Reviewer’s report

Title: Preservation of Biomolecules in Breast Cancer Tissue by a Formalin-Free Histology System

Version: 1 Date: 19 April 2007

Reviewer: Lance Liotta

Reviewer’s report:

General
This is an interesting and potentially important manuscript which documents the comparison between a precipitating fixative and formalin crosslinking fixative for the analysis of HER2 and ER in breast cancer specimens. The yield and staining intensity for 62 cases of breast cancer was compared.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)
1. The IH staining and RNA/DNA data were not scored blindly by independent observers following the same protocol. Thus the experimentalist could have been unconsciously favoring the molecular fixative.
2. The authors state that the immunohistochemistry staining intensity for both ER and the RNA/cDNA/DNA yield, were all higher in the molecular precipitating fixative compared to the crosslinking fixative. No charts or tables are presented comparing the full set of cases. Individual IHC scoring for several independent observers blinded to each others scoring should be presented for each case. The level of yield or staining intensity is not the most clinically significant parameter for comparison. The most important comparison is not even presented or done as follows. The most important question is are the low, medium, high and very high cases in the formalin the same as those in the precipitating fixative? A chart comparing the blinded independent observer scoring will show whether the lowest staining or lowest copy number cases in one fixative are the same in both groups and so on for the other groups. Another way to show this would be to plot all the cases individually one fixative on one axis and the other fixative on the other axis, or compared and ranked in a heat map. In this way the intensity of staining or the copy number can be evaluated along a continuum and shown to be proportional. This is critical because a decision to treat a patient can be based on the result and it is completely unknown whether a scoring system cut off can be made for the precipitating fixative so that the patients selected for therapy using formalin would be the same ones selected for the precipitating fixative. In the results it is stated that the staining is “similar” but in the conclusions it is stated that it is “identical”. This demonstrates substantial subjectivity. Thus the subjective claim “identical” and “equal sensitivity” are not supported by presented data.
3. Information must be provided as follows: chemical composition of the molecular fixative, time of fixation in the molecular fixative, time from procurement to immersion in the fixative. The time in the formalin fixative is quite variable 6 to 48 hours. This could contribute to significant variability in the outcome. The authors should mention the uniformity of the tissue pieces being fixed. Are the tissue pieces larger in the formalin fixative compared to the molecular fixative? For proper comparison the tissue volumes should be approximately the same.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
Was the formalin fixed tissue run on the same processor as the molecular fixed tissue?

Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:

I declare that I have no competing interests