

A Microarray-Based Gene Expression Test as an Aid to Tumor Diagnosis Using Formalin-Fixed Paraffin-Embedded (FFPE) Specimens



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ABSTRACT

Context:

Tumors that are poorly differentiated, undifferentiated, or metastatic represent 5-10% of all new cancer cases. The Pathwork[®] Tissue of Origin Test is the first FDA-cleared gene expression test to aid in the diagnosis of tumors using frozen specimens. Here we report on the validation of a version of the test that works with formalin-fixed paraffin embedded (FFPE) specimens.

Design:

Poorly differentiated and metastatic FFPE human tumor specimens with available diagnoses representing the 15 different tissue of origin sites on the Tissue of Origin Test panel were blinded and processed at three independent labs to generate microarray data files. A prespecified classification model using >1500 genes was applied to each data file to yield Similarity Scores corresponding to the 15 tissues on the test panel. Results were un-blinded and compared with the available diagnoses.

Results:

Of 549 specimens processed to data files, 462 (84%) yielded qualified data files. Based on the top Similarity Score, the overall agreement with available diagnoses was 89%. Metastatic and poorly differentiated primary specimens showed similar performance. In addition to the positive test results ("rule-ins"), an average of 12 out of 15 tissues for each specimen could be ruled out with >99% probability.

Conclusions:

The large size of this study allows an accurate estimate of the confidence of test results for both ruling in and ruling out tissues as likely sites of origin. The Tissue of Origin Test makes the potential benefits of microarray-based gene expression tests for tumor diagnosis available for use with the most common type of histology specimen, FFPE.

STUDY DESIGN

Specimens for this study were selected to represent the 15 tumor types in the Tissue of Origin Test panel. Over 500 FFPE specimens were distributed over the range of 15 tumor types for the measurement of performance of FFPE specimens, with at least 25 specimens per tumor type. Specimens were from metastatic tumors or from primaries that were poorly differentiated or undifferentiated.

MATERIALS AND METHODS

Sample source and entry criteria:

FFPE specimens were obtained from human tumor tissue banks under IRB-approved protocols. All human tumor samples were selected from the 15 tumor tissue types (covering 58 morphologies) included in the Pathwork Origin – FFPE Test panel. These types include: bladder (BL), breast (BR), colorectal (CO), gastric (GA), testicular germ cell (GC), kidney (KI), hepatocellular (LI), non-small cell lung (LU), non-Hodgkin's lymphoma (LY), melanoma (ME), ovarian (OV), pancreas (PA), prostate (PR), thyroid (TH) and soft tissue sarcoma (SC). At least two 10-µm-thick sections ("paraffin curls") and one 5-µm-thick H&E stained section were available from each specimen. All specimens were estimated to contain ≥ 60% viable tumor tissue.

MATERIALS AND METHODS (continued)

Sample processing:

Specimen processing
Total RNA was isolated from the specimens used for this study using the Agencourt Formapure method. The RNA was used for target preparation with a two-cycle amplification procedure followed by array hybridization to Pathchip microarrays. The arrays were washed and scanned using Affymetrix fluidics stations and scanners respectively. Positive/negative controls (total RNA) were run with every target preparation batch.

METHODS OF REPRODUCIBILITY

Reproducibility Study

The reproducibility of the test was established in a study performed at three laboratories using 60 tumor specimens representative of those intended for use with the product. A set of 60 specimens was sent to each site. All 15 tissue types on the test panel were represented by four specimens each. Each tumor specimen was divided into adjacent 10-µm-thick "curls". Each specimen received full processing at each laboratory, from tissue curl specimen to reported result.

CLINICAL VALIDATION RESULTS

Sample processing: 598 tumor specimens were processed in this study. 552 specimens provided adequate amounts of total RNA.
These 552, as well as 11 additional specimens producing < 150 ng total RNA were moved forward into the assay, bringing the total number of specimens processed to 563. Of the 563, processing was continued to microarray on 549.
Of 549 specimens for which microarray data were available, 462 (84%) passed the Data Verification quality tests.

Table 1: Origin-FFPE Clinical Validation Results

Available Diagnosis (15 tumor types)	No. of specimens	Agreement with available Dx	Non-agreement	Percent Agreement	Percent Non-agreement
Bladder (BL)	29	23	6	79%	21%
Breast (BR)	57	55	2	96%	4%
Colorectal (CO)	36	33	3	92%	8%
Gastric (GA)	25	18	7	72%	28%
Testicular Germ Cell (GC)	25	21	4	84%	16%
Kidney (KI)	28	25	3	89%	11%
Hepatocellular (LI)	25	24	1	96%	4%
Non-small Cell Lung (LU)	27	23	4	85%	15%
Non-Hodgkin's Lymphoma (LY)	29	26	3	90%	10%
Melanoma (ME)	25	21	4	84%	16%
Ovarian (OV)	45	40	5	89%	11%
Pancreas (PA)	28	24	4	86%	14%
Prostate (PR)	25	24	1	96%	4%
Soft-tissue Sarcoma (SC)	27	24	3	89%	11%
Thyroid (TH)	31	28	3	90%	10%
Total	462	409	53	89%	11%

The Overall Agreement with the available diagnosis for Tissue of Origin Test results generated from 462 specimens that have passed Data Verification is 88.5%, with the lower and upper bounds of the 95% confidence intervals at 85.3 and 91.3 respectively. The Positive Percent Agreement (PPA) is 88.5, and the Negative Percent Agreement (NPA) is 99.1.
Rule-outs: Any tissue type with a Similarity Score less than or equal to 5 had a 99.8% probability of not being the Tissue of Origin. Thus, the Tissue of Origin Test can be used to exclude origins from specific tissues on the Test Panel if their Similarity Scores are less than or equal to 5. The average number of rule outs per tissue is 12.

CLINICAL VALIDATION RESULTS (continued)

Table 2: Distribution of Tissue of Origin Test Result by Tissue

Result/Specimen	No. of Specimens	Tissue of Origin Test Result														
		BL	BR	CO	GA	GC	KI	LI	LU	LY	ME	OV	PA	PR	SC	TH
BL	29	23	1	1											4	
BR	57		55	2											2	
CO	36			33	1						1	1				
GA	25			5	18									1	1	
GC	25		1	1		21									2	
KI	28						25								3	
LI	25							24				1			1	
LU	27		1				1	23			1				1	
LY	29				2				26						1	
ME	25								2	21					2	
OV	45		1		1						40	2			1	
PA	28			2	1							24			1	
PR	25				1								24			
SC	27			1			1				1			24		
TH	31		1								2				28	
Total	462	23	59	46	24	21	27	24	23	28	21	45	29	24	40	

Distribution of available diagnosis vs. Tissue of Origin Test test results in 462 FFPE specimens. The confusion matrix highlights, on the diagonal, the high level of overall agreement (89%) of the test result with the available diagnosis and indicates possible trends in nonagreements.

Table 3: Tissue of Origin Test test performance according to metastatic vs. primary tumor status

Metastasis or Primary	Agreement (% , ratio)		Non-Agreement (% , ratio)	
	Agreement	Non-Agreement	Agreement	Non-Agreement
Metastatic Tumors	91.1%	(163/179)	8.9%	(16/179)
Poorly & Undifferentiated Primary Tumors	86.9%	(246/283)	13.1%	(37/283)
Overall	88.5%	(409/462)	11.5%	(53/462)
	[85.3, 91.3]		[8.7, 14.7]	

These results demonstrate that performance for metastatic tumors is as high as that observed with primary tumors.

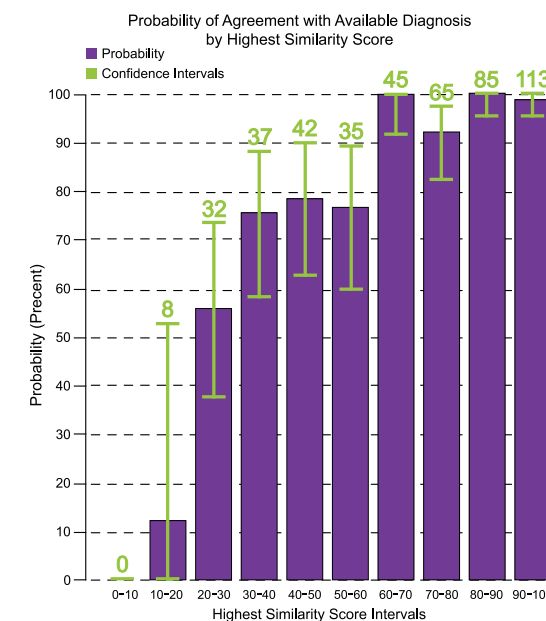
Table 4: Tissue of Origin Test test performance according to biopsy site

Biopsy Site	Agreement (% , ratio)		Non-Agreement (% , ratio)	
	Agreement	Non-Agreement	Agreement	Non-Agreement
Colorectal	100.0%	(5/5)	0.0%	(0/5)
Gastric	83.3%	(5/6)	16.7%	(1/6)
Kidney	100.0%	(1/1)	0.0%	(0/1)
Liver	100.0%	(9/9)	0.0%	(0/9)
Lung	60.0%	(3/5)	40.0%	(2/5)
Lymph Node	92.0%	(104/113)	8.0%	(9/113)
Ovary	100.0%	(6/6)	0.0%	(0/6)
Soft Tissue	86.7%	(13/15)	13.3%	(2/15)
Other	89.5%	(17/19)	10.5%	(2/19)
Overall, metastatic tumors only	91.1%	(163/179)	8.9%	(16/179)

For metastatic specimens, data were collected regarding the biopsy site from which the specimen was obtained. The data do not indicate any apparent difference in performance related to biopsy site.

CLINICAL VALIDATION RESULTS (continued)

Figure 1: Probability of Agreement with Available Diagnosis by Highest Similarity Score



The graph shows the probability of a test result being in agreement with available diagnosis as a function of the highest Similarity Score. Similarity Scores are binned in intervals of 10 with the number of test results in the bin shown above the bar.

REPRODUCIBILITY RESULTS

Table 5: Tissue of Origin Test test reproducibility: Concordance between laboratories

Stratification by Lab	# Specimens, paired	Tissue of Origin Test Kit – FFPE results	
		% Concordant	% Nonconcordant
Site 1 vs. Site 2	49	85.7% [72.8%, 94.1%] (42/49)	14.3% [5.9%, 27.2%] (7/49)
Site 1 vs. Site 3	49	89.8% [77.8%, 96.6%] (44/49)	10.2% [3.4%, 22.2%] (5/49)
Site 2 vs. Site 3	51	92.1% [81.1%, 97.8%] (47/51)	7.8% [2.2%, 18.9%] (4/51)
All Sites; (pair-wise)	149	89.3% (133/149)	10.7% (16/149)

Concordance was strictly defined as an identical test result between two laboratory sites for a paired specimen. Percent concordance is calculated as the number of results from one site for which the Test result is the same as the Test result from the other site, divided by the total number of pairs.

REPRODUCIBILITY RESULTS (continued)

Pancreas (Site 1)

TISSUE	SIMILARITY SCORE	LOW 0 5	HIGH 100
Pancreas	79.5		
Gastric	4.2		
Ovarian	3.7		
Colorectal	3.1		
Kidney	1.6		
Non-Hodgkin's Lymphoma	1.2		
Soft Tissue Sarcoma	1.2		
Breast	1.1		
Hepatocellular	0.9		
Thyroid	0.9		
Non-Small Cell Lung	0.7		
Bladder	0.6		
Melanoma	0.6		
Testicular Germ Cell	0.4		
Prostate	0.2		

Pancreas (Site 2)

TISSUE	SIMILARITY SCORE	LOW 0 5	HIGH 100
Pancreas	76.3		
Colorectal	4.6		
Soft Tissue Sarcoma	3.8		
Gastric	3.7		
Ovarian	2.7		
Breast	1.9		
Kidney	1.3		
Non-Small Cell Lung	1.3		
Hepatocellular	1.0		
Thyroid	0.9		
Melanoma	0.7		
Bladder	0.7		
Non-Hodgkin's Lymphoma	0.6		
Testicular Germ Cell	0.4		
Prostate	0.3		

Test reports for a pancreas specimen processed at 2 sites.

SUMMARY AND CONCLUSIONS

- Routine formalin-fixed paraffin-embedded specimens can be reliably processed to high-quality gene expression microarray data files.
- The Pathwork Tissue of Origin Test has shown a high degree of agreement with the available diagnosis of primary site (88.5% positive percent agreement, 95% CI: 85.3% to 91.3%).
- Tissues on the panel with Similarity Score results ≤ 5 can be ruled out as the primary site with > 99% likelihood.
- A multi-site interlaboratory study has shown that the Tissue of Origin Test results are highly reproducible with 89.3% concordance between labs.
- Specimens up to four years old were tested, and performance was found to be acceptable.
- The ability to obtain high-quality whole genome gene expression data from routine clinical specimens and to convert this data into accurate and reproducible results indicating the likely primary site of a malignancy will be highly useful as an aid in the diagnosis of human malignancies.