Reviewer's report

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

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Reviewer: Timothy O'Leary

Reviewer's report:

General

This is an interesting short manuscript which suggests that a tissue fixative, UMFIX, when used together with a microwave-based tissue processing instrument, provides RNA preservation that is superior to that provided by formalin-fixation. The histologic results appear to be very nice, and it appears that the fixative is quite useful for DNA and RNA. Nevertheless, there are some ways in which I believe the paper should be improved.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

In the introduction the authors imply that the fixative they have evaluated is "safe." The only information relayed about the composition of this fixative is that it is "alcohol-based." I suspect that allegations about safety should not be made. Virtually all the alcohols are flammable, some are capable of facilitating transport of toxic molecules across various epithelia, odd-chain alcohols tend to be rather neurotoxic (due to two-carbon elimination reactions that ultimately give rise to methanol).

The authors found that they were only able to amplify HER2 RNA in 60-70% of fixed samples, and imply that this is typical for DNA and RNA. In my experience, this is very low for fragments <200 bases, in which characterizable amplification products are generally seen in well over 90% of cases for both DNA and RNA. The major exception is for RNA transcripts present in very small abundance, such as hTERT. Perhaps the authors could explain in more detail why their results differ from those elsewhere in the literature, such as in the Specht paper they cited? I think it is also difficult to conclude that these techniques have not received widespread acceptance. Almost every diagnostic molecular pathology lab I know is willing to work from FFPE tissue.

Also, while the approach that the authors use to determine the copy number is formally correct, it is a little strange to compare this with FISH or HercepTest. In general, if one were trying to do such a comparison, one would use an internal control for nucleic acid integrity, thus "normalizing" for the fixative. Perhaps the authors should be a little less terse on this point.

More importantly, is there anything that makes the results with this fixative better than that of other non-cross-linking fixatives? I think it is generally accepted that the yields of RNA are better with alcohol fixation than with formalin-fixation. The most interesting finding of the paper is the ability to amplify a 450-bp segment of GAPDH. Amplification of such large segments of DNA or RNA is a major potential advantage, but data from only three cases is presented in Figure 6, and this only for DNA. This finding would be more convincing with more cases, and with RNA as well as DNA.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

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Discretionary Revisions (which the author can choose to ignore)

When discussing immunocytochemisty, the authors state that they used an antigen-retrieval protocol for both UMFIX and formalin-fixed specimens, noting that the results become identical when the antigen-retrieval step is eliminated from the UMFIX protocol. Why would one use antigen-retrieval for a non-cross-linking fixative? One would expect that the major effect in the absence of cross-linking would be protein denaturation (with generally unpredictable effects on IHC staining), rather than reversal of
cross-linking, which dominates the process when the tissue has been cross-linked.

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

**Level of interest**: An article whose findings are important to those with closely related research interests

**Quality of written English**: Acceptable

**Statistical review**: No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests**:

I am funded to work in areas relevant to this paper, but have no personal financial interests. My employer likewise has no financial interests.