



variants (across 22,300 probesets) which in turn represent 14,500 of the best characterized human genes. Each transcript is represented by a probeset, comprised of 11-16 pairs of oligonucleotide probes. The probesets are spatially distributed over the array and are used to measure the level of transcription of each sequence represented on the array. For each array, there are 2000 probesets representing 2000 human genes which are used by the Origin Test Kit-FFPE algorithm as markers to identify the tissue of origin of the specimen being tested. These probesets were selected using machine learning methods and each set has between 11 and 16 probe pairs of 25 bases whose sequences are matched to mRNA species that are found in human tissue. In addition, the array has 29 probesets that are used for normalization and data verification.

The algorithm of the Origin Test Kit-FFPE was developed using a database of 2196 specimens, divided into independent training and test datasets. The test development used a machine learning approach based on marker selection to build a predictive model. The model consists of a list of markers, a set of reference (support) samples and a set of coefficients. These components are combined to produce 15 *Similarity Scores*, one for each of the possible tissues on the test panel. Each Similarity Score ranges from 0 to 100, with a higher score being associated with a higher likelihood that the input specimen has a molecular signature of the corresponding tissue of origin. The 15 Similarity Scores are scaled to sum up to 100. Each is based on the microarray standardized expression (SE) values of selected biomarkers. The process consists of the following steps:

1. Read SE values for the biomarkers used in the Tissue of Origin Test Kit-FFPE from the input file.
2. Compute a decision function ("score") for each of 105 possible pairings of the 15 tissues on the test panel with respect to the sample described by the input SE values.
3. Convert the 105 pairwise scores into pairwise probabilities.
4. Reduce the 105 pairwise probabilities to 15 Similarity Scores, one for each Tissue of Origin. These are the final 15 Similarity Scores presented in the Tissue of Origin Test Kit-FFPE Report.

The Pathwork Specimen Processing Guide (SPG) contains instructions for the user to process tissue specimens in the manner specimens were processed during the clinical validation of the Origin Test Kit-FFPE.

The specimen used for the Origin Test Kit-FFPE (Origin Test Kit-FFPE) is a formalin-fixed, paraffin-embedded (FFPE) specimen. Prior to processing for the Origin Test Kit-FFPE test, the user must ensure, by routine H&E stain, that the specimen contains at least 60% viable tumor. The specimen is sectioned to yield a 10-um-thick curl with a minimum tissue area of at least 25 mm sq or equivalent. Total RNA must be isolated per the SPG. A total RNA yield of at least 150 ng is required. The procedural steps after isolation of total RNA from the specimen begin with a two-cycle RNA amplification: 1) round 1 reverse transcription (RT)

of total RNA with dT and random RT primers; 2) purification of round 1 cDNA; 3) tailing of first-strand cDNA with terminal deoxynucleotidyl transferase; 4) synthesis of T7/T3 promoter sequence; 5) T7 in vitro transcription 6) round 2 RT of sense RNA with a dT and random primers; 7) T3 in vitro transcription (linear amplification); 8) purification of round 2 sense RNA; 9) RT of round 2 sense RNA with random primers; and 10) purification and fragmentation of biotinylated DNA. This is followed by hybridization of the biotin-labeled cDNA target to the Pathchip microarray; washing and scanning of the hybridized Pathchip microarray; data acquisition (signal intensity per feature; CEL file), data verification, standardization of the signal intensities; determination of similarity to 15 tissues of origin and generation of the Report.

The CEL file from each laboratory is transported from the Affymetrix Workstation with GeneChip® Operating System (GCOSDx) using the Pathwork File Transfer Software (PFTS) through a secure FTP transfer protocol to Pathwork Diagnostics for analysis. The Pathwork System Software (PSS) then converts the CEL file to gene expression measurements, performs data verification, normalizes (standardizes) the data to correct for technical sources of variation, performs a series of multiplex statistical tests, and produces a report summarizing these results. Each specimen analyzed will produce 15 Similarity Scores, one for each tissue on the panel. Each Similarity Score is a measure of the similarity of the gene expression profile of the specimen to the profile of the indicated tissue, ranging from 0 (very low similarity) to 100 (very high similarity). Similarity Scores for all 15 tissues sum to 100. For each Origin Test Kit-FFPE test performed, a test report is generated that quantifies the similarity of the RNA expression pattern found in a tumor specimen (poorly or undifferentiated primary tumors, as well as metastatic tumors) to expression patterns found in tumor specimens from 15 known tissues of origin and provided back to the laboratory over a secure internet connection in pdf format. Clinical laboratory customers are expected to use the resulting report in either its printed or electronic form for incorporation into the surgical pathology report delivered to the requesting physician by the laboratory.

The report quantifies the similarity of a poorly differentiated, undifferentiated or metastatic tumor specimen to 15 cancers of known tissue of origin, for interpretation by the clinician. The report presents 15 computed Similarity Scores in a graphical format, one for each tissue on the test panel. The ordering physician interprets the Origin Test Kit-FFPE Report using the Guide to Report Interpretation (GRI) which is provided as a component of the report are located directly below the results for Similarity Scores.

**Components included in the kit**

Microarray: Pathchip microarrays, packaged in boxes of 5 or 10.

Gene Expression Reagents: Reagents for specimen processing (extraction, amplification, labeling, hybridization, staining and washing).

**Report**

The Origin Test Kit-FFPE Report is produced by the Pathwork System Software by licensed agreement. The Pathwork® System Software receives data from the scanned Pathchip™ microarray and performs data quality control and analysis. The System Software then generates an Origin Test Kit-FFPE Report which provides a Similarity Score for each of the 15 tissues on the test panel. The test report is accessible for clinical interpretation via a secure password protected website.

**807.92 (a)(5): Intended Use**

Intended use: The Pathwork® Tissue of Origin Test is an in vitro diagnostic intended to measure the degree of similarity between the RNA expression patterns in a patient's formalin-fixed, paraffin-embedded (FFPE) tumor and the RNA expression patterns in a database of fifteen tumor types (poorly differentiated, undifferentiated and metastatic cases) that were diagnosed according to then current clinical and pathological practice. This test should be evaluated by a qualified physician in the context of the patient's clinical history and other diagnostic test results.

Limitations: The Pathwork Tissue of Origin Test is not intended to establish the origin of tumors (e.g. cancer of unknown primary) that cannot be diagnosed according to current clinical and pathological practice. It is not intended to subclassify or modify the classification of tumors that can be diagnosed by current clinical and pathological practice, nor to predict disease course or survival or treatment efficacy, nor to distinguish primary from metastatic tumor. Tumor types not in the Pathwork Tissue of Origin Test database may have RNA expression patterns that are similar to patterns in the database; therefore, results cannot be used to distinguish tumor types in the database from tumor types not in the database.

**807.92 (a)(6): Technological Similarities and Differences to Predicate****Table 6.1**

<b>Similarities</b>		
<b>Item</b>	<b>Tissue of Origin Test Kit - FFPE</b>	<b>Tissue of Origin- Frozen (K080896)</b>
Function	To determine the degree of similarity of a patient's test result to patterns of known characterized samples in a database	Same
Technology	Gene expression microarray; Computer based, software driven, data driven algorithm	Same
Indications for use	Tumor types	Same
Algorithm technology	Support Vector Machine (SVM)	Same
Required platform	Affymetrix GeneChip® GCS3000Dx Scanner and FS450Dx Fluidics Station	Same
Output	Similarity of RNA expression patterns found in tumor specimens to 15 known tissues of origin	Same
<b>Differences</b>		
<b>Item</b>	<b>Tissue of Origin Test Kit - FFPE</b>	<b>Tissue of Origin- Frozen (K080896)</b>
Test Sample	Formalin-fixed, paraffin-embedded tissues	Frozen biopsy tissues
Procedure	Two cycle amplification (polydT and random primers)	One cycle amplification (polydT primer)
Software	Pathwork System S/W 4.0 Algorithm Version 42.4	Pathwork System S/W 2.1 Algorithm Version 21.2

**807.92 (b)(1): Brief Description of Non-clinical data***a. Precision/Reproducibility*

Replicate samples from 60 individual tumors were distributed among the three laboratories for a total of 177 tissue samples (Site 1 did not receive two specimens and Site 3 did not receive one specimen due to insufficient stocks at the time specimen processing began). Of the 60 specimens, 27 (45%) were metastases and 33 (55%) were primaries classified as poorly differentiated to undifferentiated. This study required each laboratory to perform the full protocol from FFPE curls. The fifteen tumor tissue types (bladder, breast, colorectal, gastric, hepatocellular, kidney, non-small cell lung, ovarian, pancreatic, prostate, and thyroid carcinomas,

melanoma, testicular germ cell tumor, non-Hodgkin's lymphoma (not otherwise specified), and soft tissue sarcoma (not otherwise specified) were represented by four specimens each, with the exception of breast (n = 3) and soft tissue sarcoma (n = 5). All specimens had at least 60% viable tumor.

**Extraction of total RNA:** Of the 177 FFPE tissue curls under study, 176 yielded sufficient total RNAs (99%). Tissue curls were distributed over 20 batches and no batch failures were noted. **Amplification and labeling of total RNA:** Of the 176 total RNAs, 173 yielded sufficient sense RNAs (99%). Of the 173 sense RNAs, 164 yielded sufficient labeled cDNAs (95%). Of nine repeats, nine yielded sufficient labeled cDNAs, leading to an overall success rate of 173/176 or 98%. Total RNAs were distributed over 15 batches and one batch failure was noted. All positive/negative controls were in specification, with the exception of the control in the batch failure. **Microarray analysis:** Of the 173 labeled cDNAs, 155 yielded Tissue of Origin - Test FFPE Reports without data verification errors (90%). Note that errors were either low percent present or low overall signal or both. Of 16 repeats, seven yielded reports without errors. All positive/negative controls were in specification, with the exception of one in a batch failure. **In summary:** Of the 177 tissue curls under study, 162 Origin Test Kit-FFPE Reports without errors were produced (91%). Processing was performed by a different operator at each laboratory, and was performed in batches, extending over a several week period at each laboratory.

#### **Reproducibility of Similarity Scores:**

Matched pairs of specimens which were successfully processed at both laboratories were evaluated using linear regression and correlation analysis to evaluate the reproducibility of Similarity Score values. Across all three laboratories, three pair-wise comparisons showed slopes of 0.93 to 0.97 and "r" of 0.92 to 0.93.

Similarity Scores were also stratified to demonstrate reproducibility across the dynamic range of the test. For each specimen an average of the Similarity Scores associated with the actual tissue of origin (available diagnosis; AD) was calculated, along with standard deviation and percent coefficient of variation (% CV). For each Similarity Score range shown below (Table 6.2), the observed average standard deviation and average % CV results are provided.

**Table 6.2 Origin Test Kit-FFPE:  
Reproducibility of Similarity Scores across Laboratories**

Average Available Diagnosis Similarity Score	Specimens, n	Replicates*	Std. Deviation, avg.	CV%, avg.
0 to 20	7	20	6.5	54.5
20 to 40	11	31	8.7	25.8
40 to 60	11	32	16.9	36.2
60 to 80	14	36	12.9	18.5
80 to 100	14	41	6.6	7.8

\*Each specimen was tested as n = 3 (Laboratories 1-3). Missing replicates are due to depleted aliquots, insufficient yields, or data verification errors (low overall signal).

**Lab-to-Lab Concordance:**

In the lab-to-lab concordance analysis, concordance was strictly defined as an identical Pathwork test result between two laboratory sites for a paired specimen. The Pathwork test result was obtained by applying the Guide to Report Interpretation (see User Guide for details). For each specimen, an average of the highest Similarity Scores was calculated. For each Similarity Score range shown below (Table 6.3) the observed lab-to-lab concordance of Origin Test Kit-FFPE results is shown.

**Table 6.3 Origin Test Kit-FFPE: Lab-to-Lab Concordance as a function of Average Highest Similarity Scores**

Average Highest Similarity Score	Specimens, n	Pairs*	Concordant Pairs	Concordant Pairs as %
0 to 20	1	3	0/3	0
20 to 40	11	29	27/29	93.1
40 to 60	17	47	36/47	76.6
60 to 80	14	30	30/30	100
80 to 100	14	40	40/40	100
Overall	57	149	133/149	89.3

\*Each specimen was tested as n = 3 (Laboratories 1-3). Missing replicates are due to depleted aliquots, insufficient yields, or data verification errors (low overall signal).

To assess the accuracy of the results reported by each laboratory, the Origin Test Kit–FFPE results were compared to available diagnosis established for each specimen. Each laboratory was expected to achieve the overall acceptance criteria of at least 80% agreement with the available diagnosis, with no more than 20% nonagreement. As can be seen in Table 6.4, all three laboratory sites achieved the criteria. No laboratory reported more than 20% non-agreement. It is important to note that accuracy results in Table 6.4 are based on a relatively small sample set. Accuracy is best assessed by the multi-site clinical validation study (See Table 6.5 Origin Test Kit – FFPE Performance), which is based on n = 462 specimens.

**Table 6.4 Origin Test Kit - FFPE agreement with available diagnosis in lab-to-lab concordance study**

Tissue of Origin Test Kit - FFPE					
Stratification by Lab	# Specimens	% Agreement		% Non- Agreement	
		%	ratio	%	ratio
		[95% CI]		[95% CI]	
Site 1	52	82.7%	(43/52)	17.3%	(9/52)
		[69.7%, 91.8%]		[8.2%, 30.3%]	
Site 2	55	81.8%	(45/55)	18.2%	(10/55)
		[69.1%, 90.9%]		[9.1%, 30.9%]	
Site 3	55	81.8%	(45/55)	18.2%	(10/55)
		[69.1%, 90.9%]		[9.1%, 30.9%]	
All Sites (cumulative)	162	82.1%	(133/162)	17.9%	(29/162)
		[74.0%, 86.6%]		[13.4%, 26.0%]	

- b. *Linearity/assay reportable range:*  
Linearity is not applicable for this range of assay.
- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

#### Quality Control

A suitable total RNA sample should be run with every batch of specimens to serve as a positive/negative control as described in the Pathwork Specimen Processing Guide.

#### Specimen Processing Controls:

The following quality control checks are required during specimen processing to assure reliable results:

- 1) Amount of total RNA extracted from tissue specimens: minimum of 150 ng.
- 2) Amount of fragmented, labeled cDNA used for hybridization to Pathchip microarrays: 3.0 ( $\pm$  0.5)  $\mu$ g.

To ensure that a sufficient quantity of high quality labeled cDNA is obtained for hybridization to Pathchip microarrays, it is recommended that:

- 1) All procedures in the Pathwork Specimen Processing Guide are followed efficiently to reduce RNase degradation.
- 2) FFPE block size is sufficient to take a 5  $\mu\text{m}$  section for H&E staining and a 10  $\mu\text{m}$  +/- 1  $\mu\text{m}$  section (curl) with a minimum tissue area of at least 25 mm sq or equivalent for the test.
- 3) Specimen contain at least 60% viable tumor.
- 4) Absorbance measurements of total RNA give an A260/A280 ratio of greater than 1.0 (typically 1.0 to 2.2).
- 5) The minimum amount of labeled cDNA used for fragmentation is 2.5  $\mu\text{g}$ .

#### Endogenous Pathchip Controls:

In addition to these external controls, the Origin Test Kit–FFPE test utilizes endogenous mRNA markers that are found in human tissue specimens and are captured on the Pathchip microarray to perform a series of data verifications that detect laboratory processing anomalies found to unfavorably influence the accuracy of the Origin Test Kit – FFPE test. These data verifications include the detection of regional discontinuities and low overall signal. When a submitted file fails one or more of the data verifications, the system software will return an Origin Test Kit – FFPE Report flagged as “Unacceptable”, with caution messages appropriate for the specific type of failure that occurred.

#### Device stability:

Storage conditions for the Origin Test Kit - FFPE reagents range from -20° C to ambient temperature. Please refer to the Pathwork® Specimen Processing Guide for Formalin-Fixed, Paraffin-Embedded Specimens, as well as product inserts for individual storage requirements.

#### *d. Detection limit:*

##### Dilution study – Labeled cDNA:

A dilution study was performed to demonstrate that the Origin Test Kit – FFPE test is robust against reasonable variations in the amount of labeled cDNA used in hybridization. The study used three different FFPE specimens. RNA was extracted and processed to the point of labeled cDNA. For each specimen, Pathchip arrays were hybridized in triplicate with each of the five dilutions (1, 2, 4, 6 and 7.5  $\mu\text{g}$ ) of labeled cDNA. Subsequent processing of all 3 specimens x 5 dilutions x triplicates (= 45 arrays) was performed per the recommended protocol. One Pathchip array failed the percent present requirement, thus 44 arrays were used for further analysis. The data was analyzed using linear regression and correlation analysis to assess equivalence. Based on the data, the Pathwork® Tissue of Origin Test Kit–FFPE User Guide specifies  $3.0 \pm 0.5$   $\mu\text{g}$  labeled cDNA.

- e. *Analytical specificity:*  
Several potential interfering substances in tumor biopsies were evaluated for potential adverse effects.

**RNases:** Performance of the test using specimens derived from biopsies of the pancreas, a tissue known to contain high levels of endogenous ribonuclease (RNase), was equivalent to performance of the test for specimens derived from other tissue types. Nonetheless, Pathwork recommends that care be taken to limit specimen exposure to RNases during storage, extraction and processing of specimens.

**Necrotic tissue:** To examine whether necrotic tissue produce adverse effects in use of the Origin Test Kit-FFPE, the results from the FFPE specimens used in the clinical validation study were stratified by percent necrosis. Based on the % agreement, the Origin Test Kit-FFPE demonstrates adequate performance with up to 40% necrotic tissue in the sample.

- f. *Assay cut-off:*

#### Guide to Report Interpretation

The Similarity Score (SS) is a measure of the similarity of the RNA expression pattern of the specimen to the RNA expression pattern of the indicated tissue. Similarity Scores range from 0 (very low similarity) to 100 (very high similarity) and sum to 100 across all 15 tissues on the panel.

- The highest Similarity Score indicates the likely tissue of origin.
  - In male patients, a highest SS for ovarian, followed by a second highest SS for testicular germ cell, corresponds to testicular germ cell cancer.
- A Similarity Score less than or equal to 5 rules out that tissue type as the likely tissue of origin.
- Performance has not been established for results where the highest Similarity Score is less than 20.

**Performance:** Performance characteristics for the Tissue of Origin Test Kit-FFPE were established in a clinical validation study that included 462 specimens. Results matched corresponding available diagnoses 89% of the time. Similarity Scores less than or equal to 5 ruled out the corresponding tissue types as the tumor of origin 99% of the time.

**807.92 (b)(2): Brief Description of Clinical Data***a. Method comparison with available diagnosis:*

The clinical validation involved a total of three different processing laboratories. The study included 25 to 57 specimens per tissue on the panel, with an average of 31 specimens per tissue. The specimens included poorly differentiated, undifferentiated and metastatic tumor specimens. Of the total 598 tumor specimens processed in this study, 462 specimens met the labeling limitations for tumor grade and available diagnosis and passed the data verification quality tests. To assess the accuracy of the results reported by each laboratory, the Origin Test Kit–FFPE results were compared to available diagnosis established for each specimen. Based on the  $n = 462$  results, the probability that a true positive tissue call was obtained when a Similarity Score reported was 88.5%, 95% CI [85.3, 91.3]. Any tissue type with a Similarity Score less than or equal to 5 had a 99.8%, 95% CI [99.7, 99.9] probability of not being the tissue of origin. Thus, the Origin Test Kit–FFPE can be used to exclude origins from specific tissues on the Origin Test Kit–FFPE Panel if their Similarity Scores are less than or equal to 5. Each laboratory was expected to achieve the overall acceptance criteria of at least 80% agreement with the available diagnosis, with no more than 20% non-agreement.

The detailed results are presented in Tables 6.5 – 6.6.

**Positive Percent Agreement (PPA)** –  $100 \cdot TP/POS$ , where TP is the number of test results that match the available diagnoses for the given tissue of origin and POS is the total number of positive specimens as per available diagnosis for the given tissue of origin.

**Negative Percent Agreement (NPA)** –  $100 \cdot (1 - (FP/NEG))$ , where FP is the number of test results that are false positive (as per the available diagnoses) for the given tissue of origin and NEG is the number of negative specimens as per the available diagnosis for the given tissue of origin.

**Non-Agreement (%)** – the percent of POS specimens in which the Pathwork test result does not agree with the available diagnosis..

Table 6.5 ORIGIN TEST KIT - FFPE TEST PERFORMANCE\*

Available Diagnosis	Agreement		Non-Agreement		Negative Percent Agreement	Area Under ROC curve
	%	ratio	%	ratio	%	
	[95% CI]		[95% CI]		[95% CI]	
Bladder (BL)	79.3%	23/29	20.7	6/29	100	0.992
	[60.3, 92.0]		[8.0, 39.7]		[99.2, 100.0]	
Breast (BR)	96.5%	55/57	3.5	2/57	99	0.998
	[87.9, 99.6]		[0.4, 12.1]		[97.5, 99.7]	
Colorectal (CO)	91.7	33/36	8.3	3/36	96.9	0.989
	[77.5, 98.2]		[1.8, 22.5]		[94.8, 98.4]	
Gastric (GA)	72.0	18/25	28.0	7/25	98.6	0.978
	[50.6, 87.9]		[12.1, 49.4]		[97.0, 99.5]	
Hepatocellular (LI)	96.0	24/25	4.0	1/25	100	1.0
	[79.6, 99.9]		[0.1, 20.4]		[99.2, 100.0]	
Kidney (KI)	89.3	25/28	10.7	3/28	99.5	0.996
	[71.8, 97.7]		[0.3, 28.2]		[98.3, 99.9]	
Melanoma (ME)	84.0	21/25	16.0	4/25	100	1.0
	[63.9, 95.5]		[0.5, 36.1]		[99.2, 100.0]	
Non-Hodgkin's Lymphoma (LY)	89.7	26/29	10.3	3/29	99.5	0.997
	[72.6, 97.8]		[2.2, 27.4]		[98.3, 99.9]	
Non-small Cell Lung (LU)	85.2	23/27	14.8	4/27	100	0.998
	[66.3, 95.8]		[4.2, 33.7]		[99.2, 100.0]	
Ovarian (OV)	88.9	40/45	11.1	5/45	98.8	0.97
	[75.9, 96.3]		[3.7, 24.1]		[97.2, 99.6]	
Pancreas (PA)	85.7	24/28	14.3	4/28	98.8	0.993
	[67.3, 96.0]		[4.0, 32.7]		[97.3, 99.6]	
Prostate (PR)	96.0	24/25	4.0	1/25	100	0.997
	[79.6, 99.9]		[0.1, 20.4]		[99.2, 100.0]	
Sarcoma (SC)	88.9	24/27	11.1	3/27	96.3	0.967
	[70.8, 97.6]		[2.4, 29.2]		[94.1, 97.9]	
Testicular Germ Cell (GC)	84.0	21/25	16.0	4/25	100	0.998
	[63.9, 95.5]		[4.5, 36.1]		[99.2, 100.0]	
Thyroid (TH)	90.3	28/31	9.7	3/31	100	0.98
	[74.2, 98.0]		[2.0, 25.8]		[99.1, 100.0]	
Overall	88.5	409/462	11.5	53/462	99.1	
	[85.3, 91.3]		[8.7, 14.7]		[97.6, 99.7]	

**Table 6.6 Overall Tissue of Origin Test Performance:  
Stratification by Metastatic and Primary Tumor Specimens**

Metastasis or Primary	Agreement		Non-Agreement	
	%	ratio	%	ratio
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
	95% CI [85.3, 91.3]		95% CI [8.7, 14.7]	

b. *Matrix comparison:*  
Not applicable

c. *Clinical studies:*

a. *Clinical Sensitivity:*

Not examined because a reference standard defining diagnostic truth was not employed for the clinical studies. Instead, positive percent agreement with the available diagnosis was considered (Tables 6.5 and 6.6).

*Clinical specificity:*

Not examined because a reference standard defining diagnostic truth was not employed for the clinical studies. Instead, negative percent agreement with the available diagnosis was considered (Tables 6.5 and 6.6).

b. *Performance of the Tissue of Origin Test for Off-Panel Specimens*

Tissue specimens that are off-panel (i.e. not one of the fifteen tissues on the Tissue of Origin Test panel) were assessed for similarity in RNA expression pattern with one of the 15 tissues on the panel. A review of published sources and interviews with practitioners were conducted to identify the cancers not included in the 15 tissues on the Origin Test Kit–FFPE test panel that should be evaluated in this study. Criteria for selection included:

- Commonly known to metastasize
- Challenging or difficult to diagnose
- Likely to present as an uncertain primary cancer

This review selected the following: cancer of the uterine cervix, endometrium, esophagus, small cell lung, and squamous cell carcinoma of the head & neck. The study involved 101 off-panel tissue specimens from tissues from tumors of CNS origin, adult (CN, n = 13), endometrium (EN, n = 12), esophagus (ES, n = 11), head and neck, squamous, (HN, n = 10), mesothelioma (MT, n = 10), neuroendocrine (NE, n = 12), ovarian germ cell (OG, n = 11), small cell lung (SL, n = 11), and uterine cervix (UC, n = 11), of which 82/101 (81.2%) yielded Origin Test Kit – FFPE results.

### Results:

Table 6.7 shows off-panel specimen results and how highest Similarity Scores are distributed across the 15 tissues on the Origin Test Kit–FFPE panel. For off-panel tissue specimens, this indicates a false positive association with that cancer. For each off panel tissue type, a high false positive percentage was observed, especially for several cancers (CN, EN, MT, OG) with ovarian and HN and SL with non-small cell lung.

**Table 6.7 Off-panel tumor types with RNA expression patterns that are similar to patterns in the database.**

Result Specimen	Total Specimens (n = 82)	Distribution of Results across the 15 tissues on the Origin Test Kit - FFPE panel														
		BL	BR	CO	GA	GC	LI	KI	LY	LU	ME	OV	PA	PR	SC	TH
Test Result	n															
CNS Origin (CN)	13					1						7			5	
Endometrium (EN)	8											7			1	
Esophagus (ES)	7	1		3	1					2						
Head & Neck, sq. (HN)	9		1	2				1		5						
Mesothelioma (MT)	9											9				
Neuroendocrine (NE)	10			1							4	2		2	1	
Ovarian Germ Cell (OG)	10											9			1	
Small Cell Lung (SL)	10			1						5		4				
Uterine cervix (CX)	6	2		1						2					1	
Total	82	3	1	8	1	1	0	1	0	14	0	40	2	0	10	1

- d. *Other clinical supportive data (when a. and b. are not applicable):*  
None

### 807.92 (b)(3): Conclusions from Clinical Testing

The results of the clinical validation demonstrated that the Pathwork Tissue of Origin Test Kit-FFPE is substantially equivalent to the Pathwork Tissue of Origin Test (K080896)



DEPARTMENT OF HEALTH & HUMAN SERVICES

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Food and Drug Administration  
10903 New Hampshire Avenue  
Document Mail Center – WO66-0609  
Silver Spring, MD 20993-0002

Pathwork® Diagnostics  
c/o Ms. Anna Longwell, MS, JD  
Regulatory Consultant  
595 Penobscot Drive  
Redwood City, CA 94063

JUN 09 2010

Re: k092967

Trade/Device Name: Pathwork® Tissue of Origin Test Kit.- FFPE  
Regulation Number: 21 CFR §862.3100  
Regulation Name: Amphetamine Test System  
Regulatory Class: Class II  
Product Code: OIW  
Dated: June 7, 2010  
Received: June 8, 2010

Dear Ms. Longwell:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of

Page 2 – Ms. Anna Longwell, MS, JD

requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to <http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm> for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address <http://www.fda.gov/cdrh/industry/support/index.html>.

Sincerely yours,



Maria M. Chan, Ph.D  
Director  
Division of Immunology and Hematology Devices  
Office of *In Vitro* Diagnostic Device Evaluation and Safety  
Center for Devices and Radiological Health

Enclosure

## Indications for Use

510(k) Number (if known): k092967

Device Name: Pathwork® Tissue of Origin Test Kit - FFPE

### Indications For Use:

The Pathwork® Tissue of Origin Test is an in vitro diagnostic intended to measure the degree of similarity between the RNA expression patterns in a patient's formalin-fixed, paraffin-embedded (FFPE) tumor and the RNA expression patterns in a database of fifteen tumor types (poorly differentiated, undifferentiated and metastatic cases) that were diagnosed according to then current clinical and pathological practice. This test should be evaluated by a qualified physician in the context of the patient's clinical history and other diagnostic test results.

Limitations: The Pathwork® Tissue of Origin Test is not intended to establish the origin of tumors (e.g. cancer of unknown primary) that cannot be diagnosed according to current clinical and pathological practice. It is not intended to subclassify or modify the classification of tumors that can be diagnosed by current clinical and pathological practice, nor to predict disease course or survival or treatment efficacy, nor to distinguish primary from metastatic tumor. Tumor types not in the Pathwork® Tissue of Origin Test database may have RNA expression patterns that are similar to patterns in the database. Therefore, results cannot be used to distinguish tumor types in the database from tumor types not in the database.

Prescription Use    
 (Part 21 CFR 801 Subpart D)

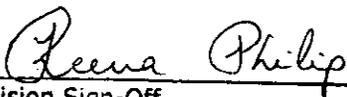
AND/OR

Over-The-Counter Use    
 (21 CFR 807 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

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Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

  
Division Sign-Off

Office of In Vitro Diagnostic  
Device Evaluation and Safety

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510K k092967