DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

May 28, 2015

Nanostring Technologies, Inc.
Sylva Krizan, Ph.D.
Regulatory Affairs Manager
530 Fairview Avenue North, Suite 2000
Seattle, WA 98109

Re: K141771
Trade/Device Name: Prosigna™ Breast Cancer Prognostic Gene Signature Assay
Regulation Number: 21 CFR §866.6040
Regulation Name: Gene expression profiling test system for breast cancer prognosis
Regulatory Class: Class II
Product Code: NYI
Dated: June 30, 2014
Received: July 1, 2014

Dear Dr. Krizan:

This letter corrects our substantially equivalent letter of November 7, 2014.

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. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. 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 Part 801); medical device reporting (reporting of medical

device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR 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 Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Reena Philip -S

Reena Philip, Ph.D.
Director
Division of Molecular Genetics and Pathology
Office of In Vitro Diagnostics and
Radiological Health
Center for Devices and Radiological Health

Enclosure:
Corrected Indications for Use Statement

510(k) Number (if known): K141771

Device Name: Prosigna™ Breast Cancer Prognostic Gene Signature Assay

Indications for Use:

The Prosigna® Breast Cancer Prognostic Gene Signature Assay is an in vitro diagnostic assay which is performed on the NanoString nCounter® Dx Analysis System using FFPE breast tumor tissue previously diagnosed as invasive breast carcinoma. This qualitative assay utilizes gene expression data, weighted together with clinical variables to generate a risk category and numerical score, to assess a patient's risk of distant recurrence of disease.

The Prosigna Breast Cancer Prognostic Gene Signature Assay is indicated in female breast cancer patients who have undergone surgery in conjunction with locoregional treatment consistent with standard of care, either as:

1. A prognostic indicator for distant recurrence-free survival at 10 years in post-menopausal women with Hormone Receptor-Positive (HR+), lymph node-negative, Stage I or II breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors.

2. A prognostic indicator for distant recurrence-free survival at 10 years in post-menopausal women with Hormone Receptor-Positive (HR+), lymph node-positive (1-3 positive nodes), Stage II breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors. The device is not intended for patients with 4 or more positive nodes

Prescription Use __x__ **AND/OR** **Over-The-Counter Use __**
(Part 21 CFR 801 Subpart D) **(21 CFR 801 Subpart C)**

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

Concurrence of CDRH, Office of In Vitro Diagnostics and Radiological Health (OIR)

Division Sign-Off
Office of In Vitro Diagnostics and Radiological Health

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K141771

B. Purpose for Submission:

Modification of device configuration and software

C. Measurand:

58 gene RNA expression profile

D. Type of Test:

Gene expression profile system based upon non-amplified RNA hybridization, visualization, and image analysis

E. Applicant:

NanoString Technologies

F. Proprietary and Established Names:

Prosigna™ Breast Cancer Prognostic Gene Signature Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.6040 Gene expression profiling test system for breast cancer prognosis

2. Classification:

   Class II

3. Product code:

   NYI, Classifier, prognostic, recurrence risk assessment, RNA gene expression, breast cancer

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   The Prosigna™ Breast Cancer Prognostic Gene Signature Assay is an *in vitro* diagnostic assay which is performed on the NanoString nCounter Dx Analysis System using FFPE

breast tumor tissue previously diagnosed as invasive breast carcinoma. This qualitative assay utilizes gene expression data, weighted together with clinical variables to generate a risk category and numerical score, to assess a patient's risk of distant recurrence of disease.

The Prosigna Breast Cancer Prognostic Gene Signature Assay is indicated in female breast cancer patients who have undergone surgery in conjunction with locoregional treatment consistent with standard of care, either as:

1. A prognostic indicator for distant recurrence-free survival at 10 years in post-menopausal women with hormone receptor-positive (HR+), lymph node-negative, Stage I or II breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors.
2. A prognostic indicator for distant recurrence-free survival at 10 years in post-menopausal women with hormone receptor-positive (HR+), lymph node-positive (1-3 positive nodes), Stage II breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors. The device is not intended for patients with 4 or more positive nodes.

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   For Prescription Use Only

4. Special instrument requirements:

   nCounter Dx Analysis System

I. Device Description:

The required components for the Prosigna™ Breast Cancer Prognostic Gene Signature Assay include the RNA Isolation kit (manufactured by Roche), Prosigna reagents (Reference Sample, CodeSet, Prep Pack, Cartridge(s) and Prep Plate) and the instruments that comprise the nCounter Dx Analysis System; the Prep Station and Digital Analyzer.

The assay requires microdissection of tumor from FFPE biopsies, isolation of RNA using a Roche RNA isolation kit, transfer of RNA to PCR tubes for hybridization before placing onto the prep station. Two sets of probes specific to each of 58 RNAs are added to the hybridization reaction. These consist of biotin-labeled magnetic probes to purify the RNAs and capture them on the assay cartridge and fluorescent "barcode" probes to detect and quantify individual RNAs. The patient sample and probes are pipetted automatically into the Prosigna test cartridge by the Prep Station. The prep station uses magnetic bead capture and washing to remove excess RNA and un-hybridized probes. The isolated and hybridized RNA species are then bound via biotin on the capture probe randomly to streptavidin on the cartridge. The fluorescent molecules are then aligned on the cartridge by addition of an electric current. The cartridge is then transferred to the Digital Analyzer where the cartridge

is scanned and digital analysis software is used to count the number of each RNA species present. The amount of each RNA is then put into a proprietary algorithm to produce a Prosigna score.

The test output is a patient specific report which includes a Prosigna score (0-100) and risk category (low/intermediate/high).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Prosigna™ Breast Cancer Prognostic Gene Signature Assay

2. Predicate 510(k) number(s):
   k130010

3. Comparison with predicate:

   Table 1: Comparison with Predicate

Similarities		
Item	Device	Predicate
Intended Use	The Prosigna™ Breast Cancer Prognostic Gene Signature Assay is an *in vitro* diagnostic assay which is performed on the NanoString nCounter Dx Analysis System using FFPE breast tumor tissue previously diagnosed as invasive breast carcinoma. This qualitative assay utilizes gene expression data, weighted together with clinical variables to generate a risk category and numerical score, to assess a patient's risk of distant recurrence of disease. The Prosigna Breast Cancer Prognostic Gene Signature Assay is indicated in female breast cancer patients who have undergone surgery in conjunction with locoregional treatment consistent with standard of care, either as:	Same

	1. A prognostic indicator for distant recurrence-free survival at 10 years in post-menopausal women with hormone receptor-positive (HR+), lymph node-negative, Stage I or II breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors. 2. A prognostic indicator for distant recurrence-free survival at 10 years in post-menopausal women with hormone receptor-positive (HR+), lymph node-positive (1-3 positive nodes), Stage II breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathological factors. The device is not intended for patients with 4 or more positive nodes.	
Prescription Use	Yes	Yes
Device Description	Prosigna™ Breast Cancer Prognostic Gene Signature Assay and nCounterDx Analysis Platform; all elements cleared by FDA as a distributed test and platform	Same
Test Sample	FFPE breast tumor tissue	Same
Extraction/amplification reagents/amplification procedures	No amplification required; procedure for processing FFPE tumor samples provided; includes RNA isolation, multiplex hybridization in solution, automated purification on a	Same

	liquid handling robot and analysis on an automated epifluorescence microscope	
Differences		
Item	Device	Predicate
Kit Stability/Shelf Life	8 months, based upon real time stability data available from original testing protocol	7 months, based on testing completed at time of clearance
Device Configuration	Reagents configured and software programmed to prepare 2 reference samples and 10 test sample -10 test configuration And Reagents configured and software programmed to prepare 2 reference samples and 4 test sample -4 test configuration	Reagents configured and software programmed to prepare 2 reference samples and 10 test sample -10 test configuration
Instrument Software	Version 1.3	Version 1.0
Instrument Functionality	FLEX configuration allows for IVD or research use of the device with different modes separated through user permissions and required log-out and log-in when changing modes	IVD use only

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

Class II Special Controls Guidance Document: "Gene Expression Profiling Test System for Breast Cancer Prognosis, issued on May 9, 2007"

L. Test Principle:

Used together, the Prosigna™ Breast Cancer Prognostic Gene Signature Assay and nCounter Dx Analysis System are a nucleic acid hybridization, visualization and image analysis system based upon coded probes designed to detect the messenger RNA transcribed from 58 genes. The test input is purified RNA from FFPE breast tumor specimens which are acquired from surgical resection. The Prosigna assay uses gene-specific probe pairs that hybridize directly to the mRNA transcripts in solution. The nCounter Dx Analysis System delivers direct, multiplexed measurements of gene expression through digital readouts of the relative abundance of the mRNA transcripts.

Specifications are included as part of the Prosigna Assay to control for sample quality, RNA quality, and process quality. The Prosigna assay utilizes prototypical expression profiles (centroids) for breast cancer. Patients RNA signatures are categorized into one of four centroids (not reported) based upon how close their gene expression pattern is to each of the centroids. The software algorithm produces a Prosigna score based on the similarity of the expression profile to each centroid, as well as the pathological tumor size and a proliferation score computed from a subset of genes. Three risk categories (low, intermediate and high) were defined based on a study with over 1007 patient samples associating Prosigna score with long-term outcome, defined by distance recurrence free survival at 10 years (DRFS) (Table 2).

Table 2: Risk Classification Scoring Algorithm Using Prosigna Score

Nodal Status	Prosigna Score Range	Risk Classification
Node-Negative	0-40	Low
	41-60	Intermediate
	61-100	High
Node-Positive (1-3 nodes)	0-40	Low
	41-100	High

M. Performance Characteristics (if/when applicable):

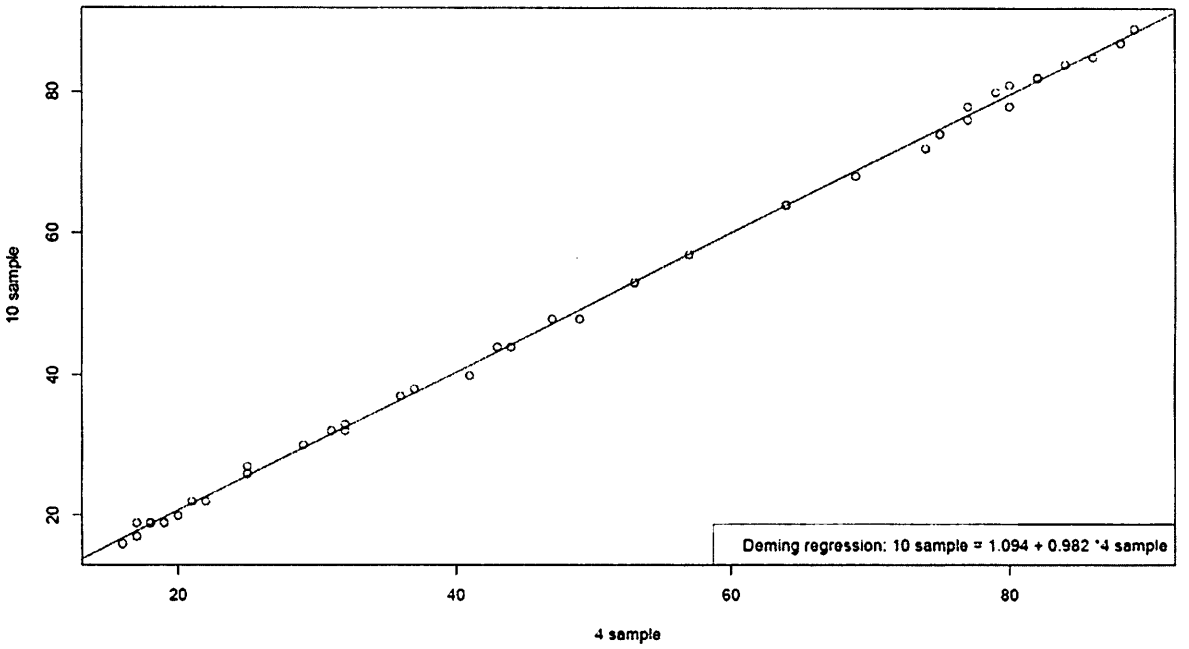
1. Analytical performance:

   See Predicate Device K130010 for Analytical Performance Data.

2. Comparison studies:

   a. *Method comparison with predicate device:*

   A comparison of Prosigna Scores was conducted using the two available configurations of the kit (4 sample and 10 sample kits). Forty samples of RNA previously extracted from FFPE breast tissue, covering a range of Prosigna scores were tested with both configurations and the data plotted using Deming regression (Figure 1 below).



The data was linear over the range of the assay with no outliers between the two methods indicating that the 4-kit and 10-kit assays produce substantially equivalent results.

No value deviated by more than 2 units from the average score when run using either configuration.

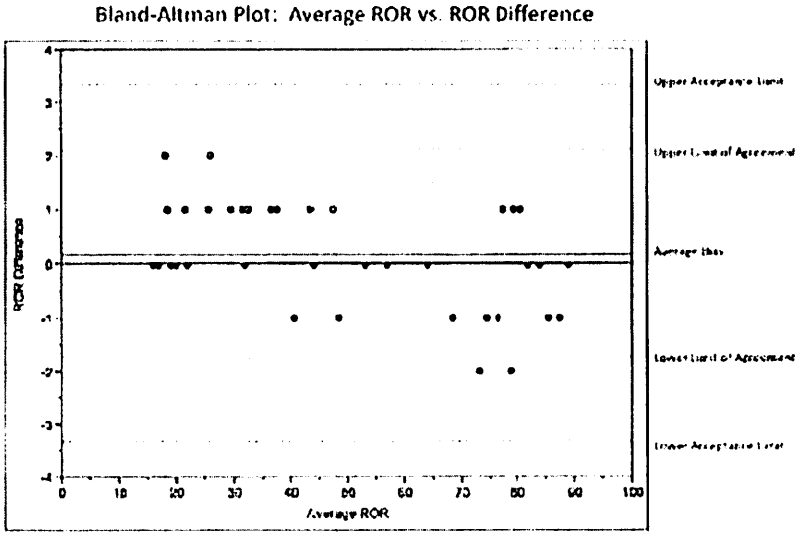


Figure 3: Bland-Altman comparison plot of ROR scores obtained from each kit configuration for the analysis data set (40 samples).

Bland Altman Analysis (above) showed that variations in data using the 4-kit versus the 10-kit configuration did not bias results and no changes in risk categorization occurred.

b. Matrix comparison:

Not Applicable. FFPE tissue is the only matrix indicated for this device.

3. Clinical studies:

See Predicate Device K130010 for Clinical Performance Data

4. Clinical cut-off:

Same as assay cut-off

5. Expected values/Reference range:

Risk assessment is reported as Low Risk, Intermediate Risk, or High Risk for node negative patients or as Low Risk or High Risk for Node positive patients (see Table 3).

Table 3: Risk Classification Scoring Algorithm Using Prosigna Score

Nodal Status	Prosigna Score Range	Risk Classification
Node-Negative	0-40	Low
	41-60	Intermediate
	61-100	High
Node-Positive (1-3 nodes)	0-40	Low
	41-100	High

N. Instrument Name:

The nCounter Dx Analysis System consists of a liquid handling robot Prep Station 5s and an epifluorescent scanner Digital Analyzer 5s.

O. System Descriptions:

1. Modes of Operation:

Automated

2. Software:

The Digital Analyzer measures and sorts multiple signals (reporter probes bound to mRNA transcript) from the clinical sample to establish an indicator (Prosigna score and risk category) to aid in determining patient prognosis. The Prep Station automates post-hybridization sample processing while the Digital Analyzer includes signal reading, raw data storage, data acquisition software and software to process the detected targets (algorithm).

The Software is a Visual C++ web-based application developed by Nanostring.

The current version of the Software is v1.3 and includes validation for the 4-test kit configuration in a dual IVD/RUO mode.

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes __x_____ or No _____

3. Specimen Identification:

   Specimen identifying information is entered into a computer application manually.

4. Specimen Sampling and Handling:

   Samples are handled individually until RNA is extracted from FFPE tissue. RNA samples are then handled in batches of 6 or12 on the instrument. These include 2 control samples and either 4 or 10 test samples, depending upon the configuration of reagent plates used.

5. Calibration:

   Installation, calibration and preventative maintenance of instrumentation are performed by the instrument manufacturer. No user calibration required.

6. Quality Control:

   Quality control includes testing of the mixed Reporter CodeSet and Capture ProbeSet for the following performance characteristics:
   - Signal level of the geometric mean of housekeeping gene probes
   - Signal levels of each of the 50 classifier genes
   - Background level of the negative controls
   - Linearity of positive controls
   - Probe cross-contamination

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

None

Q. Proposed Labeling:

1. Labeling was modified to reflect
   - the new software version,
   - to indicate the presence of the 4-test kit configuration
   - to include acceptable specifications for low volume spectrophotometers to be used with the assay
   - to alert physicians to a trend in the data noted by post-hoc analysis whereby most distant recurrence appears to occur after 5 years.

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

R. Conclusion:

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

S. Other Supportive Device and Instrument Information:

1. See predicate device K130010 for additional information concerning device performance.
2. Sponsor also included an update to the specifications for spectrophotometers that may be used to prepare RNA for the Prosigna device. The data revealed that at least two low volume spectrophotometers would provide acceptable quantitation of RNA for later use in the Prosigna assay.
3. Additional Clinical Claims
    a. Sponsor originally sought additional claims, through changes to the patient Case Report Form in order to claim the Prosigna device could distinguish risk groups by risk of early recurrence versus late recurrence. That is to say that breast cancer that is expected to recur within a 10 year period, but that had a higher risk of arising within 5 years after surgery could be distinguished from those at higher risk of arising between 5 and 10 years after surgery. The Sponsor's calculations and pre-defined acceptance criteria only included those patients in the 5-10 year (late) recurrence group and did not take into account the early recurrence group.
    b. Sponsor indicated that this information was provided to avoid confusion and were only included for "descriptive" purposes. FDA noted that the Sponsor provided precise calculations of data that had overlapping confidence intervals and was not statistically significant.
    c. FDA noted to Sponsor that the information that they intended to convey was already present in the Kaplan-Meier survival curves presented on the case report forms and that a textual instructions to take care in the interpretation of survival was truthful and accurate without being false or misleading.
    d. Sponsor removed charts of early v. late recurrence from the patient case report form.
    e. Sponsor has altered the final version of labeling to remove a specific claim of early v. late recurrence.
    f. Sponsor did include a text description of the 0-5 year early versus 5-10 year late recurrence and presented the Kaplan-Meier curve for this population
4. Additional Stability Claims
    a. Sponsor took a retrospective look at their stability data that was based upon reduction of signal of the control material geomean value once the stability study was completed.
    b. Sponsor noted that if the lot release criteria for the control material were increased from a geomean value of 2289 to 3308, the stability data previously collected would support a stability claim of 11 months.
    c. FDA noted that while this hypothesis was scientifically sound, it was not properly

validated as the new lot release criteria was neither specified, nor in place when the stability testing began. As such, the stability remains established at 8 months, as was established via an add to file for the original 510(k): K130010..

5. Additional Labeling Changes
    a. Sponsor validated a new software package that allowed the nCounter elements instrument run in two modes. The first is the cleared "IVD" mode. The other was variously described as "non-IVD" or "life Sciences" mode.
    b. FDA indicated to Sponsor that the change in software was acceptable but that all labeling and software must indicate that the FLEX device configuration is acceptable for IVD use only when used in "IVD" mode.

T. Administrative Information:

1. Applicant Contact Information:

    a. Name of applicant: Nanostring Technologies

    b. Mailing address: 530 Fairview Avenue North, Suite 2000

    Seattle, Washington 98109

    c. Phone #: (206)432-8854

    d. Fax #: (206)378-6288

    e. E-mail address (optional): skrizan@nanostring.com

    f. Contact: Sylva Krizan, Ph.D.

2. Review Documentation:

July 2, 2014	Special 510(k) received. Lead Reviewer assigned-Kevin Lorick.
July 14, 2014	RTAA designation. 510(k) accepted for review.
July 14, 2104	Special 510(k) converted to a traditional 510(k) due to new claims
July 31, 2014	Request for Additional Information
September 8, 2014	Supplement S001, Response to Request for Additional Information received
September 26, 2014	FDA sent Email Request for clarification.
September 29, 2014	FDA sent 2nd Email Request for clarification.

October 1, 2014	Sponsor sent response to September 26, 2014 FDA email
October 2, 2014	Email and phone calls to Sponsor to discuss possible disallowance of claims.
October 2, 2014	Phone call from Sponsor. Sponsor agreed in principle to attempt to modify submission by COB on October 3, 2014.
October 9, 2014	Teleconference with Sponsor to discuss timing and content of their response to FDA inquiries.
October 24, 2014	Email from Sponsor with preliminary responses to FDA inquiries.
October 30, 2014	Sponsor request to discuss proposed "Special Indications for Use"
November 3, 2014	FDA call to Sponsor to seek modification of redlines version of PI.
November 4, 2014	Sponsor email providing updates to labeling removing changes to Special Conditions for Use
November 4, 2014	FDA Email to Sponsor indicating where the new version was deficient
November 5, 2014	Email to Sponsor requesting updated data comparison figures and validation for support instrument specifications.
November 5, 2014	Sponsor email request for additional feedback based upon the November 3, PI.
November 6, 2014	Email to Sponsor with suggested PI changes.
November 7, 2014	Sponsor email with Final Labeling
November 8, 2014	SE determination made
April 17, 2015	Amendment received by DCC
April 17, 2015	Lead Reviewer Kevin Lorick Assigned
May 1, 2015	Internal Discussion about necessary documents and procedures
May 6, 2015	510(k) Amendment determined to be appropriate.
May 22, 2015	Updated labeling, 510(k) Summary and Indications for Use uploaded to DocMan.

3. Substantial Equivalence Discussion:

	Yes	No	
1. Same Indication Statement?	x		If **YES** = Go To 3
2. Do Differences Alter The Effect Or Raise New Issues of Safety Or Effectiveness?			If **YES** = Stop **NSE**
3. Same Technological Characteristics?	x		If **YES** = Go To 5
4. Could The New Characteristics Affect Safety Or Effectiveness?			If **YES** = Go To 6
5. Descriptive Characteristics Precise Enough?	x		If **NO** = Go To 8 If **YES** = Stop **SE**
6. New Types Of Safety Or Effectiveness Questions?			If **YES** = Stop **NSE**
7. Accepted Scientific Methods Exist?			If **NO** = Stop **NSE**
8. Performance Data Available?			If **NO** = Request Data
9. Data Demonstrate Equivalence?			Final Decision: SE

Note: See http://eroom.fda.gov/eRoomReq/Files/CDRH3/CDRHPremarketNotification510kProgram/0_4148/FLOWCHART%20DECISION%20TREE%20.DOC for Flowchart to assist in decision-making process. Please complete the following table and answer the corresponding questions. "Yes" responses to questions 2, 4, 6, and 9, and every "no" response requires an explanation.

a. *Explain how the new indication differs from the predicate device's indication:*
NA

b. *Explain why there is or is not a new effect or safety or effectiveness issue:*
NA

c. *Describe the new technological characteristics:*

NA

d. *Explain how new characteristics could or could not affect safety or effectiveness:*
NA

e. *Explain how descriptive characteristics are not precise enough:*
NA

f. *Explain new types of safety or effectiveness question(s) raised or why the question(s) are not new:*
N/A

g. *Explain why existing scientific methods cannot be used:*
NA

h. *Explain what performance data is needed:*
Demonstration that 4 and 10-test kit configurations produce identical results on the device with the v1.3 software are sufficient. No additional performance data is required.

i. *Explain how the performance data demonstrates that the device is or is not substantially equivalent:*

The changes to the devices are minor software and configuration changes. These effect only the sample preparation steps in terms of reagent positioning. No changes to reagents, hardware, analysis, scoring algorithm or significant changes to patient disease state result from the device modification. One additional piece of information is clarified in package insert but this was done for physician information purposes in a manner that may reduce confusion and aid patient safety.

U. Reviewer Name and Signature:

Kevin Lorick -S

Digitally signed by Kevin Lorick -S
DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Kevin Lorick -S, 0.9.2342.19200300.100.1.1=2000350803
Date: 2015.05.29 13:59:27 -04'00'

Kevin Lorick, Ph.D.
CDRH/OIR/PACB