

**Reproducibility of Quantitative Gene Expression Analysis by a New RT-PCR Assay Using Fixed Paraffin-Embedded Tissues: A Molecular Tomographic Scanning Study**

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**Background:** The reliability of using gene expression profiling in clinical practice will depend on assay reproducibility. We designed this study to determine the reproducibility of a new multi-gene RT-PCR assay (Oncotype DX) in serial sections taken from fixed paraffin embedded tumor tissue blocks.

**Design:** Tumors from two patients, both with poorly differentiated ductal carcinoma, were studied. Three blocks from each tumor were sectioned at five different levels by cutting, sequentially, sections for H E staining and for RNA extraction. Extracted RNA was assayed for 16 cancer-related genes (including ER, PR, and HER2) and 5 reference genes using RT-PCR in a 384 well format on an ABI 7900 real time thermal cycler. All reactions were performed in triplicate with both positive and negative (no-RT) controls for each sample. Expression for each gene was normalized relative to the reference genes (where 1 unit = 2-fold change in expression). Unsupervised cluster and principal component analysis were performed to examine the variance and correlation structure of gene expression profiles amongst patients, blocks within patients, and sections within tumor blocks.

**Results:** Reference-normalized gene expression measurements ranged from 2.8 to 10.3 units. Unsupervised cluster analysis and principal component analysis reveal a strong correlation between blocks from the same patient as well as between tumor samples from the same block. Standard deviations for the 16 cancer-related genes amongst the 5 different levels in each block ranged from 0.06 to 0.21 units (mean = 0.13 units). The point estimate for the average coefficient of variation was 11.2% (range 4.5% - 14.7%). This variability within block is small compared to differences between patients. For example, RT-PCR analysis for HER2 expression in other studies with this assay has observed expression of 11.5- 15 units (mean 12.9 units) in HER2 positive patients and expression of 5 to 11.5 units (mean 10.0 units) in HER2 negative patients.

**Conclusions:** RNA analysis by RT-PCR can be applied to quantitative analysis of gene expression in formalin fixed, paraffin-embedded tissue, with low variability within block.