

2006 01 T 2966 CP

IN THE SUPREME COURT OF NEWFOUNDLAND AND LABRADOR  
TRIAL DIVISION

**BETWEEN:**

VERNA DOUCETTE

PLAINTIFF

**AND:**

EASTERN REGIONAL INTEGRATED  
HEALTH AUTHORITY

DEFENDANT

Brought under the *Class Actions Act*, SNL 2001, c. C-18.1

**AFFIDAVIT**

I, Allen Michael Gown, of the City of Seattle, in the State of Washington, make oath and say as follows:

1. **THAT** I am a Medical Director and Chief Pathologist with PhenoPath Laboratories in Seattle, Washington, Medical Director of the Immunohistochemistry & Molecular Pathology Research Institute of Seattle, Affiliate Investigator of the Division of Clinical Research with the Fred Hutchinson Cancer Research Center, Clinical Professor of the Department of Pathology with the University of British Columbia, Visiting Clinical Professor of Pathology of the University of Southern California and Adjunct Professor of Pathology with MD Anderson Cancer Center. My professional qualifications and publications are set out in full in my curriculum vita, attached hereto as Exhibit A.
2. **THAT** my opinions concerning the Laboratory were formed and information obtained during a visit to St. John's, Newfoundland and Labrador, in February 2006. During this

visit I met with laboratory technologists and pathologists involved in the determination of estrogen (ER) and progesterone (PR) receptor status of breast cancers at the Laboratory and toured the facility. I also attended at the St. Clare's Hospital to view some of the slides immunostained using the Dako instrument.

3. **THAT** standard immunohistochemical (IHC) techniques were employed in the determination of estrogen (ER) and progesterone (PR) receptor status of breast cancers at the St. John's Regional Hospital laboratory ("the Laboratory") between 1999 and 2004, using standard immunochemical techniques, employing a Dako autostainer.
4. **THAT** in 2004, the Laboratory switched to a Ventana autostainer, which, while also using standard immunohistochemical techniques, employs a different tissue pretreatment, primarily antibody dilution, primary antibody and detection system compared with the Dako autostainer.
5. **THAT** I have been informed that a case of lobular carcinoma that had been read out as ER-negative with the Dako instrument was re-run and found to be ER-positive with the Ventana instrument. Following this discovery retesting was performed using the Ventana instrument on a selected set of the other ER-negative cases which had originally been tested using the Dako instrument.
6. **THAT** in reviewing the generic data presented to me it appeared that the ER positivity rate was in the range of 65-75% for breast cancers analyzed at the Laboratory during the time the Dako instrument was employed. I have been advised that the seven year average was 74% ER-positivity.

7. **THAT** there are various components of ER testing of breast cancer, each of which can pose potential problems to any laboratory performing IHC based ER testing.
8. **THAT** the ability to detect the expression of ER can be dramatically affected by factors out of the control of the testing facility usually referred to as pre-analytical factors. These include:
  - (a) time interval between removal of tissue from the patient and commencement of fixation;
  - (b) duration of fixation; and
  - (c) nature of the fixative employed.
9. **THAT** the ability to detect ER expression by IHC can be adversely affected (i.e., lead to false negative results) if tissue fixation, even of small biopsies, is less than 6-8 hours (Goldstein et al, Am J Clin Pathol 120:86-92, 2002).
10. **THAT** this is especially a potential problem in a setting like Newfoundland and Labrador where testing of tissues is performed on tissues obtained, fixed, and processed in other laboratories throughout the Province that are not under the control of the central testing facility. In Newfoundland and Labrador the Laboratory may be asked to perform testing on specimens which may or may not meet the requirements for optimal testing.
11. **THAT** all tissues for which ER IHC is performed are treated with a process known as “antigen retrieval” or “epitope retrieval”. This method generally involves the incubation of tissue sections in a specific buffer (e.g., 0.01 M citrate), use of heat applied either in the form of incubation of the tissue in a microwave oven, water bath, etc.

12. **THAT** although the vendor supplying the antibody may suggest an optimal tissue pretreatment, or one may glean this from the published literature, every laboratory needs to find the optimal pretreatment conditions for every antibody employed in that laboratory.
13. **THAT** while there are a number of antibodies to ER employed in laboratories in North American, the two most common are the ID5 clone, which is employed with the Dako instrument, and the 6F11 clone, which is employed with the Ventana instrument. With optimal epitope retrieval techniques, these two antibodies (all other variables being equal) have a high concordance rate (Caplan PA et al., Am J Clin Pathol 123:276-80, 2005).
14. **THAT** more recently a rabbit monoclonal antibody (SP1) has become available, and studies performed in conjunction between my laboratory and the British Columbia Cancer Agency (BCCA) have demonstrated that IHC studies employing the SP1 antibody identify between 5-10% additional cases as being positive than the ID5 clone (Cheang MC et al., J Clin Oncol 24:5637-44, 2006). Use of either the ID5 or 6F11 clones would be considered “standard of care” in any laboratory in North America today.
15. **THAT** the different detection systems (i.e. secondary antibody reagent) are employed in the Dako and Ventana systems that were used at the Laboratory for ER testing. Both, however, were variations of the avidin biotin immunoperoxidase technique, and both represent highly sensitive methodologies if optimized.
16. **THAT** distinction of positive from negative cases may not be straightforward in the setting of very light immunostaining, which can result from:

- (a) under or over-retrieving the tissue prior to incubation with the primary antibody;
  - (b) use of primary antibody at too high a dilution; and
  - (c) not optimizing the detection system .
17. **THAT** independent of the scoring of whether the tumor cells immunostain as positive or negative is the interpretation of the significance of this positive immunostaining.
18. **THAT** different pathologists may employ different “cutoffs” as thresholds to classify tumors as either ER-positive or ER-negative. Based on clinical outcome studies, Harvey et al (J Clin Oncol 17:1474-81, 1999) have determined that a 1% cutoff could be used to separate positive (> 1%) from negative (0 or < 1%) cases.
19. **THAT** different laboratories in North America employ a wide range of cutoffs (Layfield et al., Breast J 6:189-96, 2000). That I am informed that from 1997 until 2000 the Laboratory employed a cutoff of 30% to separate positive from negative cases, and that this cutoff was changed to 10% in 2000. However, I note that there seemed to be some disagreement between the group of pathologists and technologists with whom I met during my visit to St. John’s as to when the changes were made and I note that a cutoff of 1% has been recommended by Harvey et al. (J Clin Oncol 17:1474-81, 1999).
20. **THAT** if the cutoff employed in the interpretation of positive and negative cases changes, then some cases will be re-interpreted as ER-positive solely on the basis of the change in the cutoff employed.
21. **THAT** it appears that many of the pathologists from laboratories throughout the province, other than the Laboratory, who interpreted the slides prepared by the

Laboratory may not have employed the changed cutoffs at the time they were recommended.

22. **THAT** there are several factors which might explain the number of false negative cases tested between 1999 and 2004. Some, all or none of these factors may have contributed to each individual false negative result. Further, some of these factors may have been present in individual tests that were properly determined to be ER-positive. In fact, it is possible that some of these factors may have been present where false negative results were determined but the factor or combination of factors that caused the particular false negative result to occur may vary among individuals. Therefore, an examination of each individual's testing is required to determine what caused each false negative result.

23. **THAT** the factors referred to in paragraph 20 include, but are not limited to:

- (a) Nature of biopsy – needle core vs. resected tumor;
- (b) Interval between tissue removal and immersion into fixative;
- (c) Nature of fixative (E.G., composition, pH, etc.)
- (d) Duration of fixation;
- (e) Tissue processing;
- (f) Epitope retrieval – method of heat delivery (e.g., water bath vs. microwave vs. steamer);
- (g) Epitope retrieval – buffer employed;
- (h) Epitope retrieval – duration;
- (i) Epitope retrieval – cooldown time;
- (j) Choice of primary antibody;
- (k) Length of antibody incubation;
- (l) Detection system;

- (m) Chromogen;
- (n) Nuclear counterstaining intensity (if strong can mask weak immunostaining);
- (o) Pathologists assessment of percentage of immunostained nuclei; and
- (p) Cutoff for positivity.

24. **THAT** based on my experience with breast cancer ER and PR testing, I would say that, with respect to the ER and PR IHC performed at St. John's Regional Hospital from 1997 to the current time, the quality of the pathologists, the quality of the technical support, and the overall quality of the immunostains employed, are all within the range of what would be found in the vast majority of comparable laboratories in North America today. However, that is not to say that optimal ER and PR IHC is being performed in all these laboratories; indeed, I would not doubt that retesting of any North American laboratory's ER IHC would result in the detection of a significant number of "false negative" tests, especially if different primary antibodies, tissue pretreatments and interpretation rules were to be employed.
25. **THAT** I have reviewed the Affidavit of Charles Hutton filed in support of the Interlocutory Application for Class Certification and in particular paragraph 24 thereof. With respect, I disagree with Dr. Hutton's assertion therein that studies done on inter-laboratory differences have identified antigen retrieval as the Achilles heel of the ER/PR identification procedure. Published studies have shown that this is one of the parameters that can account for inter-laboratory differences, but the published data do not support the implication that the 'antigen retrieval' methods are the most critical.

26. **THAT** further, with respect to paragraph 24, I am not aware of any vendor who would make a guarantee of “an allowable limit of error 0-5%” given the effect of so many variables including pre-analytical factors relating to tissue preparation, as well as the interpretation of the result, which can lead to changes in what is perceived as positive or negative.
27. **THAT** , with respect to paragraph 28 of Dr. Hutton’s Affidavit, I am not familiar with the NIH Consensus statement to which he refers. The only major published study in which cutoffs of positivity were related to clinical outcomes was the study of Harvey JM et al., (J Clin Oncol 17:1474-81, 1999), in which a 5% cutoff for positivity was shown to be the best “cutpoint” to distinguish responders from nonresponders to tamoxifen therapy.
28. **THAT** in addition to the variables noted by Dr. Hutton at paragraph 30 of his Affidavit, there are many more variables which may account for differences in test results between laboratories a few of which are:
- (a) the preanalytical factors (what kind of fixative was employed, for how long the tissue was fixed, the interval between tissue removal and emersion into formalin, etc....)
  - (b) the detection system employed (e.g., avidin biotin immunoperoxidase versus indirect immunoperoxidase);
  - (c) chromogen employed (e.g., DAB vs. AEC); and
  - (d) rules for assessment of tumor (e.g., edge vs. central part of tumor assessed).
29. **THAT**, with respect to paragraph 33 of Dr. Hutton’s Affidavit, I am not aware of any published studies that confirm Dr. Hutton’s conclusion that if “all laboratories use the

same procedural methods and the pathologist the same cut off points between negativity and positivity, then inter-laboratory concurrence should be excellent.”

30. **THAT** I provide this Affidavit to the Court for the purpose of responding to the Application for Certification filed on behalf of the Plaintiff.

**SWORN TO** at Seattle, in the state of  
Washington, this day of February, 2007,  
before me:

---

Allen Gown