METRICS OF TISSUE QUALITY AND MOLECULAR PROFILING YIELD.

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ABSTRACT

Background: The ability to gather molecular data using large scale techniques such as expression profiling and sequencing offer great potential to molecularly characterize tumors. A rate limiting step that has become apparent is the availability of human samples. Traditionally, molecular analysis is performed on moderately sized specimens; these are not available in non-resectable cases from which molecular data is desired nonetheless. In addition, advances in diagnostic techniques allow tumor detection at an early stage. In these instances, it would be advantageous to isolate RNA and DNA from paraffin fixed tissues or from small samples such as those obtained with core needle biopsies or fine needle aspirates. To effectively leverage these alternate types of samples, it is critical to establish quality parameters that can be used to validate the utility of the underlying data.

Materials and Methods: We have undertaken a study to compare the quality of 29 breast tissues fresh frozen in liquid nitrogen within 15 minutes of extirpation (FF) with that of 22 breast tissues fixed in 10% formalin for 8 hours and paraffin embedded (FFPE), and that of 86 breast tissues collected into RNA Later and frozen in liquid nitrogen within 15 minutes of extirpation (RL). The QC process included evaluation of both gene specific 5'-3' ratios as a measure of RNA integrity and total numbers of transcripts detected as a measure of representation.

Results:

RNA harvested from tissues obtained from flash frozen, formalin fixed paraffin embedded, or RNALater staurated specimens was of sufficient quality to obtain quality hybridizations using Affymetrix GeneChips and standard amplification methods. There was little difference in yield of total RNA as a result of preservation method. There was a greater difference between individual samples. Assessment of RNA integrity by Bioanalyzer analysis revealed detectable differences in both ratios of large and small rRNA subunits and in the Agilent RIN number. These differences did not translate into a greater rate of failure of hybridizations or differences in transcript representation as measured by percent present calls and global 5'/3' ratios.

Summary: RNA Yield from Different Methods of Preservation

	FFPE	FF	RNALATER
Total Tissue Samples	22	29	42
Tissue Samples Positive for RNA	22	29	42
Percent RNA Positive Tissue Sam	100	100	100
Average Yield (ug/mg tissue)	0.82	0.74	0.84 *
Stdev (Yield)	1.11	0.74	0.14 *

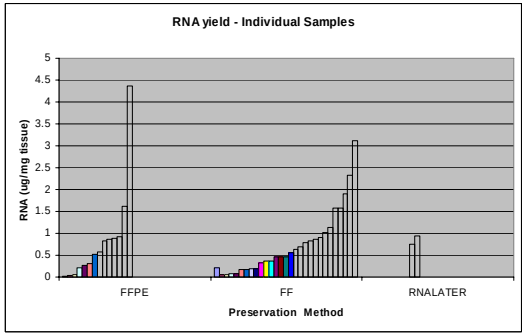

Figure 1. RNA yield from breast tissues either preserved as flash frozen (FF), formalin fixed (FFPE), or saturated with RNALater. The graph depicts the recovered material per milligram of starting tissue, and the summary for individual samples is shown in the table at top. Note that only 2 samples preserved in RNALater had tissue mass determined prior to solubilization and are reflected in the summary table. For these samples the total RNA obtained across 27 samples was 3.8 ug with a standard deviation = 3.9 ug.

Summary. Integrity of RNA Extracted From Tissue Preserved by Different Methods

	FFPE	FF	RNALATER
RNA Samples Analyzed	22	29	42
Bioanalyzer RIN Number	7.92	7.96	7.02
rRNA 28S/18S	1.42	1.38	1.11
Bioanalyzer Concentration	0.24	0.24	0.23
Overall Bioanalyzer PASS	21/22	28/29	38/42

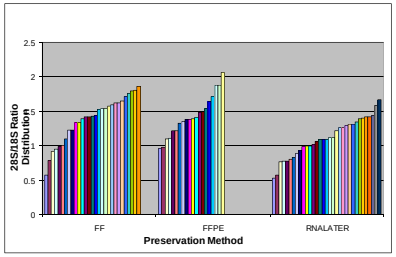

Figure 2. RNA integrity judged by Bioanalyzer analysis from samples preserved by different methods. Data for ratios of large to small rRNA subunits is plotted in the graph above for individual samples preserved as indicated. A summary of this data as well as additional measures of quality including RNA degradation using the Agilent RIN number and concentration is shown in a summary table at top.

Table 3. Amplification and Hybridization Quality from Different Preservation Methods

	FFPE	FF	RNALATER
RNA Samples Hybridized	22	29	42
Percent Present Calls	60	60	59
Scale Factor	2.25	2.55	2.26
Global 5'/3' slope (RNAdegSlope)	4.82	4.95	4.71
cRNA Production PASS	22/22	29/29	42/42
Overall Hybridization PASS	22/22	29/29	42/42

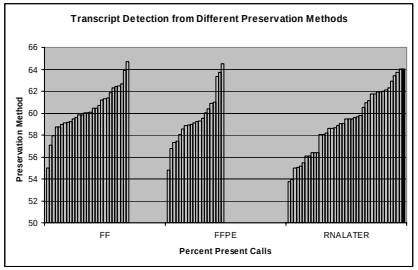

Figure 3. Transcript Detection from Different Preservation Methods. cRNA prepared from Total RNA isolated from flash frozen samples, formalin-fixed samples, or samples saturated in RNALater was hybridized to Affymetrix GeneChips (Merck Custom 2) for expression profiling. After hybridization and staining, chips were scanned to identify probesets that could be detected above background signals. This number is plotted as "percent present" in the graph above. Additional hybridization parameters are provided in the summary table at top.

Conclusion: Our data demonstrate that gene expression can be effectively profiled independently of the isolation method. It should be noted, however, that FFPE breast samples used in this study were obtained using the ASCO guidelines for formalin preservation. Typically these samples were found to perform more consistently as compared to other tumor types where fixation protocols are not standardized.