510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k080896

B. Purpose for Submission:
New device

C. Measurand:
Gene expression profile for 15 common tumor types

D. Type of Test:
Gene expression microarray

E. Applicant:
Pathwork Diagnostics Inc.

F. Proprietary and Established Names:
Pathwork® Tissue of Origin Test

G. Regulatory Information:
1. Regulation section:
   21 CFR § 862.3100 Amphetamine Test System
2. Classification:
   Class II
3. Product code:
   OIW, Software, similarity score algorithm, tissue of origin for malignant tumor types
4. Panel:
   Toxicology (91)

H. Intended Use:
1. Intended use(s):
   The Pathwork® Tissue of Origin Test is intended to measure the degree of similarity between the RNA expression pattern in a patient's fresh-frozen tumor and the RNA expression patterns in a database of tumor samples (poorly differentiated, undifferentiated and metastatic cases) that were diagnosed according to then current clinical and pathological practice. The database contains examples of RNA expression patterns for fifteen common malignant tumor types: bladder, breast, colorectal, gastric, hepatocellular, kidney, non-small cell lung, ovarian, pancreatic, prostate, and thyroid carcinomas, melanoma, testicular germ cell tumor, non-Hodgkins lymphoma (not otherwise specified), and soft tissue sarcoma (not otherwise specified). The Pathwork® Tissue of Origin Test result is intended for use in the context of the patient's clinical history and other diagnostic tests evaluated by a qualified clinician.

Limitations: The Pathwork® Tissue of Origin Test is not intended to establish the origin of tumors (e.g. carcinoma 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.
2. Indication(s) for use:
   Same as Intended use
3. Special conditions for use statement(s):
   For prescription use only
4. Special instrument requirements:
   Affymetrix GeneChip® Microarray Instrumentation System (k080995)

I. Device Description:

The Pathwork® Tissue of Origin Test is a test kit consists of the Pathchip microarray, reagents, software, Pathwork Specimen Processing Guide and Guide to Report Interpretation (GRI).

The Pathchip is a custom-designed microarray manufactured by Affymetrix, Inc. (Santa Clara, CA) per Pathwork design requirements and functions in a manner similar to GeneChip HG-U133A. The Pathchip has over 500,000 unique oligonucleotide features (18-micron in size), covering over 18,400 transcript 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 1550 probesets representing 1550 human genes which are used by the Tissue of Origin Test 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 121 probesets that are used for normalization and data verification.

The algorithm of the Pathwork® Tissue of Origin Test was developed using a database of 2039 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 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 Report.

The Pathwork Specimen Processing Guide contains instructions for the user to process tissue specimens in the manner specimens were processed during the clinical validation of the

Tissue of Origin Test.

J. Substantial Equivalence Information:
1. Predicate device name(s):
   The BioPlex™ 2200 Medical Decision Support Software (MDSS)
2. Predicate 510(k) number(s):
   k043341
3. Comparison with predicate:

Similarities		
Item	**Tissue of Origin Test**	**Bioplex 2200 Medical Decision Support Software**
Function	To determine the degree of similarity of a patient's test result to patterns of known characterized samples in a database	To associate patient results with predefined profiles that have been correlated with defined diseases
Technology	Computer based, software driven, data driven algorithm	Same
Differences		
Item	**Tissue of Origin Test**	**Bioplex 2200 Medical Decision Support Software**
Indications for use	Tumor types	Autoimmune diseases
Algorithm technology	Support Vector Machine (SVM)	"k-nearest neighbor" (kNN) statistical techniques
Input	Intensity data file from RNA expression array	Results from serological analysis of patient serum or plasma for specific autoantibodies
Test Sample	Frozen biopsy tissues	Serum or plasma
Required Platform	Affymetrix GeneChip® GCS3000Dx Scanner and FS450Dx Fluidics Station	BioPlex 2200 Multi-Analyte Detection System
Output	Similarity of RNA expression patterns found in tumor specimens to 15 known tissues of origin	Results of MDSS analysis fall into one of three categories: Negative, No Association, or Association with Disease

K. Standard/Guidance Document Referenced (if applicable):
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Class II Special Controls Guidance Document: Gene Expression Profiling Test System for Breast Cancer Prognosis
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Class II Special Controls Guidance Document: Drug Metabolizing Enzyme Genotyping System

L. Test Principle:

The specimen used for the Pathwork® Tissue of Origin Test is a snap-frozen surgical sample. Prior to processing for the TOO test, the user must ensure that the biopsy specimen is no less than 100 mg in mass, and contains at least 60% tumor and no more than 20% necrosis. The tissue must be homogenized and total RNA must be isolated per the Pathwork Specimen Processing Guide. One microgram of total RNA is required to perform the TISSUE OF ORIGINtest. The procedural steps after isolation of total RNA from the specimen are: reverse transcription of total RNA; first and second strand cDNA synthesis; cDNA purification; cRNA synthesis and biotinylation; purification and fragmentation of the labeled cRNA; hybridization of the biotin-labeled cRNA target to the Pathchip microarray; washing and scanning of the hybridized Pathchip microarray; data acquisition (signal intensity per feature), data verification, standardization of the signal intensities; determination of similarity to 15 tissues of origin and generation of the Report.

The scanned data file from each laboratory is transported from the Affymetrix Workstation with GeneChip® Operating System (GCOSDx) for analysis using the Pathwork System Software through a secure FTP transfer protocol. The Pathwork system software converts the scanned image data to gene expression measurements, performs the 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 necessarily sum up to 100. For each Tissue of Origin test performed, a test report is generated that quantifies the similarity of the RNA expression pattern found in a tumor specimen (poorly or un-differentiated primary tumors, as well as metastatic tumors) to expression patterns found in tumor specimens from 15 known tissues of origin and provided 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 Pathwork® Tissue of Origin Test using the Guide to Report Interpretation (GRI) which is provided as a component of the report directly below the results for Similarity Scores. The Guide to Report Interpretation requires the physician to use knowledge of the location of the tumor biopsy site to correctly interpret the report.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

    a. *Precision/Reproducibility:*

    Replicate samples from 60 individual tumors were distributed among the four laboratories for a total of 234 tissue samples (Site 2 and 4 did not receive samples from gastric tumors due to insufficient tumor volume). Of the 60 specimens, 46 (77%) were metastases and 14 (23%) were primaries classified as poorly

differentiated to undifferentiated. This study required each laboratory to perform the full protocol from frozen tissue, which had been subjected to the additional handling and transport required to aliquot a frozen tissue specimen. Five tumor tissue types (breast, colorectal, non-small-cell lung, non-Hodgkin's lymphoma and pancreas) were represented by six specimens each and all other tissue types (bladder, gastric, germ cell, hepatocellular, kidney, melanoma, ovarian, prostate, soft tissue sarcoma and thyroid) were represented by three specimens each. The study was published in Journal of Molecular Diagnostics, Volume 10, Number 1, January 2008, pp 67-77.

Specimens with < 60% viable tumor or > 20% necrosis were excluded from the analyses and the resulting performance characteristics. A total of 206 tissue samples met this inclusion criterion. Fourteen (14) of the 206 samples (6.8%) admitted to the study failed one of the Quality Control steps. Five samples (2.4%) across two sites failed to yield sufficient total RNA, (a minimum of 1 μg is required), and were unavailable for further processing. Nine specimens (4.4%) across three sites failed the data verification test, reporting Low Overall Signal when analyzed by the Pathwork System Software. A total of 192 of the 206 samples (93.2%) admitted to the study met all Quality Control criteria.

Processing was performed by a different operator at each site, and was performed in batches, extending over a 2 to 5 week period. Matched pairs of specimens which were successfully processed at both sites were evaluated using linear regression and correlation analysis to evaluate the reproducibility of the underlying Standardized Expression values of the markers used in the test.

In this analysis, concordance was strictly defined as an identical Pathwork test result between two laboratory sites for a paired specimen. (An indeterminate result was concordant only with another indeterminate result at the second lab.) The Pathwork test result was obtained by applying the Guide to Report Interpretation (see Dumar et al. for details). Across all four laboratories, six pair-wise comparisons showed slopes of 0.87 to 0.92 and "r" of 0.84 to 0.90

Table 1 – Lab to Lab Concordance (Regression analysis Standardized expression (SE))

Comparison	# specimens	Total Points	Intercept	Slope	R
Site 2 vs. Site 1	45	75060	0.09 (0.09, 0.09)	0.89 (0.89. 0.90)	0.90 (0.90, 0.90)
Site 3 vs. Site 1	46	76728	0.04 (0.03, 0.04)	0.91 (0.91, 0.92)	0.90 (0.90, 0.90)
Site 4 vs. Site 1	46	76728	0.15 (0.15, 0.150	0.87 (0.87, 0.88)	0.84 (0.84, 0.84)
Site 3 vs. Site 2	47	78396	-0.02 (-0.02, -0.02)	0.92 (0.92, 0.92)	0.90 (0.90, 0.91)
Site 4 vs. Site 2	45	75060	0.10 (0.09, 0.10)	0.89 (0.89, 0.89)	0.85 (0.85, 0.86)
Site 4 vs. Site 3	44	73392	0.14 (0.14, 0.15)	0.87 (0.87, 0.88)	0.85 (0.85, 0.85)

Reproducibility of Similarity Score: In the reproducibility study, each specimen

was tested at the four sites as noted and, for each specimen, an average of the Similarity Scores associated with the actual tissue of origin was calculated, along with standard deviation and percent coefficient of variation (%CV). For each Similarity Score range shown below, the observed average standard deviation and average %CV results are provided. Reproducibility of the test is shown in bold for similarity scores between 20-40, because this interval includes 30, the cut-off given in the Guide to Report Interpretation:

Table 2: Reproducibility of Similarity Scores Across Sites

Reproducibility of Similarity Scores				
Similarity Score Range	Specimens, n	Replicates*	Std. Deviation, avg.	CV%, avg.
0 to 20	3	12	4.0	37.6
20 to 40	**4**	**12**	**9.7**	**28.9**
40 to 60	2	8	8.2	15.2
60 to 80	19	74	13.9	20.2
80 to 100	23	82	4.7	5.3

*Each specimen was tested as n = 4 (Sites 1-4). 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 Physician Guided Conclusions 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 10% non-agreement. As can be seen in the table below, all four laboratories achieved the criteria, with Site 2 as the poorest performer with 87.8% agreement. No laboratory reported more than 5% non-agreement.

Table 3 Physician Guided Conclusion (PGC) agreement with available diagnosis

Stratification	# Specimens	% Agreement	% Non-agreement	% Indeterminate
Site 1	48	93.8% (45/48)	2.1% (1/48)	4.2% (2/48)
Site 2	49	87.8% (43/49)	4.2% (2/49)	8.2% (4/49)
Site 3	48	91.7% (44/48)	2.1% (1/48)	6.3% (3/48)
Site 4	47	91.5% (43/47)	<0.1% (0/47)	8.55 (4/47)
Overall	192	91.2% (175/192)	2.1% (4/192)	6.9% (13/192)

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
   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 1.0 μg.

2) Amount of fragmented, labeled cRNA used for hybridization to Pathchip microarrays: 10 μg.

To ensure that a sufficient quantity of high quality labeled cRNA 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) No more than 60 minutes between excision and freezing of the biopsy specimen.
3) A minimum of 100 mg tissue be used as starting material for extraction of total RNA.
4) Biopsy specimen should contain at least 60% tumor and no more than 20% necrosis
5) Absorbance measurements of total RNA give an A260/A280 ratio of at least 1.7.
6) Amount of labeled cRNA used for fragmentation: minimum of 15 μg.

Endogenous Pathchip Controls:
In addition to these external controls, the Pathwork Tissue of Origin 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 Pathwork Tissue of Origin 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 a Tissue of Origin Test Report flagged as follows: "The gene expression data file submitted failed data verification. This can be caused by a poor quality specimen or an error in laboratory processing. Specifics shown in CAUTION below."

Device stability: The shelf life of Pathchip is one year from the date of manufacture when stored at 2º C to 8ºC.

d. *Detection limit:*
Dilution study _ Labeled cRNA:
Dilution study was performed to demonstrate that the Tissue of Origin test is robust against reasonable variations in the amount of labeled cRNA used in hybridization. The study used 15 different frozen human tissue (FHT) specimens, three (3) each from breast, colorectal, lung, lymphoma and pancreas tissues of origin. RNA was extracted and processed to the point of labeled cRNA. For each specimen, Pathchip arrays were hybridized with each of the five dilutions (20, 15, 10, 7.5 and 5 μg) of labeled cRNA. Subsequent processing of all 15 specimens x 5 dilutions (= 75 arrays) were performed per the recommended protocol. Four samples from one specimen were excluded because of low data verification values. The data was analyzed using linear regression and correlation analysis to assess equivalence. Based on the data, the Tissue of Origin Test User Guide specifies 10 ± 2 μg cRNA.

e. *Analytical specificity:*
Several potential interfering substances in tumor biopsy were evaluated for potential adverse effects.
Adipose Tissue: To examine whether the adipose tissue in breast tumors is a

potential interferrent and produce adverse effects in the Tissue of Origin test, Physician Guided Conclusion (PGC) from 69 breast-related specimens included in the clinical validation were compared to its available clinical diagnosis. Available clinical diagnosis from Breast-related specimens were in 94.2% agreement with PGC, compared to all specimens which had 87.8% agreement.

RNases: To examine whether RNases in pancreas tumors produce adverse effects in Tissue of Origin test, the results from FHT biopsy specimens were stratified to evaluate pancreatic tumors for RNases interference. The clinical validation included 25 pancreas-related specimens. Available clinical diagnoses from pancreas-related specimens were in 72% agreement with PGC, compared to all specimens which had 87.8% agreement. Since there is a lower performance than for all the other specimens, this is cited under Limitations in the user guide.

Fibrous material: To examine whether fibrous material in skin-related specimens, produce adverse effects in the Tissue of Origin Test, the results from FHT biopsy specimens used in the clinical validation were stratified to evaluate the effect from melanomas and skin-related biopsies. The clinical validation included 27 skin-related specimens. Available clinical diagnoses from skin-related specimens were in 81.5% agreement with PGC, compared to all specimens which had 87.8% agreement. Tissue of Origin Test does not demonstrate apparent adverse effects with this interfering substance.

Necrotic tissue: To examine whether necrotic tissue produce adverse effects in use of the Tissue of Origin Test, the results from the FHT biopsy specimens used in the clinical validation study were stratified by percent necrosis. Based on the % agreement, a cut-off of 20% necrosis or less is recommended as a quality control measure for biopsy specimen inclusion criteria for this test.

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.

$SS \geq 30$:   A single SS greater than or equal to 30 indicates the likely tissue of origin.

If two or three SS's are greater than or equal to 30, then one of those results indicates the likely tissue of origin.

$5 < SS < 30$:   If every SS is between 5 and 30, then the test result is indeterminate and no tissue of origin is indicated.

$SS \leq 5$:   An SS less than or equal to 5 rules out that tissue type as the likely tissue of origin.

2. <u>Comparison studies:</u>
   a. Method comparison with available diagnosis:
   The clinical validation involved a total of four different processing sites. The study included 25 to 69 samples per tissue on the panel, with an average of 36 specimens

per tissue. The specimens included poorly differentiated, undifferentiated and metastatic tumor specimens. Of the total 659 tumor specimens processed in this study, 545 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 Physician Guided Conclusions were compared to available diagnosis established for each specimen. Based on the n = 545 results, the probability that a true positive tissue call was obtained when a Similarity Score of $\geq$ 30 was reported was 92.9%, 95% CI [90.3, 95.0], and the probability that a true negative tissue call was obtained when a Similarity Score of $\leq$ 5 was reported was 99.7%, 95% CI [99.6, 99.8]. Each laboratory was expected to achieve the overall acceptance criteria of at least 80% agreement with the available diagnosis, with no more than 10% non-agreement.

The detailed results are presented in Tables 4 – 7.

Positive Percent Agreement (PPA) –100*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*(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 and is not indeterminate.

Indeterminate (%) – the percent of POS specimens in which the Pathwork test result is indeterminate.

Area Under ROC Curve (AUC) – is the size of the area under the Receiver Operating Characteristic (ROC) curve. The ROC curve for the Tissue of Origin Test is obtained by plotting PPA/100 (i.e., sensitivity) vs. one minus NPA/100 (i.e., one minus specificity) for different values of the Similarity Score cut-off. (AUC ranges from 0.0 to 1.0, where 0.5 is achievable by a random test and 1.0 indicates perfect agreement).

Table 4: TISSUE OF ORIGIN TEST PERFORMANCE*

Available Diagnosis	Positive Percent Agreement (%, ratio, 95% CI)	Negative Percent Agreement (%, ratio, 95% CI)	Non-Agreement (%, ratio, 95% CI)	Indeterminate (%, ratio, 95% CI)	Area Under ROC Curve
Bladder	78.6% (22/28) [59.0, 91.7]	100.0% (517/517) [99.3, 100.0]	14.3% (4/28) [4.0, 32.7]	7.1% (2/28) [0.9, 23.5]	0.996
Breast	94.1% (64/68) [85.6, 98.4]	98.5% (470/477) [97.0, 99.4]	5.9% (4/68) [1.6, 14.4]	< 0.1% (0/68) [0.0, 4.3]	0.979
Colorectal	94.6% (53/56) [85.1, 98.9]	99.2% (485/489) [97.9, 99.8]	5.4% (3/56) [1.1, 14.9]	< 0.1% (0/56) [0.0, 5.2]	0.980
Gastric	76.0% (19/25) [54.9, 90.6]	99.8% (519/520) [98.9, 100.0]	16.0% (4/25) [4.5, 36.1]	8.0% (2/25) [1.0, 26.0]	0.970
Hepatocellular	92.0% (23/25) [74.0, 99.0]	99.8% (519/520) [98.9, 100.0]	<0.1% (0/25) [0.0, 11.3]	8.0% (2/25) [1.0, 26.0]	0.999
Kidney	94.9% (37/39) [82.7, 99.4]	99.8% (505/506) [98.9, 100.0]	2.6% (1/39) [0.1, 13.5]	2.6% (1/39) [0.1, 13.5]	0.975
Melanoma	84.6% (22/26) [65.1, 95.6]	99.8% (518/519) [98.9, 100.0]	7.7% (2/26) [0.9, 25.1]	7.7% (2/26) [0.9, 25.1]	0.999
Non-Hodgkin's Lymphoma	93.9% (31/33) [79.8, 99.3]	99.4% (509/512) [98.3, 99.9]	3.0% (1/33) [0.1, 15.8]	3.0% (1/33) [0.1, 15.8]	0.999
Non-small Cell Lung	90.3% (28/31) [74.2, 98.0]	98.6% (507/514) [97.2, 99.5]	6.5% (2/31) [0.8, 21.4]	3.2% (1/31) [0.1, 16.7]	0.991
Ovarian	94.2% (65/69) [85.8, 98.4]	99.4% (473/476) [98.2, 99.9]	2.9% (2/69) [0.4, 10.1]	2.9% (2/69) [0.4, 10.1]	0.996
Pancreas	76.0% (19/25) [54.9, 90.6]	99.8% (519/520) [98.9, 100.0]	16.0% (4/25) [4.5, 36.1]	8.0% (2/25) [1.0, 26.0]	0.953
Prostate	88.5% (23/26) [69.8, 97.6]	100.0% (519/519) [99.3, 100.0]	3.8% (1/26) [0.1, 19.6]	7.7% (2/26) [0.9, 25.1]	0.998
Soft-tissue Sarcoma	83.9% (26/31) [66.3, 94.5]	99.4% (511/514) [98.3, 99.9]	9.7% (3/31) [2.0, 25.8]	6.5% (2/31) [0.8, 21.4]	0.972
Testicular Germ Cell	82.1% (23/28) [63.1, 93.9]	100.0% (517/517) [99.3, 100.0]	3.6% (1/28) [0.1, 18.3]	14.3% (4/28) [4.0, 32.7]	0.999
Thyroid	91.4% (32/35) [76.9, 98.2]	99.6% (508/510) [98.6, 100.0]	5.7% (2/35) [0.7, 19.2]	2.9% (1/35) [0.1, 14.9]	0.976
Overall	89.4% (487/545) [86.5, 91.8]	99.6% (507/509) [98.6, 100.0]	6.2% (34/545) [4.4, 8.6]	4.4% (24/545) [2.8, 6.5]	

*As described in the Guide to Report Interpretation, if two or three SS are greater than or equal to 30, then one of those results indicates the likely tissue of origin. In the clinical validation study, this occurred in 11 of 545 specimens or 2%.

In Table 4 above, if one of the two SS was the tissue of origin, this was counted as an agreement. In Table 5 below, this was counted as a non-agreement due to presence of a second tissue (non-agreement). Note that for each of the eleven specimens, one of the two SS ≥ 30 did, indeed, correspond to the actual tissue of origin.

Table 5: TISSUE OF ORIGIN TEST PERFORMANCE
(Alternative treatment of results with multiple Similarity Scores ≥ 30)

Available Diagnosis	Positive Percent Agreement (%, ratio, 95% CI)	Negative Percent Agreement (%, ratio, 95% CI)	NonAgreement (%, ratio, 95% CI)	Indeterminate (%, ratio, 95% CI)	Area Under ROC Curve
Bladder	78.6% (22/28) [59.0, 91.7]	100.0% (517/517) [99.3, 100.0]	14.3% (4/28) [4.0, 32.7]	7.1% (2/28) [0.9, 23.5]	0.996
Breast	94.1% (64/68) [85.6, 98.4]	98.3% (469/477) [96.7, 99.3]	5.9% (4/68) [1.6, 14.4]	< 0.1% (0/68) [0.0, 4.3]	0.979
Colorectal	92.9% (52/56) [82.7, 98.0]	98.8% (483/489) [97.3, 99.5]	7.1% (4/56) [2.0, 17.3]	< 0.1% (0/56) [0.0, 5.2]	0.980
Gastric	64.0% (16/25) [42.5, 82.0]	99.4% (517/520) [98.3, 99.9]	28.0% (7/25) [12.1, 49.4]	8.0% (2/25) [1.0, 26.0]	0.970
Hepatocellular	92.0% (23/25) [74.0, 99.0]	99.8% (519/520) [98.9, 100.0]	< 0.1% (0/25) [0.0, 11.3]	8.0% (2/25) [1.0, 26.0]	0.999
Kidney	94.9% (37/39) [82.7, 99.4]	99.8% (505/506) [98.9, 100.0]	2.6% (1/39) [0.1, 13.5]	2.6% (1/39) [0.1, 13.5]	0.975
Melanoma	76.9% (20/26) [56.4, 91.0]	99.8% (518/519) [98.9, 100.0]	15.4% (4/26) [4.4, 34.9]	7.7% (2/26) [0.9, 25.1]	0.999
Non-Hodgkin's Lymphoma	93.9% (31/33) [79.8, 99.3]	99.4% (509/512) [98.3, 99.9]	3.0% (1/33) [0.1, 15.8]	3.0% (1/33) [0.1, 15.8]	0.999
Non-small Cell Lung	87.1% (27/31) [70.2, 96.4]	98.4% (506/514) [97.0, 99.3]	9.7% (3/31) [2.0, 25.8]	3.2% (1/31) [0.1, 16.7]	0.991
Ovarian	92.8% (64/69) [83.9, 97.6]	98.5% (469/476) [97.0, 99.4]	4.3% (3/69) [0.9, 12.2]	2.9% (2/69) [0.4, 10.1]	0.996
Pancreas	72.0% (18/25) [50.6, 87.9]	99.8% (519/520) [98.9, 100.0]	20.0% (5/25) [6.8, 40.7]	8.0% (2/25) [1.0, 26.0]	0.953
Prostate	88.5% (23/26) [69.8, 97.6]	100.0% (519/519) [99.3, 100.0]	3.8% (1/26) [0.1, 19.6]	7.7% (2/26) [0.9, 25.1]	0.998
Soft-tissue Sarcoma	83.9% (26/31) [66.3, 94.5]	99.2% (510/514) [98.0, 99.8]	9.7% (3/31) [2.0, 25.8]	6.5% (2/31) [0.8, 21.4]	0.972
Testicular Germ Cell	75.0% (21/28) [55.1, 89.3]	100.0% (517/517) [99.3, 100.0]	10.7% (3/28) [2.3, 28.2]	14.3% (4/28) [4.0, 32.7]	0.999
Thyroid	91.4% (32/35) [76.9, 98.2]	99.6% (508/510) [98.6, 100.0]	5.7% (2/35) [0.7, 19.2]	2.9% (1/35) [0.1, 14.9]	0.976
Overall	87.3% (476/545) [84.3, 90.0]	99.4% (506/509) [98.3, 99.9]	8.3% (45/545) [6.1, 10.9]	4.4% (24/545) [2.8, 6.5]	

Table 6: Overall Tissue of Origin Test Performance:
Stratification by Metastatic and Primary Tumor Specimens

Type of Tumor Specimen	Positive Percent Agreement (%, ratio, 95% CI)	Negative Percent Agreement (%, ratio, 95% CI)	NonAgreement (%, ratio, 95% CI)	Indeterminate (%, ratio, 95% CI)
Metastasis	86.3% (221/256) [81.5, 90.3]	99.6% (238/239) [97.7, 100.0]	8.6% (22/256) [5.5, 12.7]	5.1% (13/256) [2.7, 8.5]
Primary	92.0% (266/289) [88.3, 94.9]	99.6% (269/270) [98.0, 100.0]	4.2% (12/289) [2.2, 7.1]	3.8% (11/289) [1.9, 6.7]

Table 7: Overall Tissue of Origin Test Performance:
Stratification by Metastatic and Primary Tumor Specimens
(Alternative treatment of results with multiple Similarity Scores ≥ 30)

Type of Tumor Specimen	Positive Percent Agreement (%, ratio, 95% CI)	Negative Percent Agreement (%, ratio, 95% CI)	Non-Agreement (%, ratio, 95% CI)	Indeterminate (%, ratio, 95% CI)
Metastasis	83.6 (214/256) [78.5, 87.9]	99.2% (237/239) [97.0, 99.9]	11.3% (29/256) [7.7, 15.9]	5.1% (13/256) [2.7, 8.5]
Primary	90.7% (262/289) [86.7, 93.8]	99.6% (269/270) [98.0, 100.0]	5.5% (16/289) [3.2, 8.8]	3.8% (11/289) [1.9, 6.7]

    b. *Matrix comparison:*
       Not applicable
  3. <u>Clinical studies</u>:
    a. *Clinical Sensitivity:*
       Not examined because a reference standard defining diagnostic truth was not considered for the clinical studies. Instead, positive percent agreement with the available diagnosis was considered (Tables 4 and 5).
       Clinical specificity:
       Not examined because a reference standard defining diagnostic truth was not considered for the clinical studies. Instead, negative percent agreement with the available diagnosis was considered (Tables 4 and 5).
    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 Pathwork Tissue of Origin (TOO) 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 143 off-panel tissue specimens from uterine cervix (n = 42), endometrium (n = 49), esophagus (n = 28), small cell carcinoma of lung (n = 4), ovarian germ cell (n=2), and squamous cell carcinoma of the head and neck (Sq. Head & Neck; n = 18).

Results:
Table 8 shows the performance of the Tissue of Origin (TOO) test for tissue specimens that are "off-panel" (i.e. not one of the fifteen tissues on the Tissue of Origin Test panel). For off-panel cancers, a similarity score >= 30 for a cancer on panel indicates a false positive association with that cancer, according to the Guide to Report Interpretation. For each off-panel tissue type, a high false positive percentage was observed, especially endometrial cancer with ovarian cancer.

Table 8: Off-panel tumor types with RNA expression patterns that are similar to patterns in the database.

Specimen \ TOO Result	Total Specimens (n = 143)	TOO Reports with 2 SS ≥ 30	Total Indeterminates		Total False Positives	
	n		n	%I	n	%
Endometrium	49	2	1	2.0	48	98.0
Esophagus	28	1	6	21.4	22	78.6
Small Cell Lung	4	0	0	0.0	4	100.0
Ovarian Germ Cell	2	0	1	50.0	1	50.0
Sq. Head & Neck	18	0	7	38.9	11	61.1
Uterine cervix	42	2	6	14.3	36	85.7

Table 9: Break down table of false positives in Table 8

Specimen	Total False Positives	Distribution of Similarity Scores ≥ 30 across the 15 tissues on the TISSUE OF ORIGIN panel														
	N	BL	BR	CO	GA	GC	LI	KI	LY	LU	ME	OV	PA	PR	SC	TH
Endometrium	48	1	2							2		43			2	
Esophagus	22	1	1	4	14					2			1			
Small Cell Lung	4									4						
Ovarian Germ Cell	1					1										
Sq. Head & Neck	11	2	2							6						1
Uterine cervix	36	6	9	10					1	6		5			1	

> c. *Other clinical supportive data (when a. and b. are not applicable):*
> None
4. <u>Clinical cut-off</u>**:**
   Same as Assay cut-off
5. <u>Expected values/Reference range:</u>
   Not applicable

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.