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May 16, 2007

Chesley F. Crosbie, Q.C.
Ches Crosbie Barristers
169 Water Street, 4th Floor
St. John's, NL A1C 1B1

Dear Mr. Crosbie:

Re: *Doucette v. Eastern Health – Undertakings*

This will constitute my response to the requests for further information made by Mr. Boone during my cross-examination on April 23, 2007. The references below are to the paragraph numbers of my affidavit in relation to which undertakings were given. In addition, my cross-examination was conducted before I received the information contained in Ms. Predham's Answers to Interrogatories. Accordingly, I attach a schedule summarizing the new data provided.

Para. 11

“Also, as one would expect, a high percentage of ductal carcinoma, if they are reasonably differentiated (though malignant, still retain some of the biological activities of the normal ductal cells), will be still under the influence of Estrogens for growth and thus will have Estrogen receptors and be positive for Estrogen receptors on testing.”

This first part of para. 11 is accepted by most authorities back in 1997 and today in 2007.

The second part of para. 11: The percentage of positive receptor status will vary from 73% to 100% depending on the histological type of cancer. I gave an undertaking to supply references to support this statement.

There is a spectrum of malignancy from intraductal carcinoma (carcinomas in situ) to invasive ductal carcinoma of varying degrees of differentiation. With reference to one end of the spectrum intraductal carcinoma (carcinomas in situ), I quote from Rosen (page 253): “The frequencies of Estrogen receptor positivity in non-comedo intraductal carcinoma did not differ significantly.” (He lists the histological variants of non-comedo carcinomas):

Cribiform	89%	(if including comedo, average is 80% ER+)
Solid	94%	
Micropapillary	100%	

The percentage of Estrogen positivity will fall as you pass through the various histological stages (grades) from differentiated, moderately differentiated to undifferentiated tumors. When you mix, in situ carcinomas, invasive ductal and lobular carcinomas, you would expect that in testing this mix that you would get in the range of 75% positive Estrogen plus or minus a few percentage points if your IHC assay is reasonably sensitive.

Reference 4: These authors used a cutoff point of 10% and a survey of 71 laboratories found the following in 7016 cases of breast carcinomas:

<u>June '96 – September '98</u>			<u>Other studies quoted by authors</u>			
ER+ PR+	59.1%		54%	52%	52%	58%
ER+ PR-	18.8%		19%	21%	21%	23%
ER- PR+	2.9%		3.2%	4%	4%	4%
ER- PR-	<u>17%</u>		<u>22.1%</u>	<u>23%</u>	<u>23%</u>	<u>15%</u>
	77.9% Pos.		73%	73%	73%	81%

The variations in the positivity or negativity is attributed to mixed patient populations, clinical stage, and treatment status. The mean frequency of Estrogen receptor positivity was 75% (73% to 78%). In other words, the literature seems to support that 75% Estrogen positivity would be the standard for sensitivity.

The performance of the Eastern Health Board laboratory as supplied by Ms. Predham show the following:

<u>Dako system</u>	<u>Total Tests</u>	<u>Pos.%</u>	<u>Neg.%</u>
May 1997 to December 31	137	58	42
January 1, 1998 to December 31	147	48	52
January 1, 1999 to December 31	360	68	32
January 1, 2000 to December 31	370	54	46
January 1, 2001 to December 31	374	60	40
January 1, 2002 to December 31	344	58	42
January 1, 2003 to December 31	373	76	24
January 1, 2004 to April	<u>109</u>	<u>85</u>	<u>15</u>
Totals	2214	62.8%	37.2%
<u>Ventana system</u>			
April 2004 to December 31	381	90	10
January 1, 2005 to July 31.	<u>114</u>	<u>84</u>	<u>16</u>
Totals	495	87%	13%

Interpretation

From May 1997 to December 31, 2002, a period of 5 years 8 months, the average percentage of positivity was **58.5%** (range 48% to 68%). Including the year 2003 and three months of 2004, the overall average of positivity for the period in which the Dako system was in use, was 62.8% (range 48% to 85%). See the tables compiled from the recent Answers to Interrogatories at Schedule A.

Further information provided by Ms. Predham states that of the 309 cases which were retested at Mt. Sinai and found to be false negative, 306 of these cases were originally tested under the Dako system. Further, an additional 105 of the 176 deceased patients were retested at Mt. Sinai and 36 or 34% were false negative.

If we take 34% of the remaining 71 that were not retested, then we get an additional 24 false negatives.

306	false negative confirmed by Mt. Sinai
36	false negative of 105 deceased patients confirmed by Mt. Sinai
<u>24</u>	false negatives of 71 deceased patients (not retested but calculated at 34%)
366	total false negatives

As it is admitted that these false negatives occurred while the Dako system was in use then the following summary can be made:

2214	tests on Dako system
1390	tests were positive (62.8%)
824	were negative (37.2%)
458	confirmed negative by Mt. Sinai
<u>366</u>	were false negative (44.7% false negatives of 824 cases)
16.6%	overall percentage total false negatives/total tests

Improper preservation and fixation of the breast specimen has been identified as a cause of false negatives and undoubtedly, contained within the 458 negatives confirmed by Mt. Sinai, is an unknown number or percentage of false negatives which can be added to the 366 confirmed false negatives.

Para. 24

Studies done on interlaboratory differences in test results have identified this part of the procedure (antigen retrieval) as the Achilles heel of the ER identification procedure. The studies looked at:

1. Dr. Craig Allred, M.D., Professor of Pathology, Baylor College of Medicine Breast Centre, Houston, Texas, presentation to San Antonia Breast Cancer Symposium, 2000 – abstract.

In addressing contributory factors resulting in false negatives in Estrogen Receptors analysis Dr. Allred says:

“The single biggest contributor to error is the antigen retrieval, which is an artsy part of the assay in which we try to reverse the cross linkage between the proteins caused by the initial formalin fixation.”

Another major problem is antibody selection. Dozens of antibodies are available and they are not equivalent in sensitivity and specificity.

2. Dr. Susan Lester, Harvard Medical School, *Manual of Surgical Pathology*, Chapter 15 addresses false negatives in IHC procedures for ER/PR status (page 243):

- (1) Low sensitivity of the assay (antibody selection, antigen retrieval);
- (2) errors in performing the assay (from paraffin section to autostainer, antigen retrieval);
- (3) pre-analytic factors (ie. fixation).

[see Reference 8]

3. Parker et al, *American Journal Clinical Pathology* 2002 117: 723-728, *Assessment of Interlaboratory Variation in IHC determination of ER status*. In the discussion on the results, Dr. Parker says:

“The present study demonstrates that laboratories that use the same antibody and similar staining methods have similar results. The laboratory that used a different antibody and antigen retrieval technique had weaker staining and fewer positive cases compared with the other laboratories. One or both of these variables were certainly the major contributing factor to the observed variability in ER reporting. In another recent study, antigen retrieval was identified as the single most important factor contributing to the overall reliability of ER determination by immuno histochemical analysis.”

[see Reference 4]

4. Reference 6. The author states:

“The sensitivity of an IHC assay is determined by several parameters: these include quality and concentration of the primary antibody, the power of the antigen retrieval and secondary detection systems, and the quality of tissue fixation. Evidence to date suggests that inefficiencies in

the antigen retrieval step are the most probable cause of low IHC assay sensitivity for Estrogen receptors.”

[see Reference 6]

The second part of para. 24: “Once you give the specimen to the machine (autostainers Dako) the manufacturer can guarantee a reproducibility within a small percentage (allowable limit of error 0-5%).” This is a general statement based on my previous experience of 20 years as a director of laboratories related to buying instrumentation in a highly competitive and lucrative market. The statement is also based on studies in the enclosed references investigating interlaboratory inconsistencies in results. In none of these studies when autostainers were used was it even mentioned that the use of autostainers contributed to or was a factor leading to false negatives. Fixation, antigen retrieval, choice of antibody and dilution and cutoff points were the common causes identified. The Dako manufacturer refers to a warranty but the Defendant has not provided the bench manual with warranty as requested in a recent Interrogatory.

Para. 13

“A small percentage of lobular (ie. glandular) carcinomas are Progesterone positive and those that are, are also positive for Estrogen receptors 95-100%.” Mr. Boone asked for a reference source:

Rosen, *Breast Pathology*, Chapter 16, page 297: “Since the expression of PR is estrogen regulated, most PR positive carcinomas are also ER positive. Less than 10% of carcinomas are ER negative and PR positive.” To put it another way, lobular carcinomas that are positive for PR receptors are also 90% positive for Estrogen.

Lester, *Manual of Surgical Pathology*, Chapter 15, pages 241-242. Lester puts the PR positive and ER negative rate at 5%.

Status of Carcinoma	Percentage of Carcinomas	Percentage of Patients Responding to Tamoxifen
ER+ PR+	63%	75-80%
ER+ PR-	15%	25-30%
ER- PR+	5%	40-45%
ER- PR-	17%	<10%

Other references (see page 2 of this letter) are seen in discussion of para. 11 where the authors quote other series where PR+ ER- were reported at 4% and 2.9%. Consequently, a statement that 90 to 97% of PR+ lobular carcinomas are also ER+, is supported by the literature.

Yours truly,

Charles J. Hutton, M.D., F.R.C.P.C.

Schedule A

PREDHAM INTERROGATORIES – NEW DATA

Ventana & Dako Systems

Total Tests	2709		
Positives	1825	69.6%	67.4%
Negatives	884	30.4%	32.6%

Dako System

Total Tests	2214		
Positives	1390	62.8%	
Negatives	824	37.2%	

Dako System – May '97 to December 2002 (5 years, 8 months)

Total Tests	1732		
Positives	1013	58.5%	
Negatives	719	41.5%	

On retesting by reference lab Total false negatives on Dako

306	on the living	
36	on the deceased 105 of 176	
<u>24</u>	on the deceased 71 not retested (calculated) 34%	
366	false test totals	
41%	of 884 negatives	
16.5%	of total tests	