

Analytical Performance of a Microarray-based Gene Expression Test to Determine Tissue of Origin in Uncertain Primary Cancers

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ABSTRACT

Background: Clinical workup of metastatic cancers is often arduous and expensive and unsuccessful in 30 to 60% of cases. Accurate classification of the tumor of origin may improve with microarray-based gene expression testing. We evaluated the analytical performance characteristics of the Pathwork™ Tissue of Origin (TOO) test, which uses expression signals from 1,550 probe sets in a gene expression microarray, to quantify the similarity of tumor specimens to 15 tissues of known origin.

Materials and Methods: Sixty archived tissue specimens from poorly and undifferentiated tumors (metastatic and primary) were analyzed at four laboratory sites representing a wide range of preanalytical conditions (such as personnel, reagents, instrumentation, and protocols). Reproducibility was analyzed by cross-wise comparisons of all 4 sites for 3 categories of results: Standardized Expression values (SE), which are assay signals; Similarity Scores (SS), which are quantitative results; and Physician Guided Conclusions (PGC), which are clinical calls. For the continuous variables, SE and SS, linear regression analysis was used for cross-lab comparisons to generate correlation coefficients. Raw expression values from all replicates were also standardized using the Affymetrix MAS5 algorithm. Bland-Altman plots (difference between the SS values for the reference diagnosis TOO) from two sites versus the average of the SS values for those sites) were compared to all the other site combinations in order to test for possible systematic bias between laboratories. The inter-laboratory agreement for the categorical variable PGC was evaluated by use of the kappa statistic.

Results: Cross-laboratory comparisons showed highly reproducible results between laboratories with correlation coefficients ranging from 0.95 to 0.97 for measurements of SS, which were significantly improved (Wilcoxon one-sided paired test, $p = 0.01563$) from those obtained with MAS5 normalized values (0.65 to 0.82). Moreover, an average of 93.8% overall concordance between laboratories in terms of final TOO calls was obtained. Bland-Altman plots (mean coefficients of reproducibility of 32.48 ± 3.97) and kappa statistics ($k > 0.86$) also indicated a high level of agreement between laboratories.

Conclusions: We conclude that the Pathwork™ TOO test is a robust assay that can produce consistent results in diverse laboratory conditions reflecting the preanalytical variations found in the everyday clinical practice of molecular diagnostics laboratories.

BACKGROUND

In the initial pathologic evaluation of carcinoma, especially those found in unexpected or multiple locations or with poorly differentiated morphologies, the tissue of origin (TOO) can remain hard to identify.

Even with guideline-directed use of immunohistochemistry (IHC), electron microscopy, and advanced imaging procedures (e.g., computed tomography, magnetic resonance imaging, fluorodeoxyglucose positron emission tomography), the primary tumor is ultimately identified in only about 20 to 25% of living patients with metastatic tumors for which the primary site is not apparent after the initial workup.¹

Recently, newer diagnostic techniques have been evaluated to aid in the determination of the tissue of origin. Thus, gene expression microarrays, which capture data from tens of thousands of expressed genes in a single test, have the potential to allow a more accurate classification of tumors of unknown primary, including those with high histologic grade.

The Pathwork™ Tissue of Origin Test is an in vitro diagnostic test for evaluating the tissue of origin (TOO) in poorly differentiated or undifferentiated tumors. This microarray-based gene expression test quantifies the similarity of tumor specimens to 15 tissues on the TOO Test panel by means of a proprietary machine learning algorithm trained on 2039 well-characterized tumor specimens, acquired from 14 laboratories. The tumor types included in the Pathwork TOO Test are: bladder, breast, colorectal, gastric, germ cell, hepatocellular, kidney, non-small-cell lung, non-Hodgkin's lymphoma, melanoma, ovarian, pancreatic, prostate, soft tissue sarcoma, and thyroid.

The study evaluated the analytical performance characteristics of this microarray-based test for tumor TOO. By comparing test results from replicate specimens with reference diagnoses analyzed in 4 laboratories representing a wide range of preanalytical and analytical conditions (eg, protocols, personnel, reagents, and timing), the study primarily addresses the test's reproducibility across multiple sites and, thereby, gauges the test's potential usefulness in actual clinical environments.

MATERIALS AND METHODS

Figure 1. Specimen Workflow

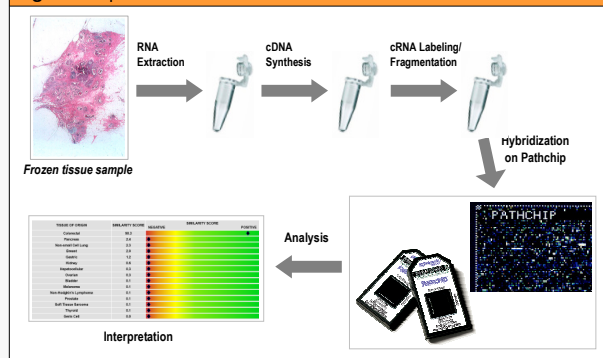
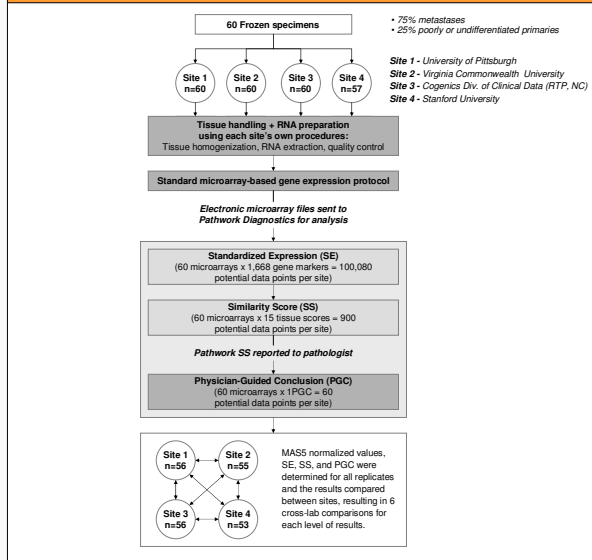


Figure 2. Clinical Validation Study Design



RESULTS

Figure 3. Reproducibility of Standardized Expression (SE)

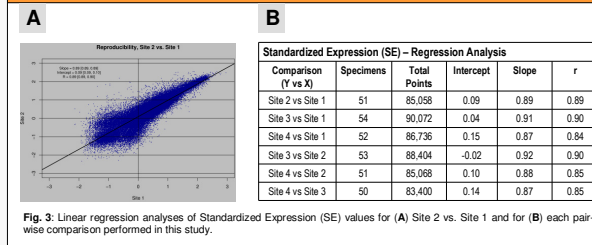


Figure 4. Reproducibility of Similarity Score (SS)

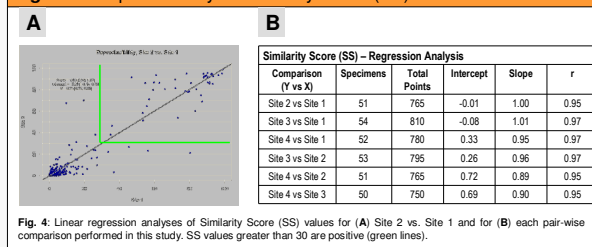


Figure 5. Overall Correlation: MAS5, SE, SS

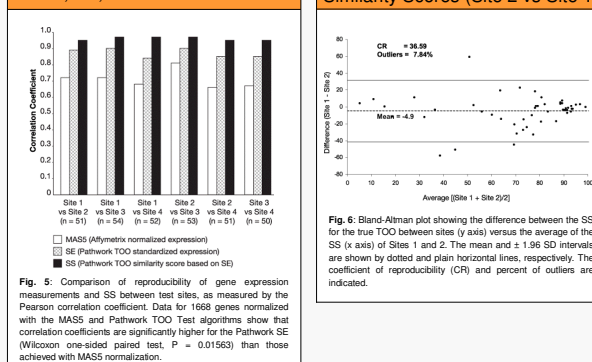


Figure 6. Agreement: Similarity Scores (Site 2 vs Site 1)

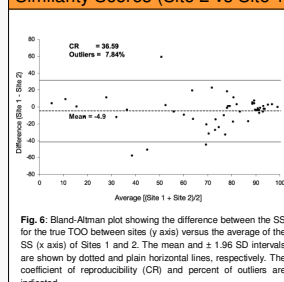


Figure 7. Reproducibility of Physician Guided Conclusion (PGC)

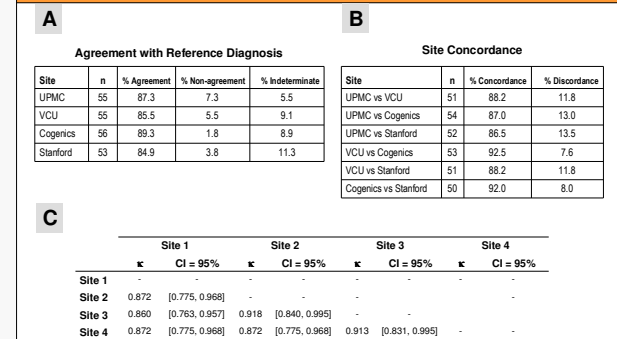
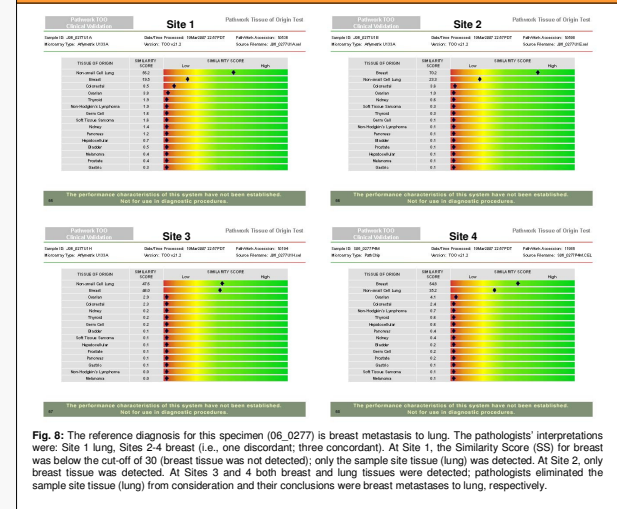


Figure 8. Examples of report interpretation



CONCLUSIONS

For a diagnostic microarray-based test to perform adequately within the clinical setting, the test's analytics must be robust enough to account for the expected variations in both laboratory technique and RNA input. Given the anticipated variations between laboratories,^{2,3} evidence of reliable results across sites is clearly a prerequisite for routine use of such tests in a clinical environment.

In this study, we assessed the robustness and reproducibility of this test through a multicenter study representing diverse laboratory environments, and we challenged the test with 60 frozen tumor specimens. Reproducibility was assessed on quantitative results (SE and SS), as well as clinical calls (PGC), from 4 laboratory sites.

The results of this study showed highly reproducible results for the Pathwork TOO Test. Thus, in all crosswise comparisons, the correlation coefficients were ≥ 0.84 for SE and ≥ 0.95 for SS. Moreover, these results were significantly ($P = 0.01563$) higher than the ones obtained with MAS5-normalized data, suggesting that the Pathwork standardization algorithm may contribute to the reproducibility of the TOO test.

Also, no evidence of a systematic site bias was observed, with a good agreement between laboratories assessed by Bland-Altman analyses (showing less than 10% outliers in all pairwise comparisons). Interestingly, some of the few discordant results corresponded to PGC calls that related to the biopsy site instead of the reference diagnosis; however in most cases the SS for the correct TOO was also >30 , suggesting that this test could be useful for clinical decisions with proper pathological interpretation.

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