Title: Consistency and Control in Clinical Assay Technology Over Time: The Onco*type* DX Recurrence Score and Assessment of Single Gene Expression Levels

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Background: ASCO® and CAP have recently highlighted the importance of consistency and control in clinical assay technology. To date the Oncotype DX® Recurrence Score® (RS) has been ordered to assist in individualized treatment decision making in over 200,000 estrogen receptor positive, early stage breast cancer patients. The assay quantifies gene expression using RT-PCR from fixed paraffin embedded tissue (FPET) and employs a large number of controls and calibrators to enhance precision and reproducibility. Over 6 years of data on the assay presents an opportunity to explore consistency over time in the RS and quantitative single gene levels (ER, PR, HER2).

Methods: All tumors successfully analyzed in the Genomic Health Laboratory from 1/1/05-3/31/11 were included. Descriptive statistics for the RS, the average reference gene expression level, and expression levels for quantitative single genes were obtained for each calendar year. This was done for the entire data set as well as for subgroups defined by histological tumor type. The associations by year between HER2 and GRB7, ER and HER2, and ER and PR expression levels were explored including scatterplots and summary statistics.

Results: There were a total of 207,691 breast cancer cases, and the number in each calendar year increased over time as shown in the Table. In general the median age increased slightly over time, as did the proportion of patients in the low RS Risk Group. There was no systematic change over time in the average reference gene expression level, or in the expression levels for the individual genes ER, PR or HER2 during the period from 2008 to 2011 when individual gene testing was provided as part of the Oncotype DX assay report. The relationships between HER2 and GRB7, ER and HER2, and ER and PR remained consistent over time.

Conclusions: Active monitoring of the Oncotype DX assay as mandated by ASCO/CAP shows a high degree of consistency in results for both the multigene Recurrence Score and the quantitative single gene results. These results and the approaches used for monitoring consistency are relevant to other institutions in their efforts to maintain and improve assay quality control

Table: Percent of Patients in each Recurrence Score Risk Group and Median Values for Age, Reference Gene Average Expression, and Expression Levels for Quantitative ER, PR and HER2

Year	2005	2006	2007	2008	2009	2010	2011
N	7123	14853	24504	39694	49224	56499	15794
Age	55	56	57	58	59	60	60
Reference Gene Avg	28.0	27.8	28.0	28.2	28.4	28.2	28.1
Recurrence Score							

% Low (<18)	50	49	52	54	56	56	53
% Int (18-30)	36	38	37	33	32	32	35
% High (≥31)	14	13	11	13	12	12	12
Quantitative ER*	n/a	n/a	n/a	9.9	10.0	10.0	9.9
Quantitative PR*	n/a	n/a	n/a	7.5	7.5	7.5	7.4
Quantitative HER2*	n/a	n/a	n/a	9.1	9.1	9.1	8.9
*Single gene reporting initiated in 2008							