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Analytical Validation for the Oncotype DX Breast Cancer Assay, a High Complexity, Multi-Analyte Molecular Diagnostic Test Service

Background

Personalized genomic analysis based on tumor expression profiling is improving oncology therapeutic decision making beyond current standard anatomic pathology measures. The *Oncotype DX Breast Cancer Assay* is a clinically validated, high complexity, multi-analyte RT-PCR genomic test that predicts likelihood of breast cancer recurrence in women with node negative, estrogen receptor positive breast cancer. Using this molecular test to analyze fixed, paraffin embedded tumor tissue gives an individual Recurrence Score, from 0-100, which correlates with a specific risk of 10-year distant recurrence. The RS provides a more precise, reproducible measurement of breast cancer aggressiveness and therapeutic responsiveness than tumor size, tumor grade and patient age. However, reporting individualized patient Recurrence Scores for clinical decision making requires very high test accuracy and precision. Strict criteria for analytical performance characteristics have been developed for the *Oncotype DX* assay based on results from a series of analytical studies. These criteria are used to monitor routine assay analytical performance in the clinical reference laboratory.

Materials and Methods

The test sample is RNA extracted from fixed, paraffin-embedded tumor tissue. DNase treated RNA is quantified with a fluorescence assay and tested for residual genomic DNA using quantitative PCR. Reverse transcription is done as a multiplexed reaction using a standard amount of RNA. Quantitative PCR analyses are done for 21 genes using well-characterized primer and probe sets.

Results

Acceptable operational assay performance ranges were defined for each gene and the Recurrence Score. Amplification efficiencies for 16 cancer related genes averaged 95.5% (range 75.4% to 112.1%) while the five reference genes had an average amplification efficiency of 87.6% (range 75.3%-101.2%). All gene assays responded linearly to at least a 1000 fold RNA concentration range with an average accuracy of 0.3% and CV's averaging 3.2% for the reference genes and 5.7% for the cancer related genes. Analytical study SD's were less than 0.5 expression units and took into account variability contributed by instruments, operators, reagents and day to day baseline variation.

Conclusions

Analytical validation and quantitative operational performance specifications have been defined which ensure high precision and accuracy for the *Oncotype DX* service which reports breast cancer recurrence risk.