510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k041875

B. Purpose for Submission:
New device

C. Analyte:
Her2/neu gene copy number on formalin-fixed paraffin-embedded breast cancer specimens

D. Type of Test:
Computer-assisted image analyzer for fluorescence in situ hybridization (FISH)

E. Applicant:
Vysis, Inc.

F. Proprietary and Established Names:
Vysis® AutoVysion™ System for PathVysion HER-2 DNA Kit

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.4700, Automated Fluorescent in situ Hybridization (FISH) Enumeration Systems
2. Classification:
   II
3. Product Code:
   NTH, System, Automated Scanning Microscope and Image Analysis for fluorescence in situ hybridization (FISH) assays
4. Panel:
   Immunology 82

H. Intended Use:

1. Intended Use:
   The Vysis® AutoVysion™ System is an automated scanning microscope and image analysis system. It is intended for *in vitro* diagnostic use with the Vysis® PathVysion® HER-2 DNA Probe Kit to aid in the detection and enumeration of FISH signals in interphase nuclei, and to determine the LSI® HER-2 to CEP® 17 signal ratio of the HER-2/*neu* gene via FISH in formalin-fixed, paraffin-embedded human breast cancer tissue specimens. The AutoVysion System is intended to reduce overall hands-on time by performing automated enumeration. For a small percentage of samples (less than 7%) manual enumeration may be required.

   The Vysis® AutoVysion™ System is an adjunctive computer-assisted methodology to assist in the acquisition and measurement of images from microscope slides of formalin-fixed, paraffin-embedded breast cancer tissue sections for the presence of amplified HER-2/*neu* gene. The Vysis® AutoVysion™ System is intended for use as aid in determining HER-2/*neu* amplification status, in conjunction with optional manual visualization directly through the fluorescence microscope.
2. Indication(s) for use:

When used with the Vysis® PathVysion® HER-2 DNA Probe Kit, the Vysis® AutoVysion™ System is indicated for use as

a) an adjunct to existing clinical and pathologic information currently used as prognostic factors in stage II, node-positive breast cancer patients

b) an aid to predict disease-free and overall survival in patients with stage II, node positive breast cancer treated with adjuvant cyclophosphamide, doxorubicin and 5-fluorouracil (CAF) chemotherapy; and,

c) aid in the assessment of patients for whom HERCEPTIN® (Trastuzumab) treatment is being considered (see HERCEPTIN package insert).

3. Special condition for use statement(s):
   For prescription use.
4. Special instrument Requirements:
   Vysis® AutoVysion™ System

I. Device Description:

The Vysis® AutoVysion™ System consists of an automated fluorescence microscope with motorized scanning stage, a large-format monochrome CCD camera, computer and scanning and assay specific analysis software. The microscope is equipped with a mercury arc lamp for fluorescence epi-illumination; three single-pass fluorescence filter sets for DAPI, SpectrumGreen™ (SG) and SpectrumOrange™ (SO) and a triple-pass fluorescence filter set for DAPI/SG/SO, all mounted in a motorized filter turret; 10x and 40x objectives in a motorized objective turret; 10x eyepieces; a CCD camera; and a motorized scanning stage that holds up to 8 slides. Images of single fluorescence colors are captured by the CCD camera and transferred to the computer. All functions are controlled by the System software.

J. Substantial Equivalence Information:

1. Predicate device name(s)
   None
2. Predicate K number(s):
   None
3. Comparison with predicate:
   Not applicable

K. Standard/Guidance Document Referenced (if applicable):

FDA guidance documents on software validation and off-the shelf Software use and NCCLS- EP9-A2

L. Test Principle:

A qualified user visually inspects the tumor regions of the slide, previously identified by a pathologist, identifies areas of tumor invasion with acceptable hybridization quality and records the coordinates of those areas for analysis through a point and click interface. Once the target areas have been identified, the AutoVysion™ System enters a fully automatic process, capturing extended-focus images of the marked areas at 40x magnification in each color: DAPI. SpectrumGreen and SpectrumOrange. All image data is saved to disk. The hybridization signals in each area are detected and enumerated automatically. The slide is also assessed for appropriate hybridization quality requirements that, if not satisfied, the sample may be rejected for automatic

analysis. Final review and reporting of sample results is performed by a qualified user.

The system uses a "targeted tiles" method for sampling the tumor. In this method, each field of view (FOV) in the area selected for analysis is sampled by placing a set of non-overlapping square "tiles" of equal size on the image. Each tile is comparable in size to the area of a tumor cell nucleus. The tiles are placed one by one in a way that maximizes the DAPI fluorescence contained in each tile, so that the set of tiles covers much of the nuclear material in the FOV. The spot count of a particular tile comprises the total spot count of the cell nuclei that are wholly or partly incorporated in the tile randomly reduced by truncation by tile boundary and microtome slicing. The method used to analyze the observed distribution of per-tile spot counts is the Expectation Maximization Algorithm (EM). EM is used to fit a mixture of two distributions to the observed two dimensional spot count distribution. The goodness of fit of the two-distribution model is compared with the goodness of fit of a single distribution to ensure that truly homogeneous samples are not erroneously fitted by two separate distributions. The HER-2/CEP 17 ratio is obtained directly from the parameters of the fitted distribution(s).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The Vysis® AutoVysion™ System was evaluated for inter-site and day-to-day reproducibility at 3 clinical sites. The study consisted of a total of 36 specimen slides prepared from four human breast tissue specimens with varying levels of HER-2/*neu* gene amplification (one normal, one borderline, one moderate and one high amplification). Each site received three of each of the specimens randomized over three days. The optimal number of fields of view (FOV) was determined using 5, 7 and 10 fields of view. All three numbers of FOVs gave similar results. The ten FOVs were selected to be used for all precision studies.

Day-to-day reproducibility was determined by calculating the mean observed ratio of LSI HER-2/*neu* to CEP 17, standard deviation (SD) and percent coefficient of variation (%CV) generated from 10 fields of view for each specimen across the three study days. The p-values associated with the Levene test statistics were calculated to test the homogeneity of day-to-day variances, with a 0.05 significance level. Results showed no statistically significant differences (see table below).

Expected	Observed ratios of LSI HER-2/*neu* to CEP 17									P-value
	Day 1			Day 2			Day 3			
	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	
1.33	1.17	0.33	28.04	1.11	0.14	12.3	1.14	0.06	5.12	0.1152
1.71	2.08	0.61	29.34	2.38	0.64	26.82	2.45	NR	NR	0.9049
8.14	5.70	0.86	15.01	5.55	0.33	5.87	5.47	0.66	12.09	0.2788
12.97	6.42	0.81	12.55	7.42	0.17	2.24	8.01	0.35	4.38	0.1205

NR= No result

Inter-site reproducibility was similarly determined across the three study sites. Results for the three sites were not statistically significant and are summarized in the following table:

Expected	Observed ratios of LSI HER-2/*neu* to CEP 17									P-value
	Site 1			Site 2			Site 3			
	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV	
1.33	1.24	0.26	21.09	1.05	0.05	4.52	1.14	0.18	15.97	0.1833
1.71	2.09	0.58	27.84	2.42	NR	NR	2.39	0.75	31.23	0.5436
8.14	5.41	0.58	10.70	5.88	0.06	1.02	5.44	0.88	16.12	0.1612
12.97	6.95	1.32	19.03	7.51	0.81	10.72	7.39	0.30	4.04	0.1665

NR= No result

b. Linearity/assay reportable range:
Not applicable.

c. Traceability (controls, calibrators, or method):
The analytical traceability of the system depends on the Vysis® PathVysion® HER-2 DNA Probe Kit. The AutoVysion™ System employs ProbeCheck control slides for every run to assess the accuracy of signal enumeration and to monitor the assay performance.

d. Detection limit (functional sensitivity):
Not applicable

e. Analytical specificity
The specificity of the test result is dependent on the analytical performance of the Vysis® PathVysion® HER-2 DNA Probe Kit

f. Assay cut-off:
The assay cut-off of the test result is dependent on the analytical performance of the Vysis® PathVysion® HER-2 DNA Probe Kit.

2. Comparison studies:

a. Method comparison with predicate device:
The substantial equivalence studies were based on comparison to conventional manual microscopy performed in accordance with Vysis® PathVysion® HER-2 DNA Probe Kit.

Duplicate slides from each tumor were randomized and assayed with the Vysis® PathVysion® HER-2 DNA Probe Kit according to the package insert instructions prior to shipment to the study sites. The randomized slides were enumerated by the standard and test method at each of the three study sites. Two hundred thirty-four clinical slides from 39 tumors with varying levels of HER-2/*neu* copy number were used in the study.

Concordance was evaluated as the agreement between manually enumerated and the calculated HER-2 to CEP 17 signal ratio and the Vysis® AutoVysion™ System produced HER-2 to CEP 17 signal ratio. Among all tissue specimens with informative results for both

methods, 92.5% (196/212) were correctly classified. Positive agreement was 96.0% and negative agreement was 89.2%. If samples with results in the equivocal range i.e. HER-2 to CEP 17 signal ratios between 1.5 and 3.0 were excluded from the calculation, total agreement was 98.8% (169/171) with 100% positive agreement and 97.5% negative agreement.

	Manual							
Scanner	1.5	1.5-<2.0	2.0-<2.5	2.5-<3.0	3.0-<5.0	5.0-<10	≥10	Total
<1.5	77	2	0	0	0	0	0	79
1.5-<2.0	17	3	3	1	0	0	0	24
2.0-<2.5	7	0	2	1	0	1	0	11
2.5-<3.0	3	0	0	1	0	2	0	6
3.0-<5.0	1	0	0	1	5	19	20	46
5.0-<10	0	1	0	1	5	19	20	46
≥10	0	0	0	0	0	1	3	4
Total	105	6	8	8	21	37	27	212

There were two false positive results by the AutoVysion™ System. When these two samples were repeated six times manually and by the scanner, both methods gave positive results in five of the six repeats.

The average bias for the manual enumeration ratio range of 1.18 to 4.49 was determined according to NCCLS guideline EP9-A2 and found to be 0.472 (SD = 1.24). This bias value was 11.7% of the average manual enumeration ratio of 4.05 which met the acceptable error of ±15%. The bias and % of average increased throughout the range as presented in the following table

Enumeration Ratio Range	Average Bias	% Average Enumeration Ratio
0.19-1.17	0.296	7.29
1.18-4.39	0.472	11.66
4.42-21	-2.98	-73.4
Overall	-0.742	-18.26

b. Matrix comparison:
Not applicable

3. Clinical studies:
   a. Clinical sensitivity:
   The clinical sensitivity of the test system is dependent on the analytical performance of the Vysis® PathVysion® HER-2 DNA Probe Kit.
   b. Clinical specificity:
   The clinical specificity of the test system is dependent on the analytical performance of the Vysis® PathVysion® HER-2 DNA Probe Kit.
   c. Other clinical supportive data (when a and b are not applicable)

Not applicable.

4. Clinical cut-off:
   The clinical cut-offs of the test result is dependent on the analytical performance of the Vysis® PathVysion® HER-2 DNA Probe Kit.
5. Expected values/Reference range:
   Expected values of HER-2/CEP 17 ratio were established on breast cancer tissue specimens from 524 breast cancer patients with the Vysis® PathVysion® HER-2 DNA Probe Kit. Based on a cut-off ratio of 2.0, 433 of the specimens were negative and 91 positive for HER-2/*neu* gene amplification. The distribution of the HER-2/CEP 17 ratios for the 433 non-amplified specimens is summarized below.

	Range		
Statistics	0.1-1.0	1.1-1.5	1.6-1.99
Mean	0.86	1.15	1.72
SD	0.14	0.13	0.11
N	185	226	22

The following table summarizes the distribution of HER-2/CEP 17 ratios for the 91 amplified specimens.

	Range		
Statistics	2.0-5.0	5.1-10.0	>10.0
Mean	3.35	7.39	12.77
SD	0.95	1.41	1.80
N	33	42	16

N. Instrument Name:

Vysis® AutoVysion™ System

O. System Descriptions:

See (H) Device Description.

1. Modes of Operation:
   Semi-automated computer-assisted interpretation.
2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types: Yes
3. Sample Identification:
   Slide identification is entered manually into the AutoVysion™ System before the slides are loaded into the instrument.
4. Specimen Sampling and Handling:
   The microscope slides to be examined are loaded onto the microscope stage of the AutoVysion™ System and the user records the coordinates of those areas for analysis through a point and click interface. Once the target areas have been identified, the AutoVysion™ System automatically captures images of the marked areas in each fluorescence color and enumerates the hybridization

signals in each area. The system also rejects slide that failed hybridization quality requirements for automatic analysis.

5. Assay Types:
   Computer-assisted image analysis of fluorescence in situ hybridization signals in interphase nuclei of cells in formalin-fixed paraffin-embedded tissue.
6. Reaction Types:
   Fluorescent microscopy
7. Calibration:
   The AutoVysion™ instrument is factory calibrated. Monthly calibration checks with End Switches and Movements tests should be performed. To assess accuracy of signal enumeration by the instrument, laboratory-stained Vysis ProCheck slides are used for every staining run.
8. Quality Control:
   The accuracy of the system depends on the laboratory following the quality control instructions recommended in the labeling of the fluorescence *in situ* hybridization (FISH) assay kit associated with the AutoVysion™.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "M. Performance Characteristics" Section Of The SE Determination Decision Summary.

None.

Q. Conclusion:

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.4700 with special controls. The special control guidance document " Class II Special Controls Guidance Document: Automated Fluorescence *in situ* Hybridization (FISH) Enumeration Systems" is available at WWW.fda.gov/cdrh/oivd/guidance/1550.pdf.