Reviewer’s report

Title: An exfoliation and enrichment strategy for clinical tissue procurement results in improved transcriptional profiles when compared to matched fixed and frozen samples.

Version: 1 Date: 15 April 2007

Reviewer: Scott Jewell

Reviewer’s report:

General

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

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

Is the question new and well defined?

The authors have proposed a process to sample viable cells from solid tissue as an enriched population to determine the integrity, recovery and reproducibility of RNA from both frozen and formalin fixed tissue samples. They conclude an exfoliation / enrichment technique represents a superior process for tissue procurement and RNA recovery than traditional methods and that a better understanding of clinically relevant profiles using tissues processed in this manner would be more reflective of the in vivo state than formalin-fixed or frozen tissues enriched using laser capture microdissection (LCM).

It is well known that tissues fixed in formalin are considerably less adequate or representative than frozen tissue. Unfortunately, this process is the gold standard and required for clinical application. The processing of frozen tissue is primarily provided for research purposes and it is more work for the pathologist as a separate and additional event compared to diagnostic processing. Frozen tissue, while it may be required for future clinical molecular tests because of the need for exquisite material, is still by far a research application.

The author’s hypothesis that exfoliation / enrichment would be better than formalin-fixed tissue enriched by LCM is a given based on literature. The results easily support and verify this existing knowledge. The authors show a more modest improvement in the recovery of RNA over frozen LCM-enriched tissue.

Are methods appropriate and well described with sufficient details?

The author provides very supportive methods documentation for the completed work. It is a pleasure to see the specific notes for the processes of procurement such as transport from the OR, time intervals for tissue processing, tissue mapping for the research sample, and subsequent cell collection.

Minor Essential Revision: An additional sentence briefly describing the “typical” automated processing would be helpful. What was the fixative used (10% NBF), the fixation time for the tissues prior to processing, the time of fixation on the tissue processor and interval of time and temperature for paraffin infiltration? These are additionally important facts to qualify this data for the reader since wide variations exist in tissue processing.

Minor Essential Revision: There may be a typo on page 8, next to the last sentence under “Cells procured for assessment of cell viability”. Should “hemocytometer change” be “hemocytometer chamber”?

Minor Essential Revision: Please clarify (also page 8) next to the last sentence, if LCM cap with samples are kept frozen on “ice” or “dry ice”. If wet ice is used, the word “frozen” should be removed.

Are the data sound and well controlled?
Under the “RNA recovery and Degradometer results” I have a concern that the quality of frozen tissue procured and LMC-enriched (figure 5) in misleading compared to routine methods of tissue procurement. It is well documented that frozen tissue provides good quality RNA that demonstrates very measurable 18S and 28S ribosomal peaks. Figure 5 shows that very poor quality or minimal concentration of RNA recovered from a frozen LCM-enriched process, which also is well known. Therefore these data overwhelmingly reflect issues with enrichment techniques not the quality of RNA recovered from frozen tissue.

Minor Essential Revision: The authors should provide statements to clarify that RNA collection by LCM methods does not reflect the quality of RNA that can be recovered from frozen tissue.

Are the discussion and conclusion well balanced and adequately supported by the data?

See the above comment under results. Possibly clarification on the quality of frozen tissue can be discussed here.

As a reviewer I’m not sure which tissues this technique would be applicable to (epithelial surface only?). Colonic epithelium is a loose population of cells and would seem most applicable to this technique. However, normal tissue texture is not representative of most tumors in texture and organization. Tumors are much more rigid and vary in viability from the center to the periphery.

Minor Essential Revision: Please consider some discussion on which tissues this is technique is most applicable to and in the case of a non-epithelial surface how would this technique be used to procure cells from the heterogeneous regions of tissues, like cancer.

Do the title and abstract accurately convey what has been found?

Minor Essential Revision: Some clarification could be used to relate the clinical versus research application of this technique. Is it not both? Does the title reflect both? I believe the word “clinical” could be removed.

Discretionary Revisions (which the author can choose to ignore)

What next?: Accept after minor essential revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

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

Declaration of competing interests:

I declare that I have no competing interest