. WEGRYNOWSKI:
A. Uh-hm.
THE COMMISSIONER:
Q. For the optimum period of time.
MS. WEGRYNOWSKI:
A. Uh-hm.
THE COMMISSIONER:
Q. Does the question of when it's sliced make a difference?

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MS. WEGRYNOWSKI:
A. Well -
THE COMMISSIONER:
Q. Do you know what I mean, in the sense of as I understood what Dr. O'Malley was saying yesterday that one of the concerns about slicing is to make sure that the formalin actually gets to the material which is going to be eventually placed -
MS. WEGRYNOWSKI:
A. Correct.
THE COMMISSIONER:
Q. - in your little paraffin blocks.
MS. WEGRYNOWSKI:
A. Uh-hm.
THE COMMISSIONER:
Q. And that's one of the important reasons for slicing and I guess my question is really, follows on from that and maybe I should have asked her, but is there an optimum time to slice? As I understand it, there is a period of time in which the tissue should be in formalin, you can't take it out too early and you can't take it out too late, is that -
MS. WEGRYNOWSKI:

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A. Correct.
THE COMMISSIONER:
Q. There's an optimum period and I suppose I'm asking whether within that timeframe does it matter when you slice?
MS. WEGRYNOWSKI:
A. Slicing it at the beginning would be the best.
THE COMMISSIONER:
Q. Okay, the earlier you slice -
MS. WEGRYNOWSKI:
A. The earlier the better, yes.
THE COMMISSIONER:
Q. Okay.
CHAYTOR, Q.C.:
Q. And this issue of reprocessing, how harmful can that be to the tissue?
MS. WEGRYNOWSKI:
A. It can be very harmful because you're going back up through temperature to melt that wax again and then going back through the different solvents to get it back to formalin, but if your formalin was never fixed properly at the beginning, your section, your tumor section will never be the same. You cannot have what you had originally had.

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CHAYTOR, Q.C.:
Q. And could that then result in no stain?
MS. WEGRYNOWSKI:
A. I couldn't say that as an absolute.
CHAYTOR, Q.C.:
Q. And if we continue on then, if there's anything else in this paragraph that you would want to point out, if--perhaps, however, you speak here again about, "however refrigeration storage is not available for larger unfixed specimens after hours."
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And again, you've indicated the importance of refrigeration and storage.
MS. WEGRYNOWSKI:
A. Absolutely, you need to stop the process of autolyses and putrefaction and that can be done by at least on off hours getting your specimens into refrigerators, they were just leaving it at room temperature.
CHAYTOR, Q.C.:
Q. And your recommendations, your first recommendation, of course, is "standard

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operating procedures relating specifically to the grossing/fixation procedures must exist for each tissue type to ensure the reproducibility and reliability of results."
MS. WEGRYNOWSKI:
A. Correct.
CHAYTOR, Q.C.:
Q. And you're recommending secondly that "Three pathologist assistant positions be created to facilitate this and the PA's will be responsible for quick sections. Thirdly, a refrigerator to be available in the quick section rooms." And then your next section of your report is 2.2 and it's called "Processing" and you note that "Currently each site maintains a tissue processor. All specimens grossed each day are placed in the tissue processor at that particular site, with the exception of biopsy specimens. Procedure manuals outlining the standard operating procedures for the tissue processors were not found. Neither signed documentation for the daily maintenance of the processors, nor temperature monitoring of the paraffin wax was found." And your recommendations are "To

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ensure that tissue processing is reproducible, it should be amalgamated to one site." And you're recommending that the St. Clare's tissue processor be relocated to the Health Sciences. "Signed daily cleaning and maintenance schedules for the tissue processors must be maintained and retained. Temperature of the paraffin wax must be recorded daily, this is especially important as some antibodies are heat laible. Standard operating procedures to be written for the tissue processing including identifying different processing and cleaning and maintenance of schedules, directions to load and unload the processors." And you indicate it's not a mutually exclusive list. "Blocks to remain at the Health Science and couriering of stain slides to remain at status quo." Is there anything amongst all of that that we haven't covered that you would like to comment on?
MS. WEGRYNOWSKI:
A. No, I think it's been covered.
CHAYTOR, Q.C.:
Q. The issue of the paraffin wax, I know we

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talked about temperatures in other context, but what difference would it make for the temperature of the paraffin wax, for that to be recorded daily?

MS. WEGRYNOWSKI:

A. Because you want to make sure that it fits within the optimum melting points. You don't want it to get too high where you're going to lose a heat laible of proteins and if it's not warm enough, it's not going to melt, so there's a particular reference range.

CHAYTOR, Q.C.:

Q. And is ER and/or PR an antibody that would be sensitive to that?

MS. WEGRYNOWSKI:

A. I think the heat laibility could affect anything.

CHAYTOR, Q.C.:

Q. If we go on then to the next page, it deals with the immunohistochemistry laboratory and you talk about the staffing issue, 3.1, "three registered technologists rotate through the IHC area with an additional registered technologist performing the microtomy", is that correct?

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MS. WEGRYNOWSKI:

A. Microtomy.

CHAYTOR, Q.C.:

Q. Thank you. All right. "And approximately 80 IHC tests are performed daily with majority done on the automated Ventana." You note that there's no medical section head for the IHC laboratory and you also note "Both the technologists and the pathologists I spoke with are frustrated with the present situation as there are no clear lines of communication, in addition, the technologists are overwhelmed as they do not completely understand the theory of IHC and this testing required high technical proficiency to troubleshoot the methodology." And your recommendations flowing from that are that "Three technologists be dedicated to perform IHC staining, optimization and validation. One of the three registered technologists to be given the additional responsibility and title to oversee the IHC lab and a medical section head be designated and responsible for the IHC lab." And is there anything else in any of that, Ms. Wegrynowski that you could expand upon or feel that we have not already covered?

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MS. WEGRYNOWSKI:

A. If I could just, please, when I spoke to the technologists, they were being put on rotations and they were doing one week in three different things, so they were in for one week in immunohistochemistry and then gone for another two weeks and then they would be dropped back into immunohistochemistry. Because it's a very dynamic laboratory and things had changed and the communication would not have always been delivered to them, I think that was part of the frustration, as well, so that even if Dr. Ejeckam had taken the time and the energy to train them, he was always dealing with a workforce that was always changing, and I feel that that really needs to be reiterated.

CHAYTOR, Q.C.:

Q. Okay.

MS. WEGRYNOWSKI:

A. And that a lot of the communications weren't just going through them, they were going through others and back to them.

CHAYTOR, Q.C.:

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Q. Okay. And you indicated earlier this morning in your evidence, though, that I think you sort of made a joke and said, "I have many bosses" and a lot of people communicating with you. How is that different from what you observed in St. John's?

MS. WEGRYNOWSKI:

A. It's very fluid where I come from. It's not unusual for me to see, I don't know, 10, 12 different pathologists in any given day just dropping by, ordering, asking questions, whatever. As I said, I do have a technical head and I do have a medical head, but because of the nature of the work that we do there, we work for every pathologist, so if they come to us, it is not one pathologist that goes to another pathologist, they take it upon themselves if they're not pleased with something, they come to us directly and ask the questions directly. If they're looking for a new antibody, they will come to us directly and tell us what they're looking for. And we work, it's a very large team.

CHAYTOR, Q.C.:

Q. And I take it you have your standard operating

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procedures in place so regardless, things are going to be done the same way in your lab regardless of how many channels of information may be coming at you or instructions may be coming at you?

MS. WEGRYNOWSKI:

A. That's correct. So then we have a communication board so that if something does change, if it's something a large change, that we're bringing a new antibody in or any large changes that would fit outside that, we do have a board where all that would fit on, as well. But because we work together every day there is always that communication that there's much less chance of it falling through.

CHAYTOR, Q.C.:

Q. Okay. So I take it in your day to day at Mount Sinai you have a lot of interaction with the pathologists?

MS. WEGRYNOWSKI:

A. Yes, we do.

CHAYTOR, Q.C.:

Q. Okay. And what kind of atmosphere do you work in there in terms of, well, what's the culture

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in terms of is this a team atmosphere or how does that work?

MS. WEGRYNOWSKI:

A. It's very collegial, it's very collegial. When we run into issues with troubleshooting ourselves, and everybody has them, we'd be remiss to say we don't, it's a wonderful opportunity for the team to sit together and say, okay, this is what has happened, what do we think it can be and go through the processes and try and rule out what can and cannot be done. Because in the end what we're doing is you're learning, so from the troubleshooting that's where you gain your most experience. And because there is so many people at work, not one person is handling the slides, because we all do it the same way, we must ensure that we get that final outcome.

CHAYTOR, Q.C.:

Q. Okay. And you say you have both a technical head and a medical head in your laboratory?

MS. WEGRYNOWSKI:

A. Yeah, I have a charge technologist that sits over me, she's in pathology, and then I have a medical, technical--a medical IHC director, as

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well.

CHAYTOR, Q.C.:

Q. And who do you--what, on what issues would you report to one as opposed to the other?

MS. WEGRYNOWSKI:

A. Well, that gets blurry. It depends on what the process is. If it's about completely technical, where it's to do with the staining, usually it's my medical head. My technical--my charge technologist knows very little about immunohistochemistry so it's more if I need to get large equipment purchased or whatever through capital.

CHAYTOR, Q.C.:

Q. Okay. And your recommendation here, No. 12 on page 8 of the exhibit, "Lines of authority, communication and organization to be determined prior to establishing these positions." You write, "The technologist responsible for the IHC laboratory will report to both the pathology manager and the medical section head, depending upon the issue at hand." So I take it that would be similar to what you have in Mount Sinai?

MS. WEGRYNOWSKI:

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A. That's correct.

CHAYTOR, Q.C.:

Q. And did you understand that to be different than what they had at the time in St. John's?

MS. WEGRYNOWSKI:

A. It would be unfair for me to answer that because the technologists were rotating, so I'm not sure that they even had this capability of doing this. I think everything was just going through Barry Dyer and then it was getting reflexed to wherever, or whoever was in the area.

CHAYTOR, Q.C.:

Q. "The pathologists need to establish a mechanism as to how their IHC concerns, issues will be addressed. Documentation of all concerns, issues, must be maintained with the corrective action." And there's also a note of succession planning. So if there's nothing else on that, we can move on.

MS. WEGRYNOWSKI:

A. Yes, please.

CHAYTOR, Q.C.:

Q. Okay. IHC documentation, and we've spent some time, I think, on the documentation, in

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general you say it was deficient. And then there's a list of key areas that you've identified. Is there anything there that you would like to highlight or speak to that we haven't already spoken of?

MS. WEGRYNOWSKI:

A. No. This is just a very sizable, it's very, very surprise--I reiterate, I came to St. John's and asked to look at the procedures before I came and I was very surprised that they weren't, they weren't there to see.

CHAYTOR, Q.C.:

Q. And so "No test procedure manual, including the standard operating procedures. Lacking organized current manufacturer specification data sheets." If we could just look for a second at 1605, please, page 15? And this is just a--page 15, yes, thank you. Is this what you're referring to as a specification sheet, this is an example we have here -

MS. WEGRYNOWSKI:

A. Yes, so you'll see your code there, your lot number. It's telling you your total protein concentration, it's telling you subclass, your G1, it'll tell you what you can use it for,

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it'll give you your storage conditions, how it's prepared. They often even give you, there's a little bit about the specificity, they'll even give you recommended suggestions on how you can go ahead and use it.

CHAYTOR, Q.C.:

Q. Okay. And if we could go back then, please, to 47, page 9? And so this is the kind of specification data sheet that you were suggesting should be -

MS. WEGRYNOWSKI:

A. Absolutely, so that you know and whoever comes into your laboratory, the pathologist knows what is the antibody that you are currently producing from this laboratory and let's look at the spec sheets about it.

CHAYTOR, Q.C.:

Q. And again, I take it it comes down to making sure, then, that you're doing things consistently so that you can have reproducible results?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And then "The minimal record daily keeping of

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the equipment maintenance" I think we've talked about that. "No pipette and thermometer calibration of accuracy." And we'll talk some more about that in a moment. "No reagent antibody or detection system evaluation and no validation documentation."

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. "No equipment documentation and no calibration records. Retirement of procedures or antibodies not documented. Retention of records not done. Lacking complete error log and corrective action." What's an error log and correction action?

MS. WEGRYNOWSKI:

A. Again, this comes back if the pathologist is finding something that's incorrect, that you would go ahead, we call it our error log, if it's a whatever, and then we'll go in and correct it.

CHAYTOR, Q.C.:

Q. Okay. So if there's something that's not optimal, you would record that and then figure out how to do it differently next time?

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MS. WEGRYNOWSKI:

A. Or determine what went wrong in the process to correct it, that's right.

CHAYTOR, Q.C.:

Q. Okay. And then "No distilled water monitoring and documentation." and we spoke of that earlier?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Recommendations then again is the procedural manual outlining the standard operating procedures. And you're talking about having that available at the bench?

MS. WEGRYNOWSKI:

A. Yes. I provided them with the--how the manual should be written and gave them the reference for that.

CHAYTOR, Q.C.:

Q. Okay. So you gave them material by which they would be able to draft that?

MS. WEGRYNOWSKI:

A. Yeah. And those guidelines were published in 1999.

CHAYTOR, Q.C.:

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Q. Okay.
MS. WEGRYNOWSKI:
A. Yeah.
CHAYTOR, Q.C.:
Q. 1999?
MS. WEGRYNOWSKI:
A. Um-hm.
CHAYTOR, Q.C.:
Q. Okay. And that's the Clinical Laboratory Standard Institute's Guidelines?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Okay. "It would be prudent," you say, "to send the technologist responsible for compiling and maintaining procedural manual to a computer course." And you finish by saying, "The benchmark--Ventana benchmark operator's manual is available at the workbench. It is an acceptable component of the overall IHC departmental procedure, but does not replace a procedure manual."
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:

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Q. And why is that?
MS. WEGRYNOWSKI:
A. Because these are just operator's manual, it's just how to operate the machine, it's not how you're actually making your procedures and processes work.
CHAYTOR, Q.C.:
Q. And if we go on then, your recommendation No. 15 is "Antibody data specification sheets to be available for all antibodies currently in use." So I take it that's similar to the sheet that I just showed you?
MS. WEGRYNOWSKI:
A. That's correct.
CHAYTOR, Q.C.:
Q. "Routine equipment maintenance must be performed and documented." And then you've broken this down between internal checks and external checks. Perhaps you could just speak to us a bit about this recommendation?
MS. WEGRYNOWSKI:
A. Okay. So the internal check we're talking about the Ventana staining module, which had its own maintenance programs. They told me that they were performing a daily maintenance

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but they didn't always document it, so they had to change that. And then the other portion was that it was a quarterly maintenance but they were unable to access the process. So I had suggested that they go to their information technology department and see if they could assist them with that. Internally that you must always set up your microscope up for Kohler illumination. And that should be done daily and should also be signed off because you want to ensure that you have a correct contrast and resolution. And again, the laboratory personnel are responsible for the reliability and proper function of the instruments that they work on, hence, service and repair, records or copies must be available to the technical staff operating the equipment. As well, an alternate protocol must exist in case of failure of the automated equipment.
CHAYTOR, Q.C.:
Q. Okay. And the external check?
MS. WEGRYNOWSKI:
A. These are when people can come in from the outside, these are things that you don't

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necessary take care of. So you want to make sure that you have your annual preventative maintenance records on your microtome, your weigh balance, your microscope, any other people of equipment that are not mentioned here that are annually inspected.
CHAYTOR, Q.C.:
Q. Okay. And I'm going to leave No. 27 for a moment because we're going--I understand you're going to do a demonstration for us around that.
MS. WEGRYNOWSKI:
A. I can.
CHAYTOR, Q.C.:
Q. Okay. About the Guarantee Pipette and temperature accuracy and calibration, so we'll come back to that.
MS. WEGRYNOWSKI:
A. All right.
CHAYTOR, Q.C.:
Q. No. 18, "Documented evaluation must be performed to ensure the sensitivity and specificity of the test results. Each component used in IHC staining must be optimized and validated individually to ensure

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the outcome and assist in troubleshooting. Presently this is not being done."

MS. WEGRYNOWSKI:

A. No.

CHAYTOR, Q.C.:

Q. And then you talk about the primary antibodies being pre-diluted and concentrated. "Any change in lot number of concentration, the specificity of the antibody must be verified prior to use. The staining results should be compared to the previous lot using the appropriate controls." And I believe you spoke to us today about that. And "The validation documentation must be approved and signed off by the medical section head prior to use in routine service." So I take it that was not taking place?

MS. WEGRYNOWSKI:

A. No.

CHAYTOR, Q.C.:

Q. "Stain, slides used for validation to be kept for reference."

MS. WEGRYNOWSKI:

A. Correct.

CHAYTOR, Q.C.:

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Q. And again, "The equipment selection criterion calibration is to be documented." And you say that, "No documentation for the selection criteria used for the Ventana" was, I take it, available?

MS. WEGRYNOWSKI:

A. No.

CHAYTOR, Q.C.:

Q. "For any new equipment or instruments purchased protocol and records must be maintained of the calibration performance and functional checks and verification performed and evidence of training and competency of technical staff on equipment must be available." So I take it that was not available in this case?

MS. WEGRYNOWSKI:

A. No, it wasn't.

CHAYTOR, Q.C.:

Q. "Retired procedures and antibodies information must be retained for two years and readily available." And "It is recommended that they be retained for longer for reference." Why two years?

MS. WEGRYNOWSKI:

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A. Industry standards.

CHAYTOR, Q.C.:

Q. Okay. "All issues, concerns and corrective actions again must be documented and this should be done by the technical and professional staff." And "Distilled water is available on tap in the laboratory. Documentation of the suitable quality of this water for the testing being performed was not found, nor was it known." So I take it nobody was able to give you that information?

MS. WEGRYNOWSKI:

A. No, they were not.

CHAYTOR, Q.C.:

Q. Okay. The next section then, Ms. Wegrynowski, deals with immunofluorenscence staining. And you spoke about this briefly in your slide show, some of the problems you encountered there. I take it this didn't--this doesn't have anything to do with ER/PR, as such?

MS. WEGRYNOWSKI:

A. No.

CHAYTOR, Q.C.:

Q. Okay.

MS. WEGRYNOWSKI:

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A. However, it is very valuable, as well.

CHAYTOR, Q.C.:

Q. Yes. And is there anything then, I believe you already spoke about the fact that it was being performed in a lit environment and no controls were used. And is there anything else about that that you -

MS. WEGRYNOWSKI:

A. They were not retaining their samples at all, so what they were doing is they were just taking their sections and then just letting the--they were leaving the sections inside the cryostat, the cryostat is just a frozen microtome, so but what it does every night it is comes back up to temperature and then goes back down again for decontamination. And they were just leaving their specimens in there so they were completely destroyed. These are--they would be kidney biopsies, oral biopsies, buckle, whatever. So the frozen patient samples should have been taken out, they should have been labelled and stored correctly to avoid desiccation in a frost-free freezer. And to maintain the antigenicity longer, I suggested that they were stored at minus 80.

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They did not use any controls when using this particular test. "Interpretation at every staining procedure must be substantiated with the use of controls, both positive and negative. Controls must be handled exactly in the same manner as test samples. It is also ideal that control sections used to have a composition similar to the patients samples being tested. Positive controls assure that the specimen staining was correctly performed when negative controls assess non-specific staining. The use of frozen sections previously confirmed positive and negative cases offers the easiest and most practical way to obtain controls for immunofluorenscence studies." And then again, as I had said before, that it should have been protected from light.

CHAYTOR, Q.C.:

Q. Okay. And the Her2/neu testing, what were you able to observe about how that was taking place?

MS. WEGRYNOWSKI:

A. They were using it manually on the desk, they were doing a manual pretreatment for these

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markers in the same way that was used for ER/PR testing in the past. So for myself I had an issue of reproducibility and consistency was of concern. So my recommendation is that "This methodology must be performed in the strictest of conditions. If you can automate the procedure, it was greatly suggested. Variation in pretreatment between testing batches must be guarded to ensure that all test results be reproducible. Strick adherence to temperature and time must be maintained."

CHAYTOR, Q.C.:

Q. Okay. And again, this test, like the ER/PR test is one which would be used for treatment purposes?

MS. WEGRYNOWSKI:

A. Yes, it's a class 2.

CHAYTOR, Q.C.:

Q. Class 2. And then 3.5 is about controls. And you note that "Currently positive tissue controls are placed on every patient tested slide." And that was good thing?

MS. WEGRYNOWSKI:

A. Yes.

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CHAYTOR, Q.C.:

Q. And you're somewhat envious of our tissue control bank?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. "No negative controls, however, are being used." And what would a negative control show?

MS. WEGRYNOWSKI:

A. I think I explain it to you further below. It might be--no, I didn't. You want to ensure that the test is not--is giving you reliable results, that there's no cross reaction, so you would use, like, for example, if we were using monoclonal antibodies and we use a universal mouse control which is a compilation all sorts of G markers that we buy commercially. We do that to ensure that the only marking that we are getting from using all the pretreatments and all the detection systems is actually the antibody.

CHAYTOR, Q.C.:

Q. Okay. And you note again, "No daily assessment and documentation of the controls

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is performed in the IHC laboratory. Without assessing the controls, no troubleshooting of the procedure is occuring. Presently the onus is on the individual pathologist to assess the control tissue with the patient sample. No documentation from the pathologist was found." What documentation would you have expected to see from the pathologists?

MS. WEGRYNOWSKI:

A. Well, I would expect it somewhere to see that what they were doing that day that the controls were a pass.

CHAYTOR, Q.C.:

Q. Okay. So was there any indication or any documentation to show that controls were being looked at and signed off?

MS. WEGRYNOWSKI:

A. I never found that information.

CHAYTOR, Q.C.:

Q. And again then your recommendations flowing from that are to run the negative reagent controls on every block of patient tissue?

MS. WEGRYNOWSKI:

A. Um-hm.

CHAYTOR, Q.C.:

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Q. "Negative tissue control slides to be run for each antibody." And "IHC registered technologist to be trained to interpret and make an assessment of the quality and specificity of staining for every positive and negative control daily tested. Signed documentation of this assessment must be maintained. Also, a review of tissue recognition would be an asset." So what you're suggesting here about the technologists being able to interpret and read, those are the external controls, I take it?

MS. WEGRYNOWSKI:

A. At least to understand that the test was a pass or a failure. They were very far from that point, but that would be certainly something that you could put ahead as a goal in the future.

CHAYTOR, Q.C.:

Q. Okay. So they were far from being to the point to be able to do that?

MS. WEGRYNOWSKI:

A. There were far more basic things that I felt that needed to be taken care of, but I included this in the report as a

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recommendation, yes.

CHAYTOR, Q.C.:

Q. For down the road?

MS. WEGRYNOWSKI:

A. Absolutely.

CHAYTOR, Q.C.:

Q. To be able to get them to the point where they'd be able to interpret whether or not the control had worked?

MS. WEGRYNOWSKI:

A. Correct.

CHAYTOR, Q.C.:

Q. Okay. And that's something that your technologists at Mount Sinai have been doing for quite some time?

MS. WEGRYNOWSKI:

A. Yes. And I tried to elaborate that with the sentence, "Without assessing the controls, no troubleshooting of the procedure is occuring."

CHAYTOR, Q.C.:

Q. Okay. So if I could understand it, the external controls were not being read by the technologists, then they weren't trained to do that so they weren't being read, and then where the external controls went in terms of

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pathologists signing off on them, you couldn't find any documentation to substantiate that had happened?

MS. WEGRYNOWSKI:

A. That's right.

CHAYTOR, Q.C.:

Q. And what do you mean by "A review of tissue recognition would be an asset."?

MS. WEGRYNOWSKI:

A. Just before they start reading the controls, just to go over the architecture of the different components of the different organs so they know what they should be looking at.

CHAYTOR, Q.C.:

Q. Okay. And then page 16, if there's nothing else there. Is there anything else there, Ms. Wegrynowski?

MS. WEGRYNOWSKI:

A. No.

CHAYTOR, Q.C.:

Q. "Surgical Reports" then. And you've indicated, "Presently each site had their own unique report headings on the surgical pathology reports. Also, a standard reporting format was not identified." Why would a

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standard reporting format be of any benefit?

MS. WEGRYNOWSKI:

A. Standard reporting is of benefit so that all people have the same information, so that the pathologists input the information in the same manner and all clinicians can interpret it in the same manner.

CHAYTOR, Q.C.:

Q. Okay. So everyone would use the same terminology and it would appear in the same place on the -

MS. WEGRYNOWSKI:

A. On the reports.

CHAYTOR, Q.C.:

Q. - reports?

MS. WEGRYNOWSKI:

A. Absolutely.

CHAYTOR, Q.C.:

Q. And the Commissioner--there's been some indication and they'll certainly be more said about in terms of trying to identify the patients in this case, and in terms of the fact that there wasn't a lot of standard reporting. So I take it if that were to happen and that there was a standardized

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report format, that shouldn't be as big an issue should issues arise down the road?

MS. WEGRYNOWSKI:

A. Correct.

CHAYTOR, Q.C.:

Q. So if you were to go back and do something like was done here and being able to identify which patients need to be retested, for example, if you're standardized in your reporting, that should certainly make it easier?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Okay. And you have three recommendations flowing out of that. And you talk about standard report headings and also "Standard reporting for all predictive prognostic information." And "Investigate synoptic reporting capability with Meditech Medical Information Technology Inc." What do you mean by that, reporting, standard reporting for predictive and prognostic information, can you give us an example?

MS. WEGRYNOWSKI:

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A. So that you would want to see on the report what the ER or PR was, you'd like to know what the scoring method is below that, it's that sort of thing, so that the clinician would know, by looking at it, whether it was positive, negative, what the rates were and how you came up with those rates, what your scoring basis were.

CHAYTOR, Q.C.:

Q. Okay. So instead of just using words "positive" or "negative" using actual percentages, for example, for the ER/PR test and having everyone report in the same manner?

MS. WEGRYNOWSKI:

A. And definitely what, and what scoring method you used, absolutely.

CHAYTOR, Q.C.:

Q. Was there any issue as to words used like the word "diagnosis" versus the word "interpretation" anything like that?

MS. WEGRYNOWSKI:

A. I don't recall.

CHAYTOR, Q.C.:

Q. "All IHC tests providing predictive prognostic information must include information on the

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patient record on specimen processing, antibody clone and the scoring method used."

MS. WEGRYNOWSKI:

A. Yeah.

CHAYTOR, Q.C.:

Q. So this information would actually show up on the patient's chart?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Okay. So, for example, for an ER/PR test what would we expect to see on the patient's chart?

MS. WEGRYNOWSKI:

A. Oh, you could see, I would say, 6F11, ER 6F11, which would be the clone. They would say, I think what ours say, they'll have a percentage point and then underneath it you'll see the Allred's scoring pattern.

CHAYTOR, Q.C.:

Q. Is there anything else there?

MS. WEGRYNOWSKI:

A. Synoptic reporting makes it really easy because it's almost like a Can Tac (phonetic), so they can just go in and plunk in what they need.

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CHAYTOR, Q.C.:

Q. Okay. Anything else along that then?

MS. WEGRYNOWSKI:

A. No.

CHAYTOR, Q.C.:

Q. Okay. And then "Quality Assurance" "Current neither external quality assurance nor interlaboratory comparisons, excluding the retrospective analysis which was taking place is performed. No documentation was seen concerning internal quality assurance for both the technical and professional components." So I take it what you're saying here is that there wasn't any external nor internal laboratory QA taking place and there was no documentation concerning what, if any, internal quality assurance was taking place?

MS. WEGRYNOWSKI:

A. That is correct.

CHAYTOR, Q.C.:

Q. And you say "You need to ensure the quality of your laboratory's results to determine the accuracy and reliability of the procedure. There must also be a mechanism to evaluate the interobserver variability amongst all the

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pathologists' interpretation of IHC staining."
And can you give us an example of that, such as what, what would such a mechanism be?

MS. WEGRYNOWSKI:

A. You could have a group of pathologists that are reading a particular type of marker all sit down and give them the slides and they would sit down and everybody would grade them and then collectively look together to ensure that they're in line with each other.

CHAYTOR, Q.C.:

Q. Okay. And is that the kind of thing that you understand happens at Mount Sinai?

MS. WEGRYNOWSKI:

A. Yes, you can speak to Frances and Brendan about that.

CHAYTOR, Q.C.:

Q. Thank you. And again then your recommendations, these were some of the recommendations for inter/intra laboratory comparison and for external -

MS. WEGRYNOWSKI:

A. Yeah.

CHAYTOR, Q.C.:

Q. - proficiency testing and QA. You also say,

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"It would be advantageous for the technologists to attend any medical rounds relevant to the work they are involved with to understand the larger scope of practice."
Does that happen at Mount Sinai?

MS. WEGRYNOWSKI:

A. It used to. We've gotten far too busy for that, but when we did have the opportunity, it's very valuable. I think we work behind the scenes and we very rarely get to see the patients or actually have the movement within clinicians and I think people sometimes lose sight exactly what it is that they're doing and the extent of what they're doing and how it can truly impact. So I think sometimes people, if they have an opportunity to go to rounds, they have a chance to step back and actually listen to some of the case histories and put people behind it and see how their impact goes. I mean, it's not something that we can obviously all go to medical rounds, but if the opportunity arises, I think it's something that is of great value for all members of the health care team.

CHAYTOR, Q.C.:

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Q. And when you have new technologists coming on, is that something that happens at Mount Sinai?

MS. WEGRYNOWSKI:

A. We've tried very hard. We've not always been successful, but we have tried. But if they go, they are always welcome.

CHAYTOR, Q.C.:

Q. And then under miscellaneous, you mentioned the competency testing and presently, no documentation exists relating to the training of staff to perform their assigned duties. Competency assessment for all staff is not performed in the pathology division, and the pathology manager has begun to investigate this.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And your recommendations are that staff must be provided with training and given a documented performance appraisal prior to working without supervision. Staff to be assessed for competence to perform tasks following training, identify requirements of a task, perform gap analysis and develop action

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plans.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. So were you suggesting that that needed to happen before--with the current staff or is this new staff coming on?

MS. WEGRYNOWSKI:

A. Anyone, because there was nothing there. You wanted to ensure that people knew what they were doing. It's for their own safety as well as the patient's safety. These are patient safety issues.

CHAYTOR, Q.C.:

Q. And what's gap analysis?

MS. WEGRYNOWSKI:

A. What you can do and what we need you to do and how do we get you there. We're going to develop an action plan to get you there.

CHAYTOR, Q.C.:

Q. Okay, and then we see reference again to the textbook and recommending internet and textbooks.

MS. WEGRYNOWSKI:

A. Yes.

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CHAYTOR, Q.C.:
Q. And continuing education conferences, and you indicate funding to be available for AIHC technologists to attend the NSH convention. What's the NSH convention?
MS. WEGRYNOWSKI:
A. The National Society of Histotechnology. It's an American organization and in my opinion, one of the very best in North America. It's where there's the largest group of histotechnologists and so there's a tremendous lecturing from all sorts of different people to do with not only histology, but with immunohistochemistry as well.
CHAYTOR, Q.C.:
Q. Okay, and then equipment placement, you indicated you recommended to relocate the automated special staining equipment, and why was that?
MS. WEGRYNOWSKI:
A. It was just--it was by something else and you don't want it to be disturbed. It's just very simple.
CHAYTOR, Q.C.:
Q. And there's a safety issue about acid storage?

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MS. WEGRYNOWSKI:
A. Yeah. The histocolometer wouldn't work for them simply because of the type of microtome that it was, but it certainly was meant to highlight that if the microtomist does not align and orient the block for recutting the sections in immunohistochemistry, there is always that possibility that if it's a small foci of tumor that it can be cut through.
CHAYTOR, Q.C.:
Q. Okay, and then the next page is a list of resources, and then your conclusion, and again, your conclusion, you talk about the rotating pathology assistants providing the grossing of large specimen would standardize the procedures between the two sites. Standardizing fixation time of every specimen will have a direct correlation on the quality of IHC staining, and the consolidation of tissue processing at the Health Sciences site should be implemented. The consolidation would ensure that all specimens are processed in the same manner, and the tissue processors are maintained in the same way. Standardizing the processing will positively impact the IHC

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staining. You talk about the procedure manuals being written again, and the three non-rotating registered technologists should be employed or to be employed in IHC with one appointed a level of responsibility, and the three technologists will be responsible for all aspects of IHC from validating all elements to every staining procedures, to assessing controls and a director of IHC should be appointed, and it is essential the laboratory become involved in a peer assessment program, and I take it that's the external proficiency.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And standardization of all processes will result in increased reliability and reproducibility of the IHC results, and you indicate that they are more than capable of attaining this degree of competency.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And is there anything else then in your

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report--I'm going to take you back to the pipettes, but is there anything else that we haven't covered that you feel is important to mention?
MS. WEGRYNOWSKI:
A. No, I'm pleased with it.
CHAYTOR, Q.C.:
Q. Okay. Commissioner, Ms. Wegrynowski was going to do a very brief demonstration for us, but if we could have a quick break so she could set up for that?
THE COMMISSIONER:
Q. Actually, it's near the time of the afternoon break. Why don't we do that?
CHAYTOR, Q.C.:
Q. Okay, sure.
(RECESS)
THE COMMISSIONER:
Q. Please be seated. Ms. Chaytor.
CHAYTOR, Q.C.:
Q. Thank you, Commissioner. Commissioner, we have a new exhibit please. It's P-1668, if we could have that entered. I'm sorry, 1768, thank you, Mr. Coffey.
THE COMMISSIONER:

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Q. They just slip up there so easily, don't they? 1768?

CHAYTOR, Q.C.:

Q. That's correct.

THE COMMISSIONER:

Q. All right, entered.

EXHIBIT ENTERED AND MARKED EXHIBIT P-1768

CHAYTOR, Q.C.:

Q. Thank you. Okay, Ms. Wegrynowski, if we could go back, please, Registrar, to P-0047, page 11? And number 17, your 17th recommendation was "guarantee pipette and temperature accuracy and calibration. The accuracy of the volume of the pipette delivers must be guarded to ensure the reproducibility of the antibody dilutions. A standard thermometric device or reference thermometer is to be available to check thermometers used on all temperature controlled instruments. Digital temperature readings do not suffice and thermometer readings are to be recorded." Perhaps then you could take it from there and tell us why this is of importance?

MS. WEGRYNOWSKI:

A. I'm sorry, which part of--the thermometers?

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CHAYTOR, Q.C.:

Q. Yes, about the accuracy of the volume of the pipette delivers must be guarded to ensure the reproducibility.

MS. WEGRYNOWSKI:

A. Okay. This is just a little example. I think--it's difficult to ascertain how small some of these volumes are, so it was suggested that perhaps that I bring a sample with me to show you. So this--I'm using it from a DAKO autostainer system because that's what we have at Mount Sinai and this is what was in place at Eastern Health prior to the Ventana Benchmark. I don't know if you can see, but this was the vial that they would have used, and in this vial, I have placed one ml of water. This actually would be enough to stain four slides on the autostainer, so you start understanding really how small our volumes are.

So to that, I'm going to make you 1 and 100 dilution. I'm afraid I forgot the correct tips for my other pipette, but this gives you a little bit of an idea. So this is ten microlitres and that's exactly what it looks like. So if you were to dispense that in here, it's such a small volume, I think you start understanding that if the calibration of your pipettes are not maintained that the volumes would begin to wane, that you would never have the accurate dilution that you required.

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You can also see that on the exhibit they're showing you or told you exactly what it was sent when it was from Rainin, what the volume settings were and what the expectations are. Our pipettes are guarded for that. If our pipettes are ever dropped, they certainly are--we call in maintenance right away on them. These are like our scalpel blades. This is everything that we do every day. If this falls and even though it may be saying ten and it's never been calibrated, you have never had that assurance. So I thought it was well worth bringing this today, Madam Commissioner.

THE COMMISSIONER:

Q. So that there is a special person who comes to do this?

MS. WEGRYNOWSKI:

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A. Yes, they have a company that comes in to do this for us and they calibrate the pipettes and it's supposed to be done a minimum of twice a year, but we get them done, I would say easily three to four times a year, simply because of the volume that we do.

THE COMMISSIONER:

Q. So it would be three to four times a year, and if you are concerned that one isn't working or has been treated in a way that might interfere with the calibration, you would get the person to do it again?

MS. WEGRYNOWSKI:

A. Absolutely, and it's because those volumes are so small.

CHAYTOR, Q.C.:

Q. And the amount that you're showing there is a larger amount than what you would be dealing with with some pipettes?

MS. WEGRYNOWSKI:

A. Absolutely. There are some pipettes, there are some dilutions, we're only talking about two microlitres, which is a fifth of the volume that I just showed you.

CHAYTOR, Q.C.:

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Q. And I'm just wondering, Commissioner, if you were able to see that or if we could -

THE COMMISSIONER:

Q. Actually, I could see it. I don't know if anybody else in the room could, but I could.

CHAYTOR, Q.C.:

Q. Did you want to do it again and perhaps you could just show people in the room the amount that you're talking about?

MS. WEGRYNOWSKI:

A. Okay, I can do that for you. So that's what ten microlitres would look like. So you can see that it's a green diluent, but then when you actually dilute it out, you would see absolutely nothing in it. It's really a small amount, so this is 1 to 100 dilution which is certainly not anything unusual in immunohistochemistry, so we're really talking little tiny amounts. Anybody want to see this? So it really gets small. So when you start taking it down into smaller volumes, you understand that the accuracy must be in place. When you dilute it out, there's nothing. You don't see anything.

CHAYTOR, Q.C.:

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Q. Thank you, Ms. Wegrynowski.

MS. WEGRYNOWSKI:

A. You're welcome.

CHAYTOR, Q.C.:

Q. Could you just tell us where, in doing the ER/PR test, when--at what stage are you using your pipette?

MS. WEGRYNOWSKI:

A. The pipettes are used primarily to make up the primary antibody. So the dilution that is required has been considered at validation. It's not unusual to have dilutions go from 1 in 20 up to 1 in 2-3,000. So we use them to make up our primaries. So some of our secondary and tertiary antibodies, we use them as well, but over 90 percent of it is all done for the primary antibodies.

CHAYTOR, Q.C.:

Q. Okay, and I believe that pipette that you showed us is probably five times more than the one you actually showed us in Toronto that day.

MS. WEGRYNOWSKI:

A. Yeah, this is it, because this is ten microlitres and what I showed you was two

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microlitres. Yes, it is.

CHAYTOR, Q.C.:

Q. Okay, and if we could look then at 1768? This is the latest exhibit, and could you just explain, please, for us, what this document is?

MS. WEGRYNOWSKI:

A. This is just what comes when you purchase your pipettes. So that--I'm not fluent on this, I have to admit, but it tells you what your useful volume range is. It should only be used between 100 microlitres and a thousand microlitres, which would explain why we have a wide variety of pipette measurements, so that the accuracy, that we're guarding against it, that we're using it in the actual spectrum that it should be used in. It tells you exactly what kind of pipette tip that you should be using and we only buy sterilized pipette tips so that we don't bring any contaminant into our primary antibody. It'll then tell you what your balance is and your sensitivity, your correction factor. It tells you a little bit about the weighings, what they're getting, and so once that's done, it's

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also ISO standardized. The warranty can get sent off, and then, as I say, we have our pipettes calibrated at a minimum of twice a year.

If there's any errors with these, then the company will either fix it or if it's beyond fix, they'll tell us to replace, and then the company that I use, they come on site and then on my pipette, it'll have the serial number and the date that they did it. This particular pipette was done February 6th of this year, and I need to have it re-calibrated by August 6th.

CHAYTOR, Q.C.:

Q. Okay, and I take it there's no guarantee otherwise that ten millilitres or whatever it is, is in fact ten millilitres?

MS. WEGRYNOWSKI:

A. That's right. That's right, so they have to be--and that's one of the things when you work in the area every day, you start getting a sense just visually of what they should look like. So that's another indicator as well.

CHAYTOR, Q.C.:

Q. Okay, thank you.

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MS. WEGRYNOWSKI:
A. You're welcome.
CHAYTOR, Q.C.:
Q. Commissioner, if we could take another quick break, so we can set up again quickly?
THE COMMISSIONER:
Q. Yes, of course.
CHAYTOR, Q.C.:
Q. Thank you.
(RECESS)
THE COMMISSIONER:
Q. Please be seated. Ms. Chaytor.
CHAYTOR, Q.C.:
Q. Thank you, Commissioner, and thank you, Ms. Wegrynowski, for the demonstration. So November 9th, 2005, your report was--you sent it on and submitted it to Eastern Health.
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. Did you receive any feedback after submitting your report?
MS. WEGRYNOWSKI:
A. Just a letter from Dr. Williams thanking me to come.

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CHAYTOR, Q.C.:
Q. And if we could look at, please, P-1331? And this is a letter here, private and confidential to yourself from Dr. Williams dated November 23rd, 2005. "I want to thank you very much for coming to our province and doing a review of the immunohistochemistry lab operated by Eastern Health. I want to thank you for the detailed and comprehensive report that you have provided to us. You can be assured that your recommendations will be used as a template as we move forward to improve the immunohistochemistry services that we offer. We intend to achieve the degree of competency that you have laid out in the recommendations. Again, thank you very much for your assistance. I'm sure Mr. Gulliver or Dr. Cook may wish to contact you further to get ongoing advice as we move forward in implementing the recommendations in your report."
Were you ever contacted by Mr. Gulliver or Dr. Cook in that regard?
MS. WEGRYNOWSKI:
A. No.

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CHAYTOR, Q.C.:
Q. If we could look at P-1330 please? And this is a letter, the same date, and private and confidential to Dr. Cook and to Mr. Terry Gulliver. "Dear Don and Terry, I have had an opportunity to review in detail the reports of Dr. Diponkar Banerjee and Ms. Trish Wegrynowski with respect to the immunohistochemistry services offered by Eastern Health. I wonder if you could prepare a spreadsheet to capture all the recommendations embodied in both those reports, i.e. this should include all recommendations, even ones such as refrigeration storage referenced on page four of Trish Wegrynowski's report, in preparing the spreadsheet and the current status with respect to implementation of these recommendations. You should assume that funding will be provided, based on the document you prepared for me on October 13th, 2005. Once you have the spreadsheet developed and the current status of our implementation, I would like to meet with you as soon as possible to review where we go from here,

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especially in dealing with the institution of the immunohistochemistry services."
So that's correspondence then from Dr. Williams to both Dr. Cook and Mr. Gulliver indicating that a spreadsheet would be prepared with recommendations, including all of your recommendations.
MS. WEGRYNOWSKI:
A. Okay.
CHAYTOR, Q.C.:
Q. We know that, of course, as the fall went on at Mount Sinai and actually this letter I note has a--the date is wrong, but we understand this should be November 23rd, 2005, we understand to be the date on that letter. As the fall went on, of course, the retests were taking place at Mount Sinai. Were you involved in that process at all?
MS. WEGRYNOWSKI:
A. No, I was not.
CHAYTOR, Q.C.:
Q. And if we could look at P-1739, please, Registrar? And these again are handwritten notes of Dr. Cook and I just bring this to your attention because on October 13th, 2005,

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he has a note "Spoke to Trish Wegrynowski regarding immuno"--looks like immuno machine. "She said machine should be up and running soon. No delays in consultations, expects some delays in retro cases." Do you recall having that discussion or what that's about?

MS. WEGRYNOWSKI:

A. No, it doesn't ring a bell.

CHAYTOR, Q.C.:

Q. Okay, were you involved in the ongoing consultations for ER/PR for Newfoundland?

MS. WEGRYNOWSKI:

A. I do the forward going work, yes.

CHAYTOR, Q.C.:

Q. Okay, so the work that was ongoing, the current cases as such -

MS. WEGRYNOWSKI:

A. Uh-hm.

CHAYTOR, Q.C.:

Q. You were involved in doing those.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And were you also involved in any, from the retests that were sent up as consultations?

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MS. WEGRYNOWSKI:

A. No.

CHAYTOR, Q.C.:

Q. And so you don't recall having this discussion with Dr. Cook?

MS. WEGRYNOWSKI:

A. No, I don't actually.

CHAYTOR, Q.C.:

Q. And the idea of, then again this is October, 2005, machine should be up and running soon. Were you having difficulties with your equipment at Mount Sinai?

MS. WEGRYNOWSKI:

A. I mean, anything is possible, I would have to go back and check my service records at home to determine whether or not that is so the case.

CHAYTOR, Q.C.:

Q. But this doesn't ring any bells to you at all?

MS. WEGRYNOWSKI:

A. No, I mean, I think when they first took on some of the retro work, this is something from memory, I think they were using the auto-stainers that were in the service laboratory, so there could have been--there was one that

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did have some concerns that we took down, so unless--that's the only thing I can think of, but again, I would have to check the service records.

CHAYTOR, Q.C.:

Q. Okay, and if we could look at please, P-0101? And this is not a document that I expect you to be familiar with here. It's a letter to Dr. Williams, but it's dated December 11th, 2005 and it's written by Dr. Carter, who you've met, of course and she copies it to Dr. Cook, Mr. Gulliver and Dr. Ejeckam. And in this letter she writes, "I was most recently asked by Dr. Don Cook to comment on the suggestion of Mr. Barry Dyer that stated that he felt the Ventana testing for estrogen receptor, progesterone receptor and HER2/neu could be started at any time. I find this comment quite startling in the face of the two fairly damning reports sent by Dr. Banerjee and Trish Wegrynowski on their review of our immunohistochemistry laboratory, with special emphasis on the predictive factors for breast cancer patients." And she goes on to point out several things in her letter and my point

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in showing you those things would be to ask whether or not you agree with what has been highlighted by Dr. Carter. She says "Breast pathology, indeed all of anatomic pathologies are often very difficult and is subject to a myriad of influences that can lead to poor outcomes for our patients. As stated by Dr. Banerjee and Ms. Wegrynowski and vehemently supported by me, there are multiple major issues that must be addressed prior to any breast testing being reported from our immunohistochemistry laboratory. The most important of these is organization of the immunohistochemistry laboratory. Our technologists need to be dedicated to the immunohistochemistry laboratory. My most recent ER/PR/HER2/neu validation meeting with them booked in advance, required taking one technologist from the frozen section room and one from the grossing room and one of them should be deemed the charged technologist or other equal terms. These technologists need then to be educated at an acceptable training institution, not at individual local pathologist's desks, in the theory of

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immunohistochemistry. They need to learn about the pitfalls of immunohistochemistry, troubleshooting and quality control." And I take it, Ms. Wegrynowski if I could just stop there and ask you about that, is that what you were referring to as well in your report?

MS. WEGRYNOWSKI:

A. Yes, they needed to learn a lot more about the theory.

CHAYTOR, Q.C.:

Q. "This education must be documented and competency testing carried out. Textbooks and internet references need to be made available in the laboratory; further, there must be documentation of training and competency of the staff on all equipment used in the laboratory. Continuing medical education of an improved and pertinent manner must be carried out and monies provided for it. These technologists need to document the optimization and validation of every antibody, including ER/PR and HER2/neu currently in use. All validations must be documented and readily available, format available to any staff in the absence of the technologist, signed off by

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the pathologist who acts as section head or a designate and the slides kept permanently and readily available. They need to derive standard operating procedures for the staining protocols for every antibody in the laboratory and not a copy of the Ventana manual. These SOP's need to be in agreement with well-known regulatory guidelines such as CSLI. All antibody specification sheets must be available at the workbench, again in readily available format. The technologist must set up an appropriate internal and external quality assurance program and alliance with a large volume teaching hospital should be for use as an occasional quality advisor. An appropriate organizational chart must be designed whereby the immunohistochemistry technologists are professionally responsible to the section head and designate and not to the laboratory manager and laboratory director. Of course, the technologist must also be professionally responsible to them and should not look to pathologists to solely make decisions about the technical aspects of the immunohistochemistry, rather the technologists

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must act as equal partners presenting their decision to the section head for approval. And there must be a well-documented user friendly system, whereby individual pathologist's complaints and concerns are registered and a mechanism set up by which these problems are investigated. Appropriate positive and negative controls must be selected for ER/PR and HER2/neu. The immunochemistry technologists need to be trained in the basics of interpretation of ER/PR and HER2/neu, so that appropriately stained slides only leave the laboratory. The microscope needs to be placed in the laboratory for the use of the technologists. Routine equipment maintenance schedules must be set up and carried out. Some of the equipment in the immunohistochemistry lab must be moved to ensure optimal operation. I feel that all the above recommendations must be fully in place before we can began to release ER/PR/HER2/neu status reports from our lab. I am unaware that any of these have taken place. The pathologists must standardize their approach to the accessioning and gross

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handling of all breast specimens. All tissue processing must be amalgamated and standardized. Pathologists should be educated"--and I think the rest of that is more about the pathologists. "Standardized reporting of immunohistochemistry results should be used that list the clone percentage positivity in the procedure to minimize oncologist's chance of misrepresentation. Inter- and intra- pathologist variability in reporting must be assessed." So that's basically what Dr. Carter wrote and again, this is two and a half months after you were in St. John's on December 7th, 2005. So I just ask your comment on that in terms of how consistent it is with what you had pointed out?

MS. WEGRYNOWSKI:

A. I think this letter is very consistent with what I wrote in my report.

CHAYTOR, Q.C.:

Q. If we could look, please, at P-1748? And these are handwritten notes of Dr. Cook again, February 8th, 2006. And it's a meeting from 10:30 to 11:30 a.m. And before I take you to

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that, are you aware of anybody from Eastern Health going to Mount Sinai for further training?

MS. WEGRYNOWSKI:

A. Bev Carter had been out to visit Frances at a point in time, coming to the laboratory and I had shown her around. She had wanted to see the documentation that we had and she went back and Mary Butler was sent up to Mount Sinai Hospital.

CHAYTOR, Q.C.:

Q. Okay, so Dr. Carter looked around your IHC lab.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And then out of that visit to Mount Sinai came a request for Mary Butler, one of the technologists to come up?

MS. WEGRYNOWSKI:

A. Yes, Bev commented on how many different kinds of manuals we have and just how many, just how many binders we have in the place, just recording everything that's going on.

CHAYTOR, Q.C.:

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Q. Okay, and did you have any other discussion with Dr. Carter at that time?

MS. WEGRYNOWSKI:

A. I don't recall, no.

CHAYTOR, Q.C.:

Q. And so Ms. Butler then attended?

MS. WEGRYNOWSKI:

A. Uh-hm.

CHAYTOR, Q.C.:

Q. And how long did she visit with you?

MS. WEGRYNOWSKI:

A. She was, I believe on site somewhere in the two-week vicinity, but she was not with me for that entire time.

CHAYTOR, Q.C.:

Q. So she was up there, though, in Mount Sinai for about two weeks?

MS. WEGRYNOWSKI:

A. I think so.

CHAYTOR, Q.C.:

Q. And about how long did she spend in the IHC lab with you?

MS. WEGRYNOWSKI:

A. I remember a couple of days.

CHAYTOR, Q.C.:

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Q. Okay. And what kind of things did she do while she was there? Or what did you do with her?

MS. WEGRYNOWSKI:

A. From my understanding Mary was coming up to do just an observation of the immunohistochemistry laboratory, so we do have clients that do come on board to do that, they weren't coming to do competency testing or any of the sort, so when a client comes on board for observation, I'm there to serve whatever needs that they need. So whatever she needed to look at or whatever she wanted to evaluate, I would certainly deal with her, I always provide an overview of the department, just going through the processes and the procedures and all the documentation that we have just to provide a well base of knowledge.

CHAYTOR, Q.C.:

Q. So she actually observed what you did in your day-to-day job?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And did she also review your documentation on

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how you record things?

MS. WEGRYNOWSKI:

A. Yes, she did.

CHAYTOR, Q.C.:

Q. Okay, and your SOP's?

MS. WEGRYNOWSKI:

A. She had the opportunity to read our SOP's, yes.

CHAYTOR, Q.C.:

Q. Okay, and what did you understand--what was the purpose of Ms. Butler being there?

MS. WEGRYNOWSKI:

A. I would just say just to look at how the IHC lab was run at Mount Sinai Hospital.

CHAYTOR, Q.C.:

Q. And by the time that she left then there, did you have a sense that her understanding had progressed in terms of the theory and how the lab is run, how it's set up and the importance of documentation?

MS. WEGRYNOWSKI:

A. Could you rephrase that for me please?

CHAYTOR, Q.C.:

Q. Okay. I'm just wondering whether or not by the time that Ms. Butler left, had there been

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progress in terms of her understanding of the processes and the documentation that go along with the processes?

MS. WEGRYNOWSKI:

A. I wouldn't say great strides.

CHAYTOR, Q.C.:

Q. Okay, and what did you think then needed further work?

MS. WEGRYNOWSKI:

A. I had tried to sit with Mary to assist her in writing some standard operating procedures and I don't think this was what she was most comfortable doing.

CHAYTOR, Q.C.:

Q. Okay. So the actual development of the standard operating procedures, there was a level of discomfort for her in doing that?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And if we could look please at P-1766? I'll come back to this one, Registrar, so if you just want to diminish it. And this is indicated to be a Mount Sinai document, ER/PR immunohistochemistry standard operating

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procedure.

MS. WEGRYNOWSKI:

A. Uh-hm.

CHAYTOR, Q.C.:

Q. Special histology, hard tissue procedure manual. Can you identify this document for us and tell us what this is?

MS. WEGRYNOWSKI:

A. This document would be seen on the other side of the lab, this is not part of the service laboratory and it's indicated by the terminology there, the special hard tissue procedure manual, it's not the immunohistochemistry procedure manual, although it is an operating procedure for immunohistochemistry. The supervisor, the written bio dates are all present. The Table of Contents are there. The Table of Contents always must be prefaced by a flow chart in which it is.

CHAYTOR, Q.C.:

Q. So this is from the research side of the lab, is it?

MS. WEGRYNOWSKI:

A. Yes, it is.

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CHAYTOR, Q.C.:

Q. This is a sample of one of your SOP's that have been given to us, we understand.

MS. WEGRYNOWSKI:

A. Okay. So it's just walking through the process in a schematic, that you receive the slides in HNE, in the laboratory, it was run on the auto-stainer, they do separate their runs according to antibody pretreatment detection system. They run their antibodies with controls, positive for antibody, negative for each--should be each test, each case in the detection system, and then of course they collate the slides and send them on up with the HNE.

CHAYTOR, Q.C.:

Q. And so I won't take you through the whole document, but this is the type of--it's a typical, I take it, SOP, is it?

MS. WEGRYNOWSKI:

A. It's a little bit different than mine, but yes.

CHAYTOR, Q.C.:

Q. And if we could go back then please to P-1748? And by the way, were you contacted to offer

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any further training to either Ms. Butler or anyone else?

MS. WEGRYNOWSKI:

A. No, I was not.

CHAYTOR, Q.C.:

Q. And did anyone follow up with you from Eastern Health or from the laboratory to inquire how it had gone with Mary?

MS. WEGRYNOWSKI:

A. Not to my memory, no.

CHAYTOR, Q.C.:

Q. And this is the meeting of February 8th, 2006 and it appears from this meeting that Ms. Butler has already been to Mount Sinai, it's a meeting regarding an update on the implementation of ER/PR and there's a number of people present, including pathologists Cook, Ejeckam, Carter, Fontaine, Mr. Gulliver and Mr. Dyer are present, so are three techs, Mr. Simms, Mr. Green and Ms. Butler. And the meeting started with both Ken Green and Mary giving written reports of their experiences in Mount Sinai for Mary, and Montreal Jewish General for Ken. "Ken brought back information from Montreal on types of

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antibodies used and protocols. Mary brought forth the issue on QA. She brought back document on PA's, pathology assistants and fixation protocols. Mary was not allowed to bring back documents on immuno peroxidase protocols from Mount Sinai. Both agreed on the need for documentation of activities in the lab and the establishment of manuals." so it looks like she--Mary did bring back documents on pathology assistants and fixation protocols.

MS. WEGRYNOWSKI:

A. Uh-hm.

CHAYTOR, Q.C.:

Q. And this referenced that she was not allowed to bring back documents on immuno peroxidase protocols from Mount Sinai. Can you speak to that?

MS. WEGRYNOWSKI:

A. We do not give out our standard operating procedure manuals. We don't give them out to paying clients either. Protocols are needed to be developed on site by your own people. I provided Mary with an opportunity to write them. I saw with her. In the lecture that I

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provided to and I gave you this morning, there are also templates in there about the antibody data sheets and the historical data sheets. Manuals are--the whole purpose of a manual is to scribe the process at your site. What I do at my site may not necessarily to be at your site. We don't even use the same equipment. So her manuals would have to--the manuals for Eastern Health would have to be how Eastern Health worked with the equipment and worked with the actual patient samples. Your protocols would have to include that you are a multi site; we are a single site. There are many differences, but I certainly did assist her in allowing her--well I shouldn't use that term, she certainly had the opportunity to read our procedure manual and see what was required in it and I would have been certainly able there to assist it, had she asked.

CHAYTOR, Q.C.:

Q. And I take it that with those standard operating procedures, one size doesn't fit all, obviously.

MS. WEGRYNOWSKI:

A. Not at all.

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CHAYTOR, Q.C.:

Q. And it has to be then, from what you're saying, it has to be created and developed according to the variations in each lab?

MS. WEGRYNOWSKI:

A. Yes. They refer to standard operating procedures and manuals as being living breathing documents to reflect your own needs.

CHAYTOR, Q.C.:

Q. Okay. And so you provided templates and you provided other information, but the idea of giving the standard operating procedure which pertains to Mount Sinai, you didn't think that would be prudent because it might not -

MS. WEGRYNOWSKI:

A. That's not our procedure, no.

CHAYTOR, Q.C.:

Q. Okay. It's not what should or would be relevant to St. John's.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Okay. In these notes, Dr. Cook writes, "it was agreed to test 30 - 40 case concurrently with Mount Sinai and our system in respect to

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ER and PRs and also Montreal General for correlation". In getting ready for St. John's to start up again, we understand in February of 2007, so it's a year after this is actually being written, ER/PR was initiated again here in St. John's. And were you involved at all in helping them with the correlation and having -

MS. WEGRYNOWSKI:

A. No.

CHAYTOR, Q.C.:

Q. No, okay. And if that did happen, do you think you'd be aware of it, if Mount Sinai had been used to help correlate results?

MS. WEGRYNOWSKI:

A. Only if I had been involved in it. No, I was not.

CHAYTOR, Q.C.:

Q. Okay. And if we could look please at P-1753.

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. And perhaps then you could tell us, we have this two page document and it's entitled "Optimized IHC staining condition validation"

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and perhaps you can tell us what those documents are.

MS. WEGRYNOWSKI:

A. Okay. This is from the same group that made the first document. It's very similar to the template that I gave Mary and very similar to the template that I use as well. It states on it again, the antibody and what the clone is, the catalogue number, the lot numbers that's presently in use and the expiry date. It says that it's a monoclonal antibody and who manufacturers this produce. It tells us what the isotype is. They haven't gone forward to get the protein concentration, but that certainly is available if you were to go to the manufacturer. It came in a concentrated form. They've given you what the primary dilution is under the testing conditions, what they used for it. So, they tested at 1:50-1:100 and the final results was 1:75. They gave me the name of the diluent, what the pretreatments were and what the detection system and chromogens are. It tells you the technologist's name, the pathologist's name, the date and the initial date of use and when

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it was reviewed.

CHAYTOR, Q.C.:

Q. Okay. And those templates were given, you're saying, to -

MS. WEGRYNOWSKI:

A. That was in that lecture and I left it for Mary when I was here, yes, very similar, their own.

CHAYTOR, Q.C.:

Q. Okay. Now, we understand that you had a trip back in the spring then of 2006.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Perhaps you could tell us, how did that come about? Who contacted you?

MS. WEGRYNOWSKI:

A. I received a phone call from Dr. Williams asking me to come back to re-review what I had done originally.

CHAYTOR, Q.C.:

Q. Okay. And what was to be the purpose in doing a further review?

MS. WEGRYNOWSKI:

A. If I recall correctly, it was to look at what

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I had recommended and where they were coming through the process.

CHAYTOR, Q.C.:

Q. Okay. And if we could look please P-1749. And this is an e-mail exchange between Ms. Dunn, who I believe worked as an assistant for Dr. Williams, March 6, 2006 to Dr. Cook, Mr. Gulliver, Mr. Dyer and Ms. Predham and you're the subject. "I want to advise you that Trish Wegrynowski will be coming to visit us again to review our program. She will be arriving late on the evening of March 29 and staying until March 31, 2006" and he's going to follow up on that. And then a further e-mail at 3:34 p.m. on the same day, "further to my e-mail below, Terry, I wonder if you would follow up with her directly as she would like to have some information to update her on our progress prior to her arrival. I advised her that we have a spreadsheet of issues we are working on in an updated format. Once you have talked to her, they may be sufficient to help her prepare for her visit". So, this indicate that you are looking for more information or looking for some information as to where they

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were in their progress prior to arriving in St. John's.

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Did Mr. Gulliver follow up with you?

MS. WEGRYNOWSKI:

A. I can't remember who called me, but I do recall getting the spreadsheet. I know if it was by phone or e-mail, but I did have a spreadsheet prior to arriving back at St. John's.

CHAYTOR, Q.C.:

Q. Okay. And other than the spreadsheet, did you receive anything else?

MS. WEGRYNOWSKI:

A. Not that I recall.

CHAYTOR, Q.C.:

Q. Okay. And the spreadsheet that you received, do you recall did it have all of your recommendations on it?

MS. WEGRYNOWSKI:

A. No, it did not.

CHAYTOR, Q.C.:

Q. It did not. If we could look please at P-

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1757. Could we make that a little bigger, Registrar? Thank you, it's getting late in the day. And this spreadsheet is dated, updated March 10, 2006. And if we scroll down through we'll see that there's a total, I believe, of 30 recommendations on this sheet. So, there's a total of 30 recommendations and you'll see that it's recommended by either yourself and/or Dr. Banerjee. So, there's 30 recommendations here. Does this look like the spreadsheet that you were provided?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Okay. And if we could look then, please, at-- I think it's P-0050. And this is the same spreadsheet--I don't think that's the right one though. We can look at it. This one is updated; it's been updated several times. So, this one is updated April 26, 2007 and if we scroll down through we see that there are 52 recommendations on this spreadsheet. Ms. Wegrynowski, after you came back to St. John's the second time, did you have further recommendations?

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MS. WEGRYNOWSKI:

A. Yes, there was a second report generated.

CHAYTOR, Q.C.:

Q. Okay. And so there were new recommendations plus other recommendations that had originated from your first report?

MS. WEGRYNOWSKI:

A. I don't know about new recommendations, but certainly what my premise was to do was to look at what they had done and if things had been done or not done to clarify it. There may be.

CHAYTOR, Q.C.:

Q. Yes, and perhaps I'll take you through that.

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. And you may then be able to identify whether or not there, in fact, are additional recommendations.

MS. WEGRYNOWSKI:

A. Okay. And if we could look, please, at P-1751? And this is an e-mail from yourself to Dr. Williams. The subject is IHC quality review, March 23, 2006. "Dr. Williams, I will

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be arriving" and you say when you're arriving and where you're staying. "If there are any protocols or paperwork that you would like me review prior to my arrival" and you indicate that can be faxed to you. "I do ask however that you let me know prior to sending them because it's a common fax machine" and you "look forward to reviewing your laboratory again". And you're asking about snow in March in Newfoundland which I can't understand. And then Dr. Williams forwards this onto Dr. Denic, Mr. Gulliver, Mr. Dyer, "do we have anything to send to Trish. Thanks." And then Dr. Denic replies, "can we give her our spreadsheets so she will know in advance what has been accomplished? Signed, Nash". Other than the spreadsheet, did you receive any other documentation?

MS. WEGRYNOWSKI:

A. Nothing else?

CHAYTOR, Q.C.:

Q. And were you expecting or anticipating that there might be more available?

MS. WEGRYNOWSKI:

A. I was hopeful, I believe, that is why I asked

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for the protocols, if there are any protocols or paperwork for me to review because it had been several months since I had originally been there.

CHAYTOR, Q.C.:

Q. Okay. Yes, I think it's about six months later. So you are specifically asking for protocols or paperwork. So do I take it from that that you were anticipating being able to see standards of operating procedure?

MS. WEGRYNOWSKI:

A. Something, yes.

CHAYTOR, Q.C.:

Q. And do you know, did they exist at that time, six months after you had been here the first time?

MS. WEGRYNOWSKI:

A. I only found that out when I came on site.

CHAYTOR, Q.C.:

Q. And what then did you learn when you came on site?

MS. WEGRYNOWSKI:

A. They still weren't on site.

CHAYTOR, Q.C.:

Q. There was still no standards operating

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procedure?
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. When you came back in March of 2006, the end of March 2006, what specifically were you asked to do? Was it anything different than what you had been asked to do before? And were you restricted in any way?
MS. WEGRYNOWSKI:
A. I came back doing what I thought I was going to do before, but the second time that I came back to Eastern Health, I was restricted. I was only allowed to go to the immunohistochemistry department and I was not interviewing the same--not given the same latitude to interview the pathologists and to go and look at the different sites again.
CHAYTOR, Q.C.:
Q. Okay, and was any explanation given to you as to why that would be?
MS. WEGRYNOWSKI:
A. No, I didn't ask.
CHAYTOR, Q.C.:
Q. And what then, overall, and I'll take you to

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your report, but overall then, what was your impression of the progress that had been made in those six months?
MS. WEGRYNOWSKI:
A. They had certainly listened to the recommendations and that they had started to implement some of the recommendations, but they were a long, long, long way to completing them.
CHAYTOR, Q.C.:
Q. And in terms of the progress then, being a long way from where they had to go, were you surprised by that?
MS. WEGRYNOWSKI:
A. I would have thought that there would have been much more documentation in place at that point because it was six months out, and at least a start on the standard operating procedures.
CHAYTOR, Q.C.:
Q. Were there even drafts of those procedures?
MS. WEGRYNOWSKI:
A. Not that I saw.
CHAYTOR, Q.C.:
Q. Were they doing any pHing?

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MS. WEGRYNOWSKI:
A. You know, I would feel more comfortable if we went through the report. I would prefer, because I have it documented there and I don't want to work off memory, if that's at all possible?
CHAYTOR, Q.C.:
Q. Sure, okay, we can do that. And what about the issue of not having interviews with anyone, did you meet with anyone? Did you have discussions with anyone?
MS. WEGRYNOWSKI:
A. I met with the technologists, the three technologists. I met with Catherine Parnell and Barry Dyer.
CHAYTOR, Q.C.:
Q. Okay, and who did you understand Catherine Parnell to be?
MS. WEGRYNOWSKI:
A. To be taking over or to begin with the QA.
CHAYTOR, Q.C.:
Q. And did Ms. Parnell have any particular concerns or issues that she addressed with you?
MS. WEGRYNOWSKI:

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A. She had mentioned to me that prior to the amalgamation that they had been doing quality assurance and that she had been rather frustrated that it hadn't been continued on and that she was very pleased to see that it was being looked at.
CHAYTOR, Q.C.:
Q. So that prior to the amalgamation back in 1996 or the one that had just happened in 2005?
MS. WEGRYNOWSKI:
A. I don't know that.
CHAYTOR, Q.C.:
Q. Okay, and did you understand, was she a lab technologist herself?
MS. WEGRYNOWSKI:
A. Yes, she had told me that she and Barry had applied for the same position and Barry had been successful in it, so that she had had many years of experience in the area as well.
CHAYTOR, Q.C.:
Q. Okay, and which site did you understand or which hospital did she come from? Did you know that?
MS. WEGRYNOWSKI:
A. I don't recall.

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CHAYTOR, Q.C.:
Q. Okay, and when you met with Ms. Parnell, did you make any observation as to the tools or equipment that she had available to her to be able to do her job?
MS. WEGRYNOWSKI:
A. Yes, I do. She had some relic of a computer and I remember at the exit interview telling Dr. Williams that that needed to be rectified because she needed something that certainly had some capacity to hold the information that was required. I wasn't quite sure where the lines of communication went with that.
CHAYTOR, Q.C.:
Q. And your discussions then with Mr. Dyer, the second time around, what did you discuss with him?
MS. WEGRYNOWSKI:
A. It was more to do with what had happened, the advances that they had made, the recommendations that they had looked at and where they were going with that.
CHAYTOR, Q.C.:
Q. Okay, and were there any particular concerns voiced by him at that point in time?

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MS. WEGRYNOWSKI:
A. Not that I recall.
CHAYTOR, Q.C.:
Q. And your meeting then with the technologists, you were in St. John's from the 29th to the afternoon of the 31st?
MS. WEGRYNOWSKI:
A. It was a very short visit. It was like one and a half days.
CHAYTOR, Q.C.:
Q. Okay, and when did you meet with the technologists?
MS. WEGRYNOWSKI:
A. On the first day, I had--it was Barry, Catherine Parnell, and then I met with the technologists. I remember one of them taking me over to the Janeway to look at their frozen section room and we came back. I remember looking at their paperwork. They had been enrolled in the UK NEQAS. That's basically -
CHAYTOR, Q.C.:
Q. So they had been enrolled then in external proficiency?
MS. WEGRYNOWSKI:
A. Yes.

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CHAYTOR, Q.C.:
Q. So that was obviously a very positive improvement?
MS. WEGRYNOWSKI:
A. Very positive.
CHAYTOR, Q.C.:
Q. Okay, and what did you discuss with the technologists? Were those the same three technologists that you had met with the first time?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And had their jobs or their roles changed in any way?
MS. WEGRYNOWSKI:
A. Yes, they were now permanently immunohistochemistry. They had taken the antibody spec sheets out of the cupboard and put them into alphabetical order. I don't recall there being validation sheets attached to them, but they had certainly compiled them. The antibodies were at least in the refrigerator now in alphabetical order, so that they could certainly find them much

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easier. They had talked about that they were cleaning the Ventana, and we talked about firming up the documentation on that. That they need to, you know, fill it out every day that they were doing that.
CHAYTOR, Q.C.:
Q. Okay, and I take it at this point in time, they still had not resumed their ER/PR testing. So this would be with respect to other IHC testing, but you were also there to observe what was in place on a go-forward basis, presumably to get ready for re-instituting the ER/PR.
MS. WEGRYNOWSKI:
A. Okay.
CHAYTOR, Q.C.:
Q. Did you have an exit interview this time?
MS. WEGRYNOWSKI:
A. Yes, I did.
CHAYTOR, Q.C.:
Q. And that was, I think you said, with Dr. Williams?
MS. WEGRYNOWSKI:
A. Yes, it was.
CHAYTOR, Q.C.:

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Q. And what else did you discuss with Dr. Williams besides Ms. Parnell's computer?
MS. WEGRYNOWSKI:
A. We were talking about external quality assurance and we were talking about with the UK NEQAS and we were talking about with CAP, the College of American Pathologists, and I asked him if he was interested in knowing what the differences were, and he said yes, and I explained the differences of the two to him.
CHAYTOR, Q.C.:
Q. Okay, and do you recall meeting with Drs. Denic, Fontaine, Cook, Mr. Dyer, Mr. Gulliver? Do you recall -
MS. WEGRYNOWSKI:
A. Yes, I met with them prior to going to see Dr. Williams.
CHAYTOR, Q.C.:
Q. Okay. So you had a meeting with--this would be the lab people or the senior management of the lab, I take it?
MS. WEGRYNOWSKI:
A. Um-hm.
CHAYTOR, Q.C.:
Q. So you met with them and what did you discuss

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with them?
MS. WEGRYNOWSKI:
A. We were talking about just some of the recommendations that had gone on and I remember mentioning to them that there still weren't refrigerators in the quick section rooms and there was a discussion that went on why there wasn't, and they need to -
CHAYTOR, Q.C.:
Q. And what was the explanation as to why?
MS. WEGRYNOWSKI:
A. There wasn't. There just was a lot of people saying "how come they're not there?" So I don't know.
CHAYTOR, Q.C.:
Q. Okay, and did you discuss anything else with them other than the refrigerators?
MS. WEGRYNOWSKI:
A. Just going over what I was going to be saying, but nothing stands out.
CHAYTOR, Q.C.:
Q. Okay. So going over what you had observed and what you saw having--the progress had been made on?
MS. WEGRYNOWSKI:

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A. Yes, and that some -
CHAYTOR, Q.C.:
Q. And other things that still needed to be done?
MS. WEGRYNOWSKI:
A. Absolutely.
CHAYTOR, Q.C.:
Q. Okay. So basically, I take it, you reviewed with them what ultimately ended up in your second report?
MS. WEGRYNOWSKI:
A. For the most part, yes.
CHAYTOR, Q.C.:
Q. And if we could look at, please, P-1752, page 13?
MS. WEGRYNOWSKI:
A. Okay.
CHAYTOR, Q.C.:
Q. These are notes--I think these are some of your notes and it indicates at the top that--you've got Terry, Barry, Dr. Cook, Dr. Denic, Dr. Dan Fontaine.
MS. WEGRYNOWSKI:
A. Um-hm.
CHAYTOR, Q.C.:
Q. And actually, if I take you back, there's also

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reference back here to Ms. Parnell as well.
MS. WEGRYNOWSKI:
A. Okay.
CHAYTOR, Q.C.:
Q. Here we go, at page eight of the exhibit there's reference to Ms. Parnell. I don't know if there's anything there that jogs your memory. These are fairly scant notes.
MS. WEGRYNOWSKI:
A. Yes. Controls, tech to read the external controls for the day. Path to determine validity of the stain if the patient negative or not done, patient negative, slide designated patient negative. Yeah.
CHAYTOR, Q.C.:
Q. Okay, so these are things that you were reviewing again on your second trip, I take it?
MS. WEGRYNOWSKI:
A. Uh-hm. Processing at St. Clare, all processing at--yeah, the cutting was going on there, the slides and block storage, three month there and then going back to St. Clare's for storage. They had an enhanced courier system, they had a stacked courier system

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coming four times a day. They said they had documentation for the courier. Surgical reports, they wanted to use Eastern Health standard. They still weren't amalgamated. Interpretation and diagnosis, one side wanted one and one side, the other. Even in the Ventana handout themselves, I showed them that they too talk about using the negative and positive controls and that they were available at the universal controls from that particular company, that they could purchase. I believe this is from their NEQAS, their antigen was assessed, the number of participating labs, the sections circulated, assessment of slides, score staining patterns, features optimal slide sub-groups, yes.

CHAYTOR, Q.C.:

Q. Okay, and then this was the page I originally brought you to.

MS. WEGRYNOWSKI:

A. Okay.

CHAYTOR, Q.C.:

Q. Okay, and it says I believe "Control bank IHC beginning with documented."

MS. WEGRYNOWSKI:

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A. Yeah, so they were already starting to get together different, for some controls for IHC, you're required diseased processes to be in place, so they were getting that control bank established so that they would always have a wealth of tissue. They had involved themselves in UK and NEQAS and that there was a journal, so it was assessed, the number of labs that were participating, assessment of the staining, so we had an in-house tumors, there was an introduction guidelines used in the validation. The score staining pattern, features of--the NEQAS would tell them what the features of the optimal staining was, features of sub-optimal, importance of good fixation, antigen retrieval and the conclusion from the data, there would have been references.

CHAYTOR, Q.C.:

Q. And did you understand they're participating in UK NEQAS, were they actually running ER/PR tests?

MS. WEGRYNOWSKI:

A. I don't recall what it was on.

CHAYTOR, Q.C.:

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Q. Okay.

MS. WEGRYNOWSKI:

A. This would have been taken right out of their handbook, out of NEQAS' handbook, so the primary antibody, the list of clones, the number of participants, suppliers, yeah.

CHAYTOR, Q.C.:

Q. So these would have been the main topics discussed with the doctors and Mr. Gulliver and Mr. Dyer that day, I take it?

MS. WEGRYNOWSKI:

A. Yeah, uh-hm.

CHAYTOR, Q.C.:

Q. So the concentration being on the external proficiency?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. And again your discussion that you had in your exit interview with Dr. Williams there was discussion about which of the two programs that you preferred or the differences between the two programs.

MS. WEGRYNOWSKI:

A. The differences between them, yes.

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CHAYTOR, Q.C.:

Q. Is there anything else then that you recall about your visit to St. John's or will we--I can take you right to your report?

MS. WEGRYNOWSKI:

A. I don't recall anything in addition to this.

CHAYTOR, Q.C.:

Q. Okay, if we could look then, please, at P-0048? And you sent along four copies of your report to the leadership team again. And your report this time is dated May 2nd, 2006. And you indicate under your executive summary, "In September, 2005, the pathology laboratory's practices and procedures were evaluated. The inconsistencies identified in immunohistochemistry and the total lack of standard operating procedures throughout the entire pathology laboratory for both the technical and professional staff, directly contributed to the lack of reproducibility associated with the ER/PR receptor status." So that's similar to the conclusion that you drew from your first report as well?

MS. WEGRYNOWSKI:

A. Right.

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CHAYTOR, Q.C.:
Q. And then under "Background" you indicate that "two of the registered technologists assigned to the immunohistochemistry service had been sent to other institutions to view their processes."
MS. WEGRYNOWSKI:
A. Uh-hm.
CHAYTOR, Q.C.:
Q. And that's referenced, I take it, to Mary having come to Mount Sinai and Ken having gone to Jewish General. "A reassessment of the immunohistochemistry laboratory occurred from March 30th to 31st at the request of Dr. Williams and a recommendation spreadsheet was reviewed prior to." And it was the one compiled December 16th, 2005, the updated version I showed you was March 10th, 2006, so that appears to be the document you would have had with thirty recommendations?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. And your objective this time around was to evaluate the current practices and procedure,

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comment on the progress of the immunohistochemistry laboratory and provide recommendations. And your scope this time, as you have indicated, "focus on immunohistochemistry laboratory, in particular in pathology in general." Ms. Wegrynowski, if you had--if you could have done what you deemed appropriate or necessary to do, what would you have done when you came back to St. John's the second time round?
MS. WEGRYNOWSKI:
A. I would have looked at the process in the beginning as I did the previous visit. I would have looked to see whether or not there was standard operating procedures for their specimens. I would have looked at their standard operating procedures for the fixation and as well, as for the processing. I would have gone right back down to the basics to make sure that those corner stones were in place before we moved on with immunohistochemistry.
CHAYTOR, Q.C.:
Q. And the methodology, you've indicated that "the immunohistochemistry laboratory

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technologist's processes were assessed in that time period. The process assessment was evaluated by interviews, the review of written material and observation. A brief discussion also took place with Mr. Barry Dyer and Ms. Catherine Parnell."
MS. WEGRYNOWSKI:
A. Uh-hm.
CHAYTOR, Q.C.:
Q. What written material, other than the spreadsheet, what other written material did you review?
MS. WEGRYNOWSKI:
A. It would have been definitely the UK NEQAS, back that they sent me back.
CHAYTOR, Q.C.:
Q. Okay, so the results of the UK NEQAS.
MS. WEGRYNOWSKI:
A. Yes, that I definitely recall.
CHAYTOR, Q.C.:
Q. Okay, and do you recall reviewing any other documentation?
MS. WEGRYNOWSKI:
A. Not at this point in time.
CHAYTOR, Q.C.:

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Q. And are there any other pertinent observations that you made?
MS. WEGRYNOWSKI:
A. No.
CHAYTOR, Q.C.:
Q. And I've already asked you about your discussions with Ms. Parnell and Mr. Dyer. "The histology laboratory fixation and grossing, the fixation and grossing of the surgical pathology specimen has remained site dependent." So I take it that's St. Clare's and the Health Science are still doing things independently?
MS. WEGRYNOWSKI:
A. Independently.
CHAYTOR, Q.C.:
Q. "The majority of the breast cases still originate from St. Clare's site, this review did not include a visit to St. Clare's site and did not review any processes or documentation associated with the fixation or specimens at either site." And again that was because you were restricted in what, the mandate that you were given?
MS. WEGRYNOWSKI:

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A. Correct.
CHAYTOR, Q.C.:
Q. "Frozen sections continue to be performed at both sites. I had an opportunity to inspect the quick section room at Health Science Centre. Refrigeration storage is still not available for large or own fixed specimens." And again you indicated there was some discussion in your meeting with the doctors around that issue?
MS. WEGRYNOWSKI:
A. Yes.
CHAYTOR, Q.C.:
Q. "Four pathology assistant positions have been recently created. This shall provide an opportunity to ensure that all fixation and grossing procedures at both sites are consistent and standardized. Very informal protocols and documentation exist. Standard operating procedure manuals do not exist." And then your recommendation regarding that, number one recommendation is the same as previous.
MS. WEGRYNOWSKI:
A. Absolutely.

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CHAYTOR, Q.C.:
Q. You're again recommending the standard operating procedures. Number two, regarding the pathology assistants, I take it this is a new recommendation now that they've created those positions and you're indicating that documentation must also exist as to the performance of the PA's and they are to be evaluated by the pathologists. Is that what happens in Mount Sinai, the PA's are evaluated by the pathologists?
MS. WEGRYNOWSKI:
A. Absolutely because every morning there are rounds, so the pathologists go in to visit with the pathologist assistants and Brendan Mullen would probably be the better person to ask about that.
CHAYTOR, Q.C.:
Q. And again, number three, is a refrigerator in the operating room quick section at both sites.
MS. WEGRYNOWSKI:
A. That's right.
CHAYTOR, Q.C.:
Q. And that, you indicate has not been addressed

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since the assessment. And you're suggesting a time stamp for the receipt of specimens from the courier to avoid any unforeseen circumstances and I believe that might be a new recommendation. And if we go on to the processing section of your report, "the tissue processors are now all located at the Health Science Centre." So just explain that to us, so what exactly is happening now in terms of how the processing was taking place?
MS. WEGRYNOWSKI:
A. So if the grossing was being done at site dependents, the actual blocks were being put on a processor at one particular site. So however they got from St. Clare's, I didn't view this, so I don't know, but they would have needed to come to the Health Sciences Centre and then all the blocks would have been put on the same tissue processor at the same time and handled in the same way, and the blocks created from that.
CHAYTOR, Q.C.:
Q. And you go on to say that "Procedure manuals detailing standard operating procedures for the tissue processors do not exist. I did not

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have an opportunity to review the processing area, but was informed that temperature monitoring and maintenance documentation has begun. The St. Clare's site has five pathologists who assess and evaluate the surgical breast samples. All paraffin embedded blocks are kept on the site at Health Science Centre for three months and subsequently stored at St. Clare's." And then your recommendation is previous, "Standard operating procedures are to be written for the tissue processors, also written for the embedding centres and embedding protocols for all tissue types and procedures for the handling of suboptimal specimens must be developed and documented." And in the immunohistochemistry lab under "IHC Staffing" you note that "This section is now staffed with three registered technologists dedicated to perform the staining optimization and validation." And "The staff have also absorbed the"--help me with that word?
MS. WEGRYNOWSKI:
A. "Microtomy"
CHAYTOR, Q.C.:

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Q. Thank you, "function. One of the technologists has been given the lead position." So all that, I take it, was good news and that's what you had recommended the first time down?

MS. WEGRYNOWSKI:

A. Yes.

CHAYTOR, Q.C.:

Q. Okay. And "A medical section head has been established for the immunohistochemistry laboratory." And some note there that they may need to be looking at a successor. You indicate, "Lack of communication between technical and professional staff still remains an issue, for example, recently the marker Low Molecular Weight Keratin was added to all sentinel lymph node biopsy protocols. The immunohistochemistry laboratory was not apprised of this prior to implementation. This has a direct impact on the laboratory, not only regarding the amount of primarily antibody and detection system required for staining but on the workload in general." Ms. Wegrynowski, what was that all about?

MS. WEGRYNOWSKI:

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A. They had just mentioned to me that they were still a little bit frustrated because they weren't sure where the lines of communication were coming. So you find out, I can't recall how they found out, but perhaps secondhand, that this was going to be a change. This change should have been--had there been a procedure manual, it would have been documented in that, as well. They have to ensure that they have enough product on hand to be able to maintain a fluid end product, so they would want to ensure that they either had enough lots in or whatever for their validation process as well, and it's going to impact their workload.

CHAYTOR, Q.C.:

Q. Okay. And these recommendations then about the lines of communication, of course, are carried forward. You certainly had mentioned this in your previous report. In terms of-- and you describe briefly for us, though, a little while ago about the lines of communication in Mount Sinai.

MS. WEGRYNOWSKI:

A. Um-hm.

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CHAYTOR, Q.C.:

Q. And how the pathologists and the technologists interact there. In terms of any new--anything new happening within the laboratory, what kind of consultation process takes place?

MS. WEGRYNOWSKI:

A. At Eastern Health?

CHAYTOR, Q.C.:

Q. No, at Mount Sinai.

MS. WEGRYNOWSKI:

A. At Mount Sinai. In respect to what?

CHAYTOR, Q.C.:

Q. Oh, for example, if a situation such as what you've described here were to arise, how are decisions made, what consultation -

MS. WEGRYNOWSKI:

A. Oh, if somebody was going to add, so if something was going to be added to one of the programs, the pathologist that was responsible for that program, let's say, for example, somebody was to come up with a protocol for a specific tumor, they would come and they would state that to us. We would make sure that we had all the product that they needed. They would either send up an e-mail to all their

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contemporaries or they would go through the medical section head. It's very fluid.

CHAYTOR, Q.C.:

Q. Okay. If you, at Mount Sinai if you encounter a problem with a block, for example, we discussed earlier today the issues around fixation and if you encounter a problem, what kind of communication can you have with the pathologists?

MS. WEGRYNOWSKI:

A. Okay. If we receive a block, you have to understand that more than half the work that we do at Mount Sinai presently is consult blocks, so we would be speaking about a block from the outside, we have a particular sheet that we've developed called a "Client Satisfaction Form", but what it truly is is a mechanism for the medical laboratory technologists to inform the pathologist of any concerns that they have with a particular block. So on it would be the outside case number, what their case number would be, and what the issue would be. So for example, if we got something in and it was far too small and we were concerned that we wouldn't be able

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to get enough product out of it, we would send that along, if there was staples in the block, if there anything absolutely wrong with it. One of the reasons that we do this is that as professionals we want to ensure that the pathologist recognizes that the product that they're getting may not be of the best standard. We have received blocks from all over the places and sometimes, you know, they get left out in the heat, so we get a block that is completely deformed because it's been mounted. We're not sure what that is going to do to the actual antigen. So what we will do is then we will then go ahead, we will do what we need to do, we'll melt it down, but we will give that direct information to the pathologist on this form. When the case gets signed out, we give it to the pathologist so that they have a heads up prior to reading it and then what they do, they should do, is they sign it back and then we maintain all that information in our laboratory.

CHAYTOR, Q.C.:

Q. Okay. And then you have written documentation outlining what the problem was with respect to

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any particular block?

MS. WEGRYNOWSKI:

A. That's correct.

CHAYTOR, Q.C.:

Q. Okay. And how long has Mount Sinai, how long has that been a practice?

MS. WEGRYNOWSKI:

A. Oh, we've been doing that for a number of years now.

CHAYTOR, Q.C.:

Q. Okay. And is that also done internally, the Client Satisfaction Form, is that also done if there's a problem with a block internal to Mount Sinai?

MS. WEGRYNOWSKI:

A. Yes, we would do that, as well.

CHAYTOR, Q.C.:

Q. Okay.

COMMISSIONER:

Q. Ms. Chaytor, it's getting near the close of the day, so I'm just going to invite you at a convenient place to call the break.

CHAYTOR, Q.C.:

Q. Okay. Thank you. Then the lines of communication, again, we've talked about that,

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so that's still there. And page 10, the documentation, "The process of documentation has recently begun in immunohistochemistry. Key areas identified," and again you talk about "The assemblage of the procedure manual has been initiated, however the manual is not written in compliance with Clinic and Laboratory Standard Institution Guidelines. All antibody data sheets have been compiled. However, formal documented validation is not present. Templates of examples of validation worksheets were provided during the initial assessment." So you had given those templates during the initial assessment?

MS. WEGRYNOWSKI:

A. Yes, I had.

CHAYTOR, Q.C.:

Q. Okay. "Recorded maintenance records of Ventana benchmark were present." And "No pipette and thermometer calibration of accuracy is documented."

MS. WEGRYNOWSKI:

A. None.

CHAYTOR, Q.C.:

Q. None. So they're still not calibrating the

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pipettes?

MS. WEGRYNOWSKI:

A. No. And I have no idea how old those pipettes were.

CHAYTOR, Q.C.:

Q. Okay. "Reagent and detection system validation initiated. Retirement of procedures and antibodies documentation was commenced." So those were positive, I would take it, improvements?

MS. WEGRYNOWSKI:

A. Um-hm.

CHAYTOR, Q.C.:

Q. "Ensure documentation is evident in the procedure manual as to the location of the retired documents." And you're saying that they need to "ensure that obsolete reagents are removed and only currently authorized reagents are available for use." And "Incomplete corrective action and error log was now present."?

MS. WEGRYNOWSKI:

A. Um-hm.

CHAYTOR, Q.C.:

Q. Or "Incomplete corrective action and log, and

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error log was present." What does that mean?
MS. WEGRYNOWSKI:
A. I don't know.
CHAYTOR, Q.C.:
Q. "Incomplete corrective action".
MS. WEGRYNOWSKI:
A. I'll think about that.
CHAYTOR, Q.C.:
Q. Okay. I'm not sure if that's a good thing or a bad thing.
MS. WEGRYNOWSKI:
A. No, no.
CHAYTOR, Q.C.:
Q. "Retired procedures and antibodies information has been gathered and maintain records for a minimum of two years."
MS. WEGRYNOWSKI:
A. Um-hm.
CHAYTOR, Q.C.:
Q. And then you have a number of recommendations flowing from there, which perhaps that's a good place for us to pick it up tomorrow at recommendation No. 10.
COMMISSIONER:
Q. All right, then. We'll break until 9:30 in

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the morning. Thank you.
Upon conclusion at 4:57 p.m.

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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 24th day of June, 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 24th day of June, A.D., 2008
Judy Moss

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