SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	*In Vitro* Diagnostic Device for Detection of HER-2/neu Gene Amplification in formalin-fixed, paraffin-embedded (FFPE) Human Breast Tissues using dual chromogenic *In Situ* Hybridization (Dual ISH)
Device Trade Name:	INFORM® HER2 Dual ISH DNA Probe Cocktail
Applicant's Name and Address:	Ventana Medical Systems, Inc. 1910 East Innovation Park Drive Tucson, AZ 85755 USA

Date(s) of Panel Recommendation: None.

Premarket Approval Application (PMA) Number: P100027

Date of FDA Notice of Approval: June 14, 2011

Expedited: Not applicable.

II. INDICATIONS FOR USE

The INFORM® HER2 Dual ISH DNA Probe Cocktail is intended for use in determining HER2 gene status by enumeration of the ratio of the HER2 gene to Chromosome 17. The HER2 and Chromosome 17 probes are detected using two color chromogenic *in situ* hybridization (ISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens following staining on Ventana BenchMark® XT automated slide stainers (using NexES software), by light microscopy. The INFORM® HER2 Dual ISH DNA Probe Cocktail is indicated as an aid in the assessment of patients for whom HERCEPTIN® (trastuzumab) treatment is being considered.

This product should be interpreted by a qualified reader in conjunction with histological examination, relevant clinical information, and proper controls.

This reagent is intended for *in vitro* diagnostic (IVD) use.

III. CONTRAINDICATIONS

The contraindictions can be found in the INFORM® HER2 Dual ISH DNA Probe Cocktail labeling and in the BenchMark® XT automated Slide stainer labeling.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the INFORM® HER2 Dual ISH DNA Probe Cocktail labeling and in the BenchMark® XT automated Slide stainer labeling.

V. DEVICE DESCRIPTION

The INFORM® HER2 Dual ISH DNA Probe Cocktail contains DNA probes to detect the HER2 gene and Chromosome 17 formulated together. The detection and enumeration of the HER2/Chr 17 signals on a single slide containing invasive breast carcinoma tissue is used to determine HER2 gene status. The results are reported as the ratio of copies of HER2/Chromosome 17 in tumor cells. The HER2 genomic probe is labeled with the dinitrophenyl (DNP) hapten which is conjugated to the nucleotide dCTP. The labeled probe sequences specifically hybridize to the HER2 gene region, located on human chromosome 17 (17q12-q21). This gene region is amplified in 10-30% of invasive breast carcinoma cases. The second probe is labeled with the digoxigenin (DIG) hapten and contains the alpha-satellite sequences of human chromosome 17. The labeled probe specifically hybridizes to the centromeric region to determine the copy number status of the entire chromosome (i.e., ploidy).

The probes are detected using two separate Ventana detection kits. The *ultra*View SISH DNP and *ultra*View Red ISH DIG detection kits specifically recognize DNP-HER2 and DIG-labeled Chromosome 17 probes, respectively. The assay also requires ancillary reagents, which are used on the BenchMark® XT automated slide staining instrument. The HER2 and chromosome 17 targets are visualized as discrete dots in the tumor nuclei, enumerable with 20, 40, and 60x brightfield microscopy. The scoring algorithm requires quantifying HER2 and chromosome 17 copy numbers in at least 20 tumor nuclei. The results are reported as a ratio of HER2 to Chr 17. Gene amplification is defined as a HER2/Chr 17 ratio $\geq$2.0, while HER2/Chr 17 <2.0 indicates non-amplification.

Tumors with HER2 gene amplification typically exhibit intra-nuclear silver clusters of varying sizes, multiple single silver dots, or a mixture of clusters and multiple dots. Tumors without HER2 gene amplification typically exhibit fewer single silver dots per nucleus that are easily enumerated.

The INFORM® HER2 Dual ISH DNA Probe Cocktail is a standardized formulation containing a formamide-based buffer, blocking DNA, and two probes, in a dispenser sufficient for 50 tests. Additional reagents and materials

compatible with the Ventana BenchMark® XT automated slide staining instrument are required. Troubleshooting may be aided by the use of FFPE slides (INFORM HER2 Dual ISH 3-in-1 Xenograft Slides) that contain HER2 non-amplified (negative) and amplified (positive) human cancer cell lines on each slide. A HER2 Dual ISH Interpretation Guide (containing color images of representative staining patterns and known artifacts) is included to assist in the interpretation of INFORM® HER2 Dual ISH DNA Probe Cocktail results.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are several alternatives for the detection of Her2 gene amplification: Other devices utilizing fluorescent *in situ* hybridization (FISH) or chromogenic *in situ* hybridization (CISH) methodologies for gene amplification determination in human breast cancer tissue specimens are commercially available. Immunohistochemistry (IHC) is an alternative procedure for detection of gene product over-expression in human breast. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

INFORM® HER2 Dual ISH DNA Probe Cocktail launched for distribution outside of the United States in June of 2010. This product is marketed as an in vitro diagnostic device in the following countries:

- Algeria
- Argentina
- Australia
- Austria
- Bahrain
- Belgium
- Brazil
- Bulgaria
- Canada
- Chile
- China
- Columbia
- Czech Republic
- Denmark
- Dubai
- Ecuador
- Egypt
- Finland
- France
- Germany
- Greece
- Hong Kong
- Hungary
- India
- Ireland
- Israel
- Italy
- Japan
- Jordan
- Kuwait
- South Korea
- Latvia
- Lebanon
- Lithuania
- Libya
- Luxembourg
- Malaysia
- Mexico
- Morocco
- Netherlands
- New Zealand
- Norway
- Peru
- Philippines
- Poland
- Portugal
- Romania
- Russia

- Saudi Arabia
- Singapore
- Slovenia
- South Africa
- Spain
- Sweden
- Switzerland
- Taiwan
- Thailand
- Tunisia
- Turkey
- Uruguay
- United Kingdom
- Vietnam

This represents product sold since international market launch on June 2010. INFORM® HER2 Dual ISH DNA Probe Cocktail has never been withdrawn from the market.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

A potential risk associated with misuse of the assay or a false positive test result is to assign patients to receive a more aggressive adjuvant therapy regimen than needed, possibly exposing the patient to serious side effects and, in rare cases, death. Alternatively, a false negative test result may exclude a patient who might benefit from more aggressive therapy from a treatment regimen, potentially resulting in a poor clinical outcome.

IX. SUMMARY OF NON-CLINICAL STUDIES

Several studies were conducted in order to determine the robustness of the assay and test the ranges of the selectable options in the automated staining procedure. All non-clinical studies were performed with the INFORM® HER2 Dual ISH DNA Probe Cocktail assay on the BenchMark® XT platform, with specimens including at least one non-amplified, one amplified and one "borderline" human invasive carcinoma case whenever possible. A "borderline" case is one that, based upon the instructions for use, requires the user to count 40 nuclei, rather than 20 to come to a final diagnosis.

A. Laboratory Studies

1. Analytical Specificity and Sensitivity

a. Hybridization Efficiency

Specificity of the INFORM Dual ISH probes is partially guaranteed by the use of DNA segments identical to those already in use, though in a different form, for PathVysion HER2 FISH.

To verify that the INFORM® HER2 Dual ISH DNA Probe Cocktail probes specifically detect their target sequences, the INFORM® HER2 Dual ISH DNA Probe Cocktail was used to stain human metaphase spreads. All probe conditions (denaturation, stringency, and hybridization) were the same for the metaphase spreads as used in other studies for breast tissue samples.

The INFORM® HER2 Dual ISH DNA Probe Cocktail assay was used to stain human metaphase spreads on the BenchMark® XT. One hundred (100) metaphase spreads were analyzed for specific co-hybridization of both probes.

.Specificity of the probes was measured by percentage of the metaphases with no cross-hybridization to any other chromosome loci.

Sensitivity of the probes was measured by percentage of the metaphases with co-localization of HER2 probe and Chromosome 17 probe on the same chromosome.

The specific co-localization of the HER2 DNA and Chr 17 probes to a single chromosome was verified 100% of the time on human metaphase spreads. No cross hybridization to other chromosomes was detected

b. **Analytical Sensitivity (quantitative)**

The analytical sensitivity of the INFORM® HER2 Dual ISH DNA Probe Cocktail was verified in a quantitative manner. The ability of the probe cocktail to detect specific targets was verified by comparing copy numbers obtained from FISH.

Two slides (containing each of the 3 cores) from three different lots of MCF7, ZR75-1 and Calu-3 cell lines were quantified for HER2 and Chr 17 by Vysis PathVysion FISH. The data were compared with copy numbers obtained after staining with the INFORM® HER2 Dual ISH DNA Probe Cocktail assay.

The copy numbers for HER2, Chr 17 and ratios were in agreement between the HER2 Dual ISH DNA Probe Cocktail assay and PathVysion FISH for all three cell lines. Linear repeated measures analysis was used to compare copy numbers obtained from the two assays; the %CVs were less than 20%. The data are shown in Table 1.

Table 1 Comparison of Copy Numbers for HER2, Chr 17 and Ratios Between HER2 Dual ISH DNA Probe Cocktail Assay and PathVysion HER-2 FISH for All Three Cell Lines.

Xenograft core	Mean HER2/Chr17 Ratio by Dual ISH assay (SD) and corresponding Gene Status	Mean HER2/Chr17 Ratio by FISH assay (SD) and corresponding Gene Status
Calu-3	5.53 (0.564) Amplified	5.01 (0.786) Amplified
MCF7	0.85 (0.097) Non-amp	1.00 (0.067) Non-amp
ZR-75-1	1.34 (0.121) Non-amp	1.36 (0.124) Non-amp

3. **Assay Robustness Studies**

a. Tissue Thickness

The effect of tissue thickness on the performance of the INFORM® HER2 Dual ISH DNA Probe Cocktail was studied using one each of non-amplified, "borderline", and amplified breast carcinoma cases and HER2

Dual ISH 3-in-1 Xenograft Slides. Each specimen was sectioned at 2, 4, 6, and 8 µm, and stained with the INFORM® HER2 Dual ISH DNA Probe Cocktail assay, in duplicate. The specimens were enumerated using the scoring algorithm. For verification of repeatability, 10 sections (cut at 4 µm) from each specimen were stained and enumerated by one reader. Acceptance criteria were %CV <20%.

All specimens sectioned at 4 µm passed slide adequacy, stained appropriately, and were in agreement with FISH results. The sections cut at 2, 6, and 8 µm also stained appropriately and consistently with slides cut at 4 µm (%CV<14%). The 10 total replicates cut at 4 um all exhibited %CVs <11%. The data are summarized in **Table 2** and Error! Reference source not found.. Human specimen SERA 78 was non-amplified, SERA 94 which was near the borderline, and SERA 4 exhibited HER2 gene clusters. FISH staining of these cases resulted in the following HER2/Chromosome 17 ratios and HER2 status: SERA 4; 3.88 (Amplified), SERA 78: 0.98 (Non-amplified) and SERA 94: 1.17 (Non-amplified).

Table 2. INFORM® HER2 Dual ISH DNA Probe Cocktail assay results on specimens cut at different thickness. Duplicate slides were stained for each specimen, at each thickness. NA=Non-Amplified for HER2; A=Amplified for HER2.

Table 2 Specimen and Mean HER2/Chr 17 ratio for each tissue thickness and corresponding HER2 status (2 slides for each specimen)

Specimen	2 µm		4 µm		6 µm		8 µm	
	Mean HER2/Chr17 ratio (SD, %CV)	HER2 Status	Mean HER2/Chr17 ratio (SD, %CV)	HER2 Status	Mean HER2/Chr17 ratio (SD, %CV)	HER2 Status	Mean HER2/Chr17 ratio (SD, %CV)	HER2 Status
Calu3	6.8 (0.40, 5.9)	A, A	6.3 (0.12, 1.9)	A, A	6.1 (0.59, 9.6)	A, A	6.6 (0.90, 13.6)	A, A
MCF7	0.8 (0.06, 7.6)	NA, NA	0.8 (0.07, 9.2)	NA, NA	0.9 (0.06, 6.8)	NA, NA	0.9 (0.06, 6.2)	NA, NA
ZR751	1.4 (0.01, 1.0)	NA, NA	1.3 (0.06, 4.9)	NA, NA	1.2 (0.11, 9.4)	NA, NA	1.5 (0.07, 4.4)	NA, NA
SERA 78	1.1 (0.03, 2.5)	NA, NA	1.1 (0.03, 2.5)	NA, NA	1.1 (0.04, 4.0)	NA, NA	1.2 (0.02, 1.3)	NA, NA
SERA 94	1.6 (0.03, 2.1)	NA, NA	1.4 (0.01, 0.4)	NA, NA	1.4 (0.02, 1.2)	NA, NA	1.5 (0.01, 0.7)	NA, NA
SERA 4	9.0 (0.08, 0.9)	A, A	8.2 (0.33, 4.0)	A, A	8.8 (0.30, 3.4)	A, A	9.5 (0.50, 5.3)	A, A

Table 3. INFORM® HER2 Dual ISH DNA Probe Cocktail assay reproducibility on specimens cut at the same thickness. Ten slides were stained for each specimen. NA=Non-Amplified for HER2; A=Amplified for HER2.

Table 3 Specimen and Repeatability: Mean HER2/Chr 17 ratio (and gene status) of 10 sections per specimen, cut at 4 µm thickness	
Specimen	**Repeatability: Mean HER2/Chr 17 ratio (and gene status) of 10 sections per specimen, cut at 4 µm thickness (SD, %CV)**
Calu3	6.8 (10/10 amplified) (0.71, 10.4)
MCF7	0.8 (10/10 non-amplified) (0.08, 10.0)
ZR751	1.4 (10/10 non-amplified) (0.12, 8.8)
SERA 78	1.2 (10/10 non-amplified) (0.09, 7.4)
SERA 94	1.4 (10/10 non-amplified) (0.07, 5.1)
SERA 4	8.2 (10/10 amplified) (0.80, 9.7)

b. Tissue Fixation

The impact of pre-analytical conditions, (i.e. tissue fixation type and time) on HER2 Dual ISH DNA Probe Cocktail assay performance was evaluated using xenograft tumors generated from the MCF7 human carcinoma cell line. The MCF7 xenograft tumors were harvested and processed under several different conditions (six different fixations at six different times each). Sections were cut and stained in duplicate with the HER2 Dual ISH DNA Probe Cocktail. Specimens fixed in 10% NBF for 6-48 hours (recommended conditions for HER2 testing by ASCO/CAP) stained appropriately. Specimens fixed in alcoholic formalin, zinc formalin, and Prefer also stained appropriately. Fixation in Davidson's AFA or Bouin's did not result in appropriate staining and specimens processed using this fixative are not recommended for use with the INFORM® HER2 Dual ISH DNA Probe Cocktail assay.

c. Pretreatment (Cell Conditioning)

The effect of changing incubation times and or/temperatures for the available selectable options in the staining procedure was evaluated. For these studies, one each of non-amplified, "borderline" (containing multiple copies of HER2 and/or Chromosome 17, but not clustered), and amplified breast carcinoma cases and HER2 Dual ISH 3-in-1 Xenograft Slides were used. FISH staining of the cases resulted in the following HER2/Chromosome 17 ratios and HER2 status: Amplified case= 6.61, Non-amplified case: 0.99; and "Borderline" (Multiple copies of HER2 and/or Chromosome 17): 1.35.

Table 4 describes the results from the study, including the range of conditions that are selectable in the staining procedure, the "default" (nominal) conditions recommended by Ventana, and the conditions tested. Note that Ventana did not test all possible iterations of conditions possible, and does not recommend using combined extreme ranges for all conditions.

Table 4 Robustness Study Results			
Selectable Protocol Step	**Purpose of the Step and range of conditions available**	**Selectable conditions tested (Nominal = recommended by Ventana and used in all studies)**	**Staining results (HER2 gene status determined for each specimen)**
Deparaffiniza tion	Using heat (75 C) and detergent (EZ prep), the paraffin in the sample is melted and rinsed from the sample.	NA. The deparaffinization step was always selected.	NA. The specimens were always subjected to deparaffinization.
Cell Conditioning (CC2 buffer incubation on the slide)	Up to three cycles of CC2 buffer incubation for a selectable range of times (One cycle is "Mild", Two cycles are "Standard" and three are "Extended). The washes enable probe and antibody penetration in the tissue. The incubation times selectable for each cycle are 8, 12, or 16 minutes.	**Test**: Mild (8 min), which is only one cycle with the minimum incubation time available. **Test**: Extended (3 cycles), with maximum incubation time (16 min, 16 min, and 16 min). This is the maximum cell conditioning time allowed. **Nominal**: Extended (3 cycles) of 8, 12, and 8 minutes. This is the default procedure.	**Test for Mild**: All 6 xenograft specimens stained for appropriate HER2 gene status. Five out of six human tissue specimens exhibited weak staining and therefore were not adequate for enumeration. One of the human tissue specimens stained appropriately for HER2 gene status (amplified). Ventana does not recommend using the mild condition for human tissue specimens. **Test for Extended**: All specimens except for one xenograft slide stained appropriately for HER2 gene status. One xenograft slide exhibited slide drying and was not adequate for enumeration. **Test for nominal**: All specimens stained appropriately for HER2 gene status.
ISH Protease	Protease treatment immediately follows cell conditioning. The enzyme cleaves proteins to enable probe and antibody penetration. Protease 3 (4 min-32 min: in 4 min. increments) And Protease 2 (4 min-32 minutes: in 4 min. increments) are available to select	**Test**: Protease 2, 4 min **Test**: Protease 3, 4 min (this is the mildest protease treatment) **Test**: Protease 3, 32 Min **Nominal**: Protease 3, 16 min (this is the default condition for tissue specimens)	**Test Protease 2, 4 min:** All 12 specimens stained appropriately. **Test Protease 3, 4 min**: All 6 human specimens were too weak to enumerate but 5 out of 6 xenograft specimens stained appropriately (one xenograft core dried and was not enumerated). **Test Protease 3, 32 min**: All 12 specimens stained appropriately for HER2 gene status. **Nominal**: All 12 specimens exhibited their appropriate HER2 amplification status.

Table 4 Robustness Study Results			
Selectable Protocol Step	**Purpose of the Step and range of conditions available**	**Selectable conditions tested (Nominal = recommended by Ventana and used in all studies)**	**Staining results (HER2 gene status determined for each specimen)**
Denaturation	For the HER2 and Chr 17 probes to bind (hybridize) to complementary target sequences, the DNA must be denatured. This occurs in the presence of formamide and heat. The range of denaturation time is 12 min.- 32 minutes (in 4 min. increments)	**Test**: 12 minutes (minimum time) **Test**: 32 minutes (maximum time) **Nominal**: 20 minutes is the default time.	**Test 12 minutes**: All 6 human specimens exhibited their appropriate HER2 amplification status. One xenograft slide stained appropriately, and one dried and was not enumerated. **Test 32 minutes**: All 6 human specimens exhibited their appropriate HER2 amplification status. One xenograft slide stained appropriately, and one dried and was not enumerated. **Nominal**: All 12 specimens stained appropriately.
Hybridization :	The HER2-DNP labeled probe and the Chr17-DIG labeled probe must bind to their targets. This hybridization step is selectable in 1 hour increments, up to 12 hours.	**Test**: 3 hours **Test**: 12 hours (maximum) **Nominal**: 6 hours	**Test 3 hours:** All 12 specimens exhibited their appropriate HER2 amplification status. **Test 12 hours**: Eleven specimens exhibited appropriate HER2 amplification status except one human case that and was not enumerated. **Nominal**: All 12 specimens stained for appropriate HER2 gene status.
Stringency Wash:	Following probe hybridization, salt-containing buffer (2x SSC buffer) and high temperature are used to mitigate non-specific hybridization of the HER2 and Chr17 probes. The temperature is selectable at 72, 74, and 76 degrees C.	**Test**: 76 C is nominal for xenografts (this is the maximum) which was tested on tissue **Test**: 72 (the least stringent) is nominal for tissue and was tested on xenografts **Nominal**: 72 C on tissue and 76 degrees on xenografts	All 12 specimens exhibited their appropriate HER2 gene status for all conditions tested.

Table 4 Robustness Study Results			
Selectable Protocol Step	**Purpose of the Step and range of conditions available**	**Selectable conditions tested (Nominal = recommended by Ventana and used in all studies)**	**Staining results (HER2 gene status determined for each specimen)**
SISH HRP incubation time	A hardcoded step involves incubating the slide with anti-DNP antibody, which is not selectable. Next, the slide is incubated with the anti-Rabbit secondary antibody that is conjugated to the enzyme, HRP. The incubation time is known to affect signal intensity. The range of conditions are from 12-60 minutes (in 4 min. increments).	**Test** : 24 min **Test**: 40 min **Nominal**: 16 minutes (this is the default condition	All 12 specimens exhibited their appropriate HER2 gene status for all three conditions tested.
Silver C incubation time	Following incubation with SISH-HRP, the slide is sequentially incubated with the three silver chromogens: A, B, and C. C is the substrate for the HRP enzyme, hydrogen peroxidase. Its incubation time is selectable because it impacts signal intensity. The selectable incubation times are 4 min, 8 min., and 12 min.	**Test 35**: 8 min **Test 36**: 12 min (maximum) **Nominal**: 4 min (default)	All 12 specimens stained appropriately for HER2 gene status for the nominal conditon. **Test 8 min**: All 6 human cases stained appropriately for HER2 gene status. One xenograft slide dried and was inadequate for enumeration. The other xenograft slide stained appropriately for all three specimens. **Test 12 min**: All 6 human cases stained appropriately for HER2 gene status. One xenograft slide was inadequate for enumeration due to background staining. The other xenograft slide stained appropriately for all three specimens

Table 4 Robustness Study Results			
Selectable Protocol Step	**Purpose of the Step and range of conditions available**	**Selectable conditions tested (Nominal = recommended by Ventana and used in all studies)**	**Staining results (HER2 gene status determined for each specimen)**
RED ISH DIG AP incubation time	The anti-DIG antibody detects the hapten on the Chr 17 probe and is not selectable. Next, the anti-mouse secondary antibody recognizes the anti-DIG antibody. It is conjugated to the enzyme, alkaline phosphatase (AP). Its selectable range of incubation times is 12 min to 60 minutes (in 4 min. Increments).	**Test**: 16 min **Test**: 40 min **Nominal**: 24 minutes (default)	All 12 specimens exhibited appropriate HER2 gene status for all the conditions tested.
Fast Red incubation time	In the presence of the substrate for AP, Naphthol, the chromogen (Fast Red) is incubated on the slide. The Fast Red is deposited at the site of probe hybridization. The range of selectable incubation times is 4 min, 8 min, and 12 min.	**Test**: 4 min (minimum) **Test**: 12 min (maximum) **Nominal**: 8 min. (default)	All 12 specimens exhibited appropriate HER2 gene status for all conditions tested.
Counterstain	The last steps in the staining procedure are designed to stain the nuclei in the tissue to create contrast to discern the signals for Red and SISH within the cell boundaries. Hematoxylin II binds to the nuclear material. The range of selectable incubation times is 4 min to 32 min, in 4 min. Increments.	**Test**: Hematoxylin II, 4 Min (minimum) **Test**: Hematoxylin II, 12 Min **Nominal:** 8 min.	All 12 specimens exhibited appropriate HER2 gene status for all conditions tested, except for one human specimen that dried and could not be enumerated.

Table 4 Robustness Study Results			
Selectable Protocol Step	**Purpose of the Step and range of conditions available**	**Selectable conditions tested (Nominal = recommended by Ventana and used in all studies)**	**Staining results (HER2 gene status determined for each specimen)**
Post Counterstain	The post-counterstain incubation is designed to enhance the contrast of the Hematoxylin stain. The range of selectable incubation times is 4 min to 32 min, in 4 min. increments	**Test**: Bluing Reagent, 12 min **Nominal**: 4 min.	All 12 specimens exhibited appropriate HER2 gene status for all conditions tested.

4. Kit Stability

a. Probe Cocktail, SISH and Red detection kits, Real time

This study was designed to evaluate the stability of one Design lot and two Process Validation lots of the HER2 Dual ISH DNA Probe Cocktail assay, under different storage conditions. Stability testing was conducted on the HER2 Dual ISH DNA Probe Cocktail assay components using a Stability Master Lot (SML) of the detection and HER2/ Chromosome 17 cocktail probe components. Three lots of components, which include *ultra*View Red ISH DIG Detection kit, *ultra*View SISH DNP Detection Kit, HybReady Solution, INFORM® HER2 Dual ISH DNA Probe Cocktail, and HER2 3-in-1 Xenograft Slides, were combined to form these Stability Master Lots. All of the same lots of reagents were used for each testing time point. **Table 5** provides a summary of the stability testing data of HER2 Dual ISH DNA Probe Assay through month six (13).

Table 5 Summary of stability testing data for the HER2 Dual ISH DNA Probe Cocktail			
Intended Storage (2-8°C)			
Time Point	**SML 1** Results (Pass/Fail)	**SML 2** Results (Pass/Fail)	**SML 3** Results (Pass/Fail)
0	Pass	Pass	Pass
3 mos	Pass	Pass	Pass
6 mos	Pass	Pass	Pass
13 mos	Pass	Pass	Pass

Table 5 Summary of stability testing data for the HER2 Dual ISH DNA Probe Cocktail			
Ship Stress- SML 2			
	Hot Ship Stress 30C (CAT. A) Results (Pass/Fail)	**Hot Ship Stress 15C (CAT. B)** Results (Pass/Fail)	**Ship Stress Freeze/Thaw -20C** Results (Pass/Fail)
0	Pass	Pass	Pass
3 mos	Pass	Pass	Pass
6 mos	Pass	Pass	Pass
13 mos	Pass	Pass	Pass

Stability data from three master lots supports product stability through 12 Months for the HER2 Dual ISH DNA Probe Cocktail assay components.

5. Specimen Cut Slide Stability

This section describes stability testing conducted for cut, unstained tissue sections intended for staining with the INFORM® HER2 Dual ISH DNA Probe Cocktail. Target DNA is known to be stable in formalin-fixed, paraffin embedded tissue. This study was conducted to verify the stability of cut, unstained sections from human breast carcinoma specimens. One case, SERA 4 is amplified. The other case, 94S42615 is non-amplified. The study also used HER2 Dual ISH 3-in-1 Xenograft slides. All specimens were stored at room temperature (20-25 °C). **Table 6** contains a summary of the stability testing data through month 19. For xenografts, Pass = all cores exhibited appropriate staining.

Table 6 Summary of specimen cut-slide stability testing.			
Time Point	**Tissue case ID/ Xenograft Lot Number**	**Ratio**	**Resulting Gene Status**
Day 0	Tissue: SERA 4	10.31	Amp
	Tissue: 94S42615	1.22	Non-Amp
	Xeno: CR0958I	NA	Pass
Month 1	Tissue: SERA 4	6.00	Amp
	Tissue: 94S42615	1.19	Non-Amp
	Xeno: CR0958I	NA	Pass
Month 3	Tissue: SERA 4	10.00	Amp
	Tissue: 94S42615	1.00	Non-Amp
	Xeno: CR0958I	NA	Pass
Month 6	Tissue: SERA 4	9.76	Amp

Table 6 Summary of specimen cut-slide stability testing.			
Time Point	**Tissue case ID/ Xenograft Lot Number**	**Ratio**	**Resulting Gene Status**
	Tissue: 94S42615	1.27	Non-Amp
	Xeno: CR0958I	NA	Pass
Month 9	Tissue: SERA 4	13.10	Amp
	Tissue: 94S42615	1.19	Non-Amp
	Xeno: CR0958I	NA	Pass
Month 12	Tissue: SERA 4	11.10	Amp
	Tissue: 94S42615	1.13	Non-Amp
	Xeno: CR0958I	NA	Pass
Month 13	Tissue: SERA 4	10.80	Amp
	Tissue: 94S42615	1.14	Non-Amp
	Xeno: CR0958I	NA	Pass
Month 18	Tissue: SERA 4	12.00	Amp
	Tissue: 94S42615	1.22	Non-Amp
	Xeno: CR0958I	NA	Pass
Month 19	Tissue: SERA 4	8.31	Amp
	Tissue: 94S42615	1.72	Non-Amp
	Xeno: CR0958I	NA	Pass

The testing conducted to date confirms that the target DNA on the cut specimen slides remains stable after storage at ambient temperatures in slide boxes that prevent light exposure. The current testing performed supports up to 19 months stability of cut specimen slides for breast tissue and xenografts. The additional testing time points in the protocol will be continued until month 24.

6. Lot to Lot Reproducibility

In order to verify the lot to lot reproducibility of the INFORM® HER2 Dual ISH DNA Probe Cocktail assay, three different lots each of INFORM® HER2 Dual ISH DNA Probe Cocktail, *ultra*View SISH DNP Detection Kit, and *ultra*View Red ISH DIG Detection Kit were used for testing. Three invasive breast carcinoma cases were used in this study. One case was amplified (HER2/Chr 17 ratio ≥2.0), one non-amplified with clusters (HER2/Chr 17 ratio ≥2.0), one non-amplified (HER2/Chr 17 ratio <2.0) and one case had multiple (but not clustered) copies of HER2. Each was stained with FISH. Duplicate slides of each case and duplicate xenograft slides were stained using the recommended protocol for the INFORM® HER2 Dual ISH DNA Probe Cocktail, in various lot-to-lot combinations described in **Table 7**. The slides were stained with all

permutations on three different runs, and reading was performed by one qualified reader within a week of staining.

Three different lots of probe were tested with each lot of detection kits. The permutation of probe lot with combined SISH and Red ISH detection lots resulted in nine combinations. Variability was assessed for lot-to-lot for probe, lot-to-lot for detection kit, and replicate-to-replicate (intra-run). The three human cases and xenograft slides were stained in duplicate with each permutation (24 slides per run, 8 slides stained with each probe lot).

Table 7 Summary of Lot to Lot Analysis Scheme

Run number	INFORM® HER2 Dual ISH DNA Probe Cocktail	ultraView SISH DNP Detection Kit	ultraView Red ISH DIG Detection Kit
1	Lots 1, 2, and 3 Probe	Lot 1 SISH DNP	Lot 1 Red DIG
2	Lots 1, 2, and 3 Probe	Lot 2 SISH DNP	Lot 2 Red DIG
3	Lots 1, 2, and 3 Probe	Lot 3 SISH DNP	Lot 3 Red DIG

All slides (100%) passed slide adequacy and were enumerated by one qualified reader for HER2 and Chr 17 copies in 20 nuclei/specimen.

Table 8 contains the data for HER2 gene status, for each run and lot of probe for the human cases first and then the xenografts, and demonstrate that all permutations of probe cocktail and detection kit lot resulted in appropriate HER2 gene status for all specimens tested. These data verify the lot to lot reproducibility of the INFORM® HER2 Dual ISH DNA Probe Cocktail.

Table 8 Lot-to-Lot Reproducibility of INFORM® HER2 Dual ISH DNA Probe Cocktail Assay and Detection Reagents

Dual ISH Probe Lot/Detection Kit Lot	SERA 6	SERA 20	SERA 87	Calu3	MCF7	ZR751
Probe lot 1/Detection kit 1	11.6 (0.13) Amp	1.5 (0.13) NonAmp	1.3 (0.28) NonAmp	7.6 (1.03) Amp	0.81 (0.06) NonAmp	1.3 (0.16) NonAmp
Probe lot 1/Detection kit 2	12.0 (0.51) Amp	1.4 (0.04) NonAmp	1.3 (0.07) NonAmp	7.7 (0.07) Amp	1.0 (0.12) NonAmp	1.2 (0.06) NonAmp
Probe lot 1/Detection kit 3	13.2 (1.1) Amp	1.3 (0.05) NonAmp	1.2 (0.27) NonAmp	9.1 (1.00) Amp	0.9 (0.12) NonAmp	1.2 (0.06) NonAmp
Probe lot 2/Detection kit 1	9.5 (1.3) Amp	1.4 (0.06) NonAmp	1.1 (0.08) NonAmp	6.9 (0.75) Amp	0.8 (0.15) NonAmp	1.3 (0.02) NonAmp

Table 8 Lot-to-Lot Reproducibility of INFORM® HER2 Dual ISH DNA Probe Cocktail Assay and Detection Reagents						
Probe lot 2/Detection kit 2	11.0 (0.04) Amp	1.4 (0.01) NonAmp	1.1 (0.01) NonAmp	8.2 (0.92) Amp	0.9 (0.21) NonAmp	1.4 (0.04) NonAmp
Probe lot 2/Detection kit 3	13.6 (0.26) Amp	1.4 (0.05) NonAmp	1.1 (0.13) NonAmp	9.0 (0.61) Amp	1.0 (0.06) NonAmp	1.4 (0.04) NonAmp
Probe lot 3/Detection kit 1	10.5 (0.75) Amp	1.4 (0.09) NonAmp	1.3 (0.04) NonAmp	8.1 (1.23) Amp	0.9 (0.06) NonAmp	1.3 (0.11) NonAmp
Probe lot 3/Detection kit 2	12.8 (2.69) Amp	1.3 (0.0) NonAmp	1.1 (0.06) NonAmp	7.8 (0.08) Amp	0.8 (0.01) NonAmp	1.4 (0.26) NonAmp
Probe lot 3/Detection kit 3	13.1 (1.74) Amp	1.4 (0.11 NonAmp	1.18 (0.03) NonAmp	8.6 (0.44) Amp	0.95 (0.07) NonAmp	1.46 (0.13) NonAmp
Mean HER2/Chr 17 from all 18 slides per specimen (SD: %CV)	**11.9 (1.6: 13.6%) Amp**	**1.4 (0.08: 6.0%) NonAmp**	**1.18 (0.13: 11.1%) NonAmp**	**8.14 (0.88: 10.8%) Amp**	**0.89 (0.10: 10.6%) NonAmp**	**1.34 (0.11: 8.4%) NonAmp**

B. Animal Studies

None.

C. Additional Studies

None.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The applicant performed a clinical study to establish a reasonable assurance of safety and effectiveness of INFORM® HER2 Dual ISH DNA Probe Cocktail assay for determining HER2 gene status by enumeration of the ratio of the HER2 gene to Chromosome 17. The HER2 and Chromosome 17 probes are detected using two color chromogenic in situ hybridization (ISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens following staining on Ventana BenchMark® XT automated slide stainers (using NeXES software), by light microscopy. Studies were conducted in the US under IRB approval. One central reference laboratory (LabCorp: Durham, NC) performed the Vysis FISH testing. Another central laboratory, DCL, (DCL: Indianapolis, IN) performed the IHC testing using Ventana's PATHWAY 4B5 antibody. Three laboratories performed staining with the INFORM® HER2 Dual ISH DNA Probe Cocktail on the BenchMark® XT platform (ClinPath, ClinPath: Phoenix, AZ; TriCore, TriCore Reference Laboratories: Albuquerque, NM; and Mayo Clinic, Mayo Clinic: Rochester, MN). Ventana also purchased tissue blocks from vendors to supplement bins, as needed. Data from this clinical study were the basis for the PMA approval decision. Because the INFORM® HER2 Dual ISH DNA Probe

Cocktail is indicated as an aid in the assessment of patients for whom HERCEPTIN (trastuzumab) treatment is being considered, comparison was made with the PathVysion Her2 FISH assay for which clinical data was generated. Additionally a bridging study was conducted to validate the use of Abbott/Vysis VP2000 Tissue Processor, (a semi-automated processor that is used for pre-treatment steps in the FISH assay), in place of the approved manual method for FISH (see supplemental clinical studies). A summary of the method comparison study is presented below.

Patients' samples were collected from May 2006 through November 2009. The database for this PMA reflected data collected From October 2009 through March 2010 and included 714 patient samples. The additional bridging study was performed between October and November 2010. There were 5 investigational sites. These include Ventana in Tucson, AZ; LabCorp (the central pathology laboratory) in Research Triangle Park, NC; and the three investigational sites (Mayo Clinic: Rochester, MN (Site A); TriCore Reference Laboratories: Albuquerque, NM (Site B); and ClinPath: Phoenix, AZ (Site C)).

A. Method Comparison Study

1. Study Design

This study was a randomized, multi-site study utilizing archived tissue specimens that had been de-identified and unlinked from patient information to compare HER2 amplification status determined by the INFORM HER2 Dual ISH assay to amplification status determined by the PathVysion HER-2 FISH assay. All assays were conducted on serial sections of FFPE tissue specimens. Stained slides were evaluated for HER2 and chromosome 17 copy number in at least 20 nuclei to determine HER2 gene amplification status for each case according to the device labeling. Percent positive, percent negative and percent overall agreement between INFORM HER2 Dual ISH results and PathVysion HER-2 FISH results were determined.

Each of three clinical laboratory sites provided unstained slides from approximately 200 breast cancer cases which had previously been characterized for HER2 protein levels by IHC. Sites were required to screen cases sequentially in reverse chronological order to fill pre-specified HER2 protein expression categories (0/1+, 2+, 3+) based on the historical case data for HER2/neu protein expression. The Sponsor was blinded to the historical IHC data. HER2/neu protein expression status was determined for the study at a central laboratory by staining fresh sections with PATHWAY HER2/neu (4B5) Rabbit Monoclonal Primary Antibody assay (PATHWAY HER2/neu (4B5)), an FDA approved assay for determining HER2/neu protein expression levels in formalin-fixed, paraffin-embedded normal and neoplastic tissue. Binning by the fresh PATHWAY HER2/neu (4B5) IHC score was conducted in order to 1) create approximately balanced sets of cases with amplified and non-amplified gene status, and 2) to enrich for 2+ cases, which are considered borderline positive and are typically reflexed to a validated HER2 gene amplification assay to confirm

eligibility for treatment with HERCEPTIN. When site provided cases were insufficient to fill the IHC bins, additional cases were provided by the Sponsor (Ventana).

Eleven serial sections were required in total from each case specimen. Three sections were stained with H&E, two were used for the PATHWAY HER2/neu (4B5) assay, one was used for the PathVysion HER-2 FISH assay, one was used for the INFORM HER2 Dual ISH assay, two were reserved for back-ups, and two serial sections were reserved for staining with the INFORM® HER2 Dual ISH DNA Probe Cocktail assay under an unrelated protocol. PathVysion HER2 FISH assay was performed with the VP2000. A study was performed to demonstrate equivalency in results to manual FISH testing.

Following successful H&E staining, an independent consultant pathologist confirmed the presence of invasive breast cancer on H&E stained slides. The areas to be evaluated when performing the PathVysion HER-2 FISH, PATHWAY HER2/neu (4B5), and INFORM HER Dual ISH stained slides was marked on the H&E stained slides by the independent pathologist. The cases were randomized for IHC and PathVysion HER-2 FISH staining. Binning selection was completed based upon IHC scores. The cases were randomized a second time by the Sponsor and unstained section slides received from sites were returned to the clinical laboratory sites such that each site stained and evaluated slides from the same cohorts they initially provided to the Sponsor plus any additional cases, obtained from commercial sources, that were required to fill-out each binning category.

The primary objective of this study was to determine the positive and negative percent agreement rates and the corresponding score 95% confidence intervals (CI) for the INFORM HER2 Dual ISH assay results in comparison with PathVysion HER-2 FISH assay results at three independent clinical sites.

The secondary objectives of this study were to determine overall percent agreement and its score 95% confidence interval of the INFORM HER2 Dual ISH assay results in comparison with PathVysion HER-2 FISH assay results, initial and final staining failure rates for PathVysion HER-2 FISH and INFORM HER2 Dual ISH assays, agreement rates between PATHWAY HER2/neu (4B5) and PathVysion HER-2 FISH and agreement rates between PATHWAY HER2/neu (4B5) and INFORM HER2 Dual ISH.

a. Inclusion/Exclusion Criteria

i. Inclusion Criteria

Specimens were included in the study if they met all of the following inclusion criteria:

- FFPE invasive breast cancer tissue specimens;

- Specimen block contains sufficient tissue for the study;
- Score of 0, 1+, 2+, or 3+ based on PATHWAY HER2/neu (4B5) staining.

ii. Exclusion Criteria

Specimens meeting any of the following exclusion criteria were not included in the study:

- Insufficient tissue specimen;
- Tissue fixation with a non-formalin containing fixative;
- Diagnosis is ductal carcinoma in situ (DCIS) only.

b. Primary Clinical Endpoint

HER2 and chromosome 17 stained slides from both INFORM HER2 Dual ISH and PathVysion HER-2 FISH assays were scored by counting at least 20 nuclei to determine the HER2/Chr 17 ratio for each case. When the ratio was between 1.8 and 2.2, inclusive, an additional 20 nuclei were counted, and all 40 nuclei used to arrive at the final HER2/Chr 17 ratio. When the ratio was greater than or equal to 2.0, the HER2 gene status was considered amplified. When the ratio was less than 2.0, the HER2 gene status was considered non-amplified. The safety of this endpoint was determined by comparing the INFORM Dual ISH assay to other in situ hybridization techniques with demonstrated safety and effectiveness profiles that were determined in a prospective clinical trial. This approach is acceptable as the basic methodology is DNA probe hybridization (to the same HER2 and chromosome 17 target regions) with both techniques.

Percent positive agreement and percent negative agreement were the co-primary outcomes for this study. For each agreement rate, the two-sided 95% confidence interval was calculated using the score method. Equivalent performance between the INFORM HER2 Dual ISH and PathVysion HER-2 FISH assays was established if both the percent positive agreement and the percent negative agreement were 90% or higher, with the lower bound of the two-sided score 95% CI at least 85% for each agreement rate. Acceptable performance was confirmed by concordance with Her2 IHC staining using the approved anti-Her2 4B5 antibody.

2. Specimen Accountability

A total of 714 cases were sequentially considered for inclusion in the study. This included 576 cases from the clinical sites and 138 cases from Ventana that were considered for inclusion if binning categories could not be filled with cases that were received from the sites. After exclusion of cases due to screening failure at the sites, evaluation of H&E as unacceptable by Ventana, withdrawal per site request, data entry error and inadvertent exclusion from randomization, there were 674 cases sent to a central laboratory conducting staining with PATHWAY HER2/neu (4B5). Prior to sending cases for PathVysion HER-2 FISH staining, 1 additional case was

withdrawn by the site providing that case. A total of 673 cases were sent to the central laboratory conducting PathVysion HER-2 FISH staining. The IHC and FISH staining were performed concurrently at two different central laboratories.

Of the 674 cases stained with PATHWAY HER2/neu (4B5), 32 cases were excluded due to test failure, or other reasons (control failure, illegible or incomplete case report form, broken slide) resulting in successful PATHWAY HER2/neu (4B5) results from 642 cases.

Of the 673 cases stained with PathVysion HER-2 FISH, 654 were enumerable (includes re-staining attempts).

Cases meeting the study's inclusion/exclusion criteria were sequentially selected for inclusion in the study. Cases provided by sites or by Ventana that were in excess of the enrollment goal were excluded from further analysis.

A total of 519 cases were randomized for evaluation with the INFORM HER2 Dual ISH assay. A total of 512 cases were enumerable by INFORM HER2 Dual ISH (includes re-staining attempts). For the primary analysis, in addition to meeting all inclusion/exclusion criteria, cases were required to be enumerable by both INFORM HER2 Dual ISH and PathVysion HER-2 FISH. A summary of case disposition is presented in **Table 9**. The reasons for case non-evaluability are presented in **Table** .

A total of 510 cases were enumerable by PATHWAY HER2/neu (4B5), PathVysion HER-2 FISH and by INFORM HER2 Dual ISH assays and therefore included in the primary and secondary analyses of agreement rates between the assay methods.

A total of 417 cases were included in the exploratory analyses of agreement rates where equivocal cases (HER2 (4B5) score of 2+) were excluded.

A total of 519 cases were stained with INFORM HER2 Dual ISH on the BenchMark® XT platform and therefore included in the analysis of INFORM HER2 Dual ISH case staining failure rates and background and morphology acceptability rates.

A total of 673 cases were stained with PathVysion HER-2 FISH and therefore included in the analysis of FISH case staining failure rates and background and morphology acceptability rates. It should be noted that PathVysion HER-2 FISH staining and PATHWAY HER2/neu (4B5) staining were conducted in parallel and therefore PathVysion HER-2 FISH staining was done on cases meeting inclusion/exclusion criteria prior to elimination based on PATHWAY HER2/neu (4B5) staining outcomes. Consequently, the PathVysion HER-2 FISH sample size is larger than the INFORM HER2 Dual ISH sample size.

Table 9 Case Disposition					
Status	**Site A**	**Site B**	**Site C**	**Ventana**	**All sites**
Cases considered for inclusion in the study	203	190	183	138	714
Cases acceptable based on site screening and H&E evaluation by an independent consulting pathologist	192	170	176	136	674
Cases with successful PATHWAY HER2/neu (4B5) Staining	184	153	169	136	642
Number of cases stained with PathVysion HER-2 FISH staining assigned to sites	206	208	194	N/A	608
Number of cases enumerable after initial PathVysion HER-2 FISH staining	192	191	177	N/A	560
Total number of cases with enumerable PathVysion HER-2 FISH staining (includes successful re-staining attempts)	204	205	194	N/A	603
Number of cases stained with INFORM HER2 Dual ISH staining	173	173	173	N/A	519
Number of cases enumerable after initial INFORM HER2 Dual ISH staining	167	162	142	N/A	471
Total number of cases with enumerable INFORM HER2 Dual ISH staining (includes successful re-staining attempts)	171	172	169	N/A	512
Number of all evaluable cases (meeting inclusion/exclusion criteria and enumerable by both INFORM HER2 Dual ISH and PathVysion HER-2 FISH)	171	170	169	N/A	510

Table 10 Non-evaluable Cases-All Reasons	
Number of cases	Reason for non-evaluability
25	25 cases were site screening failures (captured on H&E CRF), including 6 cases withdrawn by site following receipt of slides at Ventana and staining with H&E)
14	14 cases failed H&E examination including 1 withdrawn by Sponsor based on H&E comment little tumor left on slide
33	33 cases failed PATHWAY HER2/neu (4B5) staining including 1 case not randomized for IHC staining & 1 case withdrawn by site prior to randomization and distribution 14 cases were not stained secondary to power failure
19	19 cases failed PathVysion HER-2 FISH staining*
6	6 cases failed INFORM HER2 Dual ISH staining*
123	123 cases were not used because the enrollment goal for the binning category to which it belonged had been met

3. **Study Population Demographics and Baseline Parameters**

All cases associated with this study were collected at U.S. sites from women who have undergone surgical biopsy for breast cancer. Race and ethnicity associated with the cases in this study was not available. Demographic data (age) was available for 122 cases at Site A, 42 cases at Site B, and 141 cases at Site C. Available demographic data are summarized in **Table 11**.

Table 11 Summary of Demographics					
Demographic Category	Characteristic /Statistic	Site A	Site B	Site C	Overall
Sample size		n = 171	n = 170	n = 169	n = 510
Age (years)	n	122	42	141	305
	Mean	56.6	56.4	58.2	57.3
	Standard Deviation	13.1	11.2	12.6	12.6
	Median	54.0	56.5	57.0	56.0
	Range (Minimum – Maximum)	31 - 96	33 - 85	33 - 87	31 – 96

4. **Safety and Effectiveness Results**

Positive and negative agreement rates of PathVysion HER-2 FISH clinical assessment (amplified or non-amplified) of the breast cancer tissue specimens and INFORM HER2 Dual ISH based on pooled data for all evaluable cases from all clinical sites are presented at the bottom of **Table 12**, based on the amplification status data presented at the top of the table. The overall agreement rate is also shown.

Table 12 Amplification Status and Positive and Negative Agreement Rates for Dual ISH vs. FISH				
		PathVysion HER-2 FISH Result		
		Amplified	Non-Amplified	Total
INFORM HER2 Dual ISH Result	Amplified	216	22	238
	Non-Amplified	9	263	272
	Total	225	285	510
		n/N	% (score 95% CI)	
Percent Positive Agreement		216/225	96.0 (92.6-97.9)	
Percent Negative Agreement		263/285	92.3 (88.6-94.8)	
Percent Overall Agreement		479/510	93.9 (91.5 – 95.7)	

There were 216 cases found to be HER2 amplified by both methods and 263 cases found to be non-amplified by both methods. Nine cases were found to be amplified by PathVysion HER-2 FISH assay, but non-amplified by INFORM HER2 Dual ISH. Twenty-two cases were found to be amplified by INFORM HER2 Dual ISH, but non-amplified by PathVysion HER-2 FISH. Thus, 31 cases were discrepant in total. The percent positive and percent negative agreement rates for INFORM HER2 Dual ISH vs. PathVysion HER-2 FISH were 96.0% and 92.3%, respectively. For each agreement rate, the two-sided 95% confidence interval was calculated. Acceptance criteria for establishing equivalent performance between the INFORM HER2 Dual ISH and PathVysion HER-2 FISH assays required both the percent positive agreement and the percent negative agreement to be 90% or higher, with the lower bound of the two-sided score 95% CI at least 85% for each agreement rate. Equivalent performance between the INFORM HER2 Dual ISH and PathVysion HER-2 FISH assays was established.

Percent positive agreement, percent negative agreement, and percent overall agreement for HER2 gene amplification status from the INFORM HER2 Dual ISH and PathVysion HER-2 FISH assay results, along with two-sided score 95% CI, were calculated for each clinical site separately. The data are summarized for Site A (**Table** 13), Site B (**Table** 14), and Site C (**Table** 15).

Table 13 Site A - Dual ISH vs. FISH				
Site A		PathVysion HER-2 FISH Result		
		Amplified	Non-Amplified	Total
INFORM HER2 Dual ISH Result	Amplified	75	7	82
	Non-Amplified	4	85	89
	Total	79	92	171
		n/N		%(score 95% CI)
Percent Positive Agreement		75/79		94.9 (87.7-98.0)
Percent Negative Agreement		85/92		92.4 (85.1-96.3)
Percent Overall Agreement		160/171		93.6 (88.8-96.4)

Table 14 Site B - Dual ISH vs. FISH				
Site B		PathVysion HER-2 FISH Result		
		Amplified	Non-Amplified	Total
INFORM HER2 Dual ISH Result	Amplified	72	7	79
	Non-Amplified	1	90	91
	Total	73	97	170
		n/N		%(score 95% CI)
Percent Positive Agreement		72/73		98.6 (92.6-99.8)
Percent Negative Agreement		90/97		92.8 (85.8-96.5)
Percent Overall Agreement		162/170		95.3 (91.0-97.6)

Table 15 Site C - Dual ISH vs. FISH				
Site C		PathVysion HER-2 FISH Result		
		Amplified	Non-Amplified	Total
INFORM HER2 Dual ISH Result	Amplified	69	8	77
	Non-Amplified	4	88	92
	Total	73	96	169
		n/N		%(score 95% CI)
Percent Positive Agreement		69/73		94.5 (86.7-97.8)
Percent Negative Agreement		88/96		91.7 (84.4-95.7)
Percent Overall Agreement		157/169		92.9 (88.0-95.9)

Site B had the highest overall agreement rate at 95.3%, followed by Site A at 93.6%, and Site C at 92.9%. All within-site agreement rates (positive, negative, and overall) exceeded 90%.

The initial and final staining failure rates for both INFORM HER2 Dual ISH and PathVysion HER-2 FISH are presented in **Table 16**. Only cases that were assigned to a site were included in the analysis of slide staining failure rates. Cases which failed to be enumerable after initial staining were allowed one additional attempt for successful staining using back-up slides. The final staining failure rate incorporates information from re-staining attempts. Data presented are based on data pooled from all sites.

Table 16 Case Staining Failure Rates				
	INFORM HER2 Dual ISH		PathVysion HER-2 FISH	
	n/N	%	n/N	%
Initial Case Staining Failure Rate	31/519	6.0	48/608	7.9
Final Case Staining Failure Rate	7/519	1.3	5/608	0.8

Both assays exhibited low staining failure rates. Initial and final staining failure rates for the two assays were similar. **Table 17** summarizes reasons for staining failures for each assay. More than one reason could exist per staining

failure, and may be specific to the different sites performing FISH vs. Dual ISH.

Table 17 Staining Failures-Pooled Data From All Sites				
	INFORM HER2 Dual ISH n (%)		PathVysion HER-2 FISH n (%)	
Parameter	**Initial**	**Repeat**	**Initial**	**Repeat**
Number of Cases Stained	519	48	608	48
Number of Enumerable Cases	488	41	560	43
Number of Not Enumerable (Failed) Cases	31	7	48	5
Failure Reasons	**Initial**	**Repeat**	**Initial**	**Repeat**
No Tissue Left	**0**	**0**	**1(0.2)**	**0**
No Invasive Carcinoma In Tissue	**0**	**2(0.4)**	**0**	**0**
Unacceptable Morphology	**2(0.4)**	**0**	**0**	**0**
Unacceptable Background	**9(1.7)**	**0**	**11(1.8)**	**1(0.2)**
HER2 and Chromosome 17	5(1.0)	0	1(0.2)	0
HER2 only	3(0.6)	0	5(0.8)	0
Chromosome 17 only	1(0.2)	0	5(0.8)	1(0.2)
Internal Positive Control Signal Not Detectable	**5(1.0)**	**1(0.2)**	**n/a**	**n/a**
HER2 and Chromosome 17	3(0.6)	1(0.2)	n/a	n/a
HER2 only	2(0.4)	0	n/a	n/a
Chromosome 17 only	0	0	n/a	n/a
Weak ISH Staining	**9(1.7)**	**1(0.2)**	**26(4.3)**	**1(0.2)**
HER2 and Chromosome 17	3(0.6)	0	0	0
HER2 only	3(0.6)	1(0.2)	26(4.3)	0
Chromosome 17 only	3(0.6)	0	0	1(0.2)
No ISH Staining	**10(1.9)**	**2(0.4)**	**9(1.5)**	**3(0.5)**
HER2 and Chromosome 17	6(1.2)	2(0.4)	5(0.8)	2(0.3)
HER2 only	3(0.6)	0	4(0.7)	1(0.2)
Chromosome 17 only	1(0.2)	0	0	0
Other	**16(3.1)**	**3(0.6)**	**6(1.0)**	**1(0.2)**
Staining Failure Rate n/N (%)	31/519 (**6.0**)	7/519 (**1.3**)	48/608 (**7.9**)	5/608 (**0.8**)

The morphology and background acceptability rates for both INFORM HER2 Dual ISH and PathVysion HER-2 FISH are presented in **Table**. The acceptability rates present here incorporate information from all slides stained, including successful re-staining attempts. Data presented are based on data pooled from all sites.

Table 18 Morphology and Background Acceptability Rates				
	INFORM HER2 Dual ISH		PathVysion HER-2 FISH	
	n/N	%	n/N	%
Morphology Acceptability Rate	569/571	99.6	782/782	100.0
Background Acceptability Rate	562/571	98.4	770/782	98.5

For the following analyses, IHC assay negative status is defined as a score of 0, 1+ and 2+; positive status is defined as a score of 3+. For both ISH assays following FDA Guidance, non-amplified status is defined as a HER2/Chr 17 ratio of <2.0; amplified status is defined as a HER2/Chr 17 ratio of ≥ 2.0.

An analysis of PATHWAY HER2/neu (4B5) with PathVysion HER-2 FISH when scoring per FDA Guidance was conducted. Data from all clinical sites were pooled for this analysis (See **Table 19**).

Table 19 IHC vs. FISH Comparison				
		PathVysion HER-2 FISH Result		
		Amplified	Non-Amplified	Total
PATHWAY HER2/neu (4B5) Results	Positive	212	28	240
	Negative	13	257	270
	Total	225	285	510
			n/N	% (score 95% CI)
Percent Positive Agreement			212/225	94.2 (90.4-96.6)
Percent Negative Agreement			257/285	90.2 (86.2-93.1)
Percent Overall Agreement			469/510	92.0 (89.3-94.0)

An analysis of PATHWAY HER2/neu (4B5) with INFORM HER2 Dual ISH when scoring per FDA Guidance was conducted. Data from all clinical sites were pooled for this analysis (See **Table 20**).

Table 20 IHC vs. Dual ISH Comparison				
All Sites		INFORM HER2 Dual ISH Result		
		Amplified	Non-Amplified	Total
PATHWAY HER2/neu (4B5) Results	Positive	221	19	240
	Negative	17	253	270
	Total	238	272	510
			n/N	%(score 95% CI)
Percent Positive Agreement Dual ISH			221/238	92.9 (88.9-95.5)
Percent Negative Agreement Dual ISH			253/272	93.0 (89.3-95.5)
Percent Overall Agreement			474/510	92.9 (90.4-94.9)

Analyses also were conducted where the 2+ cases were evaluated separately, since these cases are typically reflexed to ISH for a final diagnosis (See **Table 21** and **Table**). Both methods showed similar results.

Table 21 Agreement Between IHC (Positive, Negative and Equivocal) and HER2 Dual ISH (Amplified and Non-Amplified) _All Evaluable Cases

All Sites		INFORM Dual ISH Result		
		Amplified	Non-Amplified	Total
PATHWAY 4B5 IHC Result	Positive (3+)	221	19	240
	Equivocal (2+)	12	81	93
	Negative (0, 1+)	5	172	177
	Total	238	272	510

Site A		INFORM Dual ISH Result		
		Amplified	Non-Amplified	Total
PATHWAY 4B5 IHC Result	Positive (3+)	74	7	81
	Equivocal (2+)	5	26	31
	Negative (0, 1+)	3	56	59
	Total	82	89	171

Site B		INFORM Dual ISH Result		
		Amplified	Non-Amplified	Total
PATHWAY 4B5 IHC Result	Positive (3+)	74	5	79
	Equivocal (2+)	5	26	31
	Negative (0, 1+)	0	60	60
	Total	79	91	170

Site C		INFORM Dual ISH Result		
		Amplified	Non-Amplified	Total
PATHWAY 4B5 IHC Result	Positive (3+)	73	7	80
	Equivocal (2+)	2	29	31
	Negative (0,1+)	2	56	58
	Total	77	92	169

Table 22 Agreement Between IHC (Positive, Negative and Equivocal) and FISH (Amplified and Non-Amplified) _All Evaluable Cases.				
All Sites		**PathVysion FISH Result**		
		Amplified	**Non-Amplified**	**Total**
PATHWAY 4B5 IHC Result	**Positive (3+)**	212	28	240
	Equivocal (2+)	9	84	93
	Negative (0, 1+)	4	173	177
	Total	225	285	510
Site A		**PathVysion FISH Result**		
		Amplified	**Non-Amplified**	**Total**
PATHWAY 4B5 IHC Result	**Positive (3+)**	72	9	81
	Equivocal (2+)	6	25	31
	Negative (0, 1+)	1	58	59
	Total	79	92	171
Site B		**PathVysion FISH Result**		
		Amplified	**Non-Amplified**	**Total**
PATHWAY 4B5 IHC Result	**Positive (3+)**	70	9	79
	Equivocal (2+)	3	28	31
	Negative (0, 1+)	0	60	60
	Total	73	97	170
Site C		**PathVysion FISH Result**		
		Amplified	**Non-Amplified**	**Total**
PATHWAY 4B5 IHC Result	**Positive (3+)**	70	10	80
	Equivocal (2+)	0	31	31
	Negative (0, 1+)	3	55	58
	Total	73	96	169

B. Inter-Laboratory Reproducibility Study

1. Study Design

This randomized, blinded, design validation study was an inter-site, inter-reader, inter-run (day-to-day), and intra-run reproducibility study examining performance of Ventana's INFORM® HER2 Dual ISH DNA Probe Cocktail assay using formalin-fixed, paraffin-embedded (FFPE) invasive breast carcinoma specimens that were considered redundant clinical materials.

Two readers at each of three test sites evaluated nine breast cancer tissue sections stained on the BenchMark® XT platform with the INFORM®

HER2 Dual ISH DNA Probe Cocktail assay. Human breast cancer tissue specimens were pre-screened by the Sponsor to determine their HER2/Chr 17 ratio. Specimens were categorized as follows: non-amplified (ratio < 1.8), low amplified (2.2 < ratio < 3.0), and amplified (ratio ≥ 3.0). Participating study sites were blinded to HER2 status of the specimens and the number of cases within each amplification category.

Three external sites each used a BenchMark® XT platform to conduct one staining run per day on each of five non-consecutive days over 20 calendar days such that no two testing days were adjacent. Each staining run included:

- duplicate slides (serial tissue sections) from each of six breast cancer cases, and;
- duplicate slides (serial tissue sections) from three wildcard cases (not included in the primary and secondary data analyses).

Two qualified readers at each of the testing sites read all cases in this study.

Stained slides were evaluated for HER2 and Chr 17 copy number in at least 20 nuclei to determine HER2 gene amplification status for each case according to the device labeling. If the initial 20 nuclei resulted in borderline status (1.8 – 2.2), then an additional 20 nuclei were counted. Whether 20 or 40 nuclei were counted in total, amplification status was determined as: HER2/Chr 17 ≥ 2.0 = amplified, HER2/Chr 17 < 2.0 = non-amplified.
The study objectives were to evaluate reproducibility of the INFORM HER2 Dual ISH assay across each of the following design elements:

- Inter-site (three sites each using the BenchMark® XT instrument);
- Inter-reader (two readers at each site);
- Inter-run (five non-consecutive days with a single staining run per day at each site);
- Intra-run (replicates of a given case specimen within a run).

This study utilized archived FFPE invasive breast carcinoma specimens obtained from tissue banks within Ventana or from external vendors. One qualified reader counted at least 20 nuclei and enumerated both HER2 and Chr 17 signals, and categorized cases based on HER2/Chr 17 ratio as follows: non-amplified (ratio < 1.8), low amplified (2.2 < ratio < 3.0), and amplified (ratio ≥ 3.0). Six cases (included in the analyses) and an additional three "wildcard" cases (not included in the analyses) spanning the dynamic range of HER2 gene status were identified during tissue qualification for inclusion in the study.

An additional consideration in case selection was that specimens were of sufficient size to yield the required number of tissue sections. Thirty nine sections of each case were required for the study. One H&E stained slide from each case was provided to each site to confirm the presence of primary invasive carcinoma.

2. Inclusion/Exclusion Criteria

a. Inclusion Criteria

Specimens were included in the study if they met all of the following inclusion criteria:

- FFPE invasive breast cancer tissue specimens;
- Specimen block contains sufficient tissue for the study;
- Preliminary HER2 amplification status of non-amplified, low-amplified or amplified.

b. Exclusion Criteria

Specimens meeting any of the following exclusion criteria were not included in the study:

- Insufficient tissue specimen;
- Tissue fixation with a non-formalin containing fixative;
- Diagnosis is ductal carcinoma in situ (DCIS) only.

3. Statistical Methods

Three external sites each used a BenchMark® XT platform to conduct one staining run per day on each of five non-consecutive days over 20 calendar days such that no two testing days were adjacent. Each staining run included:

- duplicate slides (serial tissue sections) from each of six breast cancer cases, and;

- duplicate slides (serial tissue sections) from three wildcard cases (not included in the primary and secondary data analyses).

Two qualified readers at each testing site read slides in this study.

All analyses and summaries were produced using SAS v. 9.2.

Mean HER2 and Chr 17 copy number of each case were calculated based on number of cells counted and total number of signals.. HER2/Chr 17 ratio was calculated using the mean HER2 and Chr 17 copy number, and the ratio was rounded to 2 decimal places using the method of rounding to the nearest even number. A HER2/Chr 17 ratio of ≥ 2.0 was reported as amplified, while a ratio of <2.0 was reported as non-amplified. Initial staining failure rate, morphology acceptability rate, and background acceptability rate were calculated and rounded to the nearest tenth.

4. Safety and Effectiveness Results

A total of 180 case slides were stained and evaluated in this study (excluding wildcard cases). Of these, a total of 164 slides were enumerable by two readers and six slides were enumerable by a single reader. Thus, a total of 170 slides were considered to be evaluable. The reasons for slides being considered non-evaluable were 1) unacceptable morphology or background staining, 2) internal

positive control signal not acceptable, or 3) weak or no ISH staining. All evaluable case slides were included in the analyses.

The descriptive statistics for each of the 6 cases are shown below in **Table 23**. The mean HER2/Chr 17 ratios are included from all reads. N = number of reads performed for each case (30 slides were stained for each case, 60 possible reads total). The FISH testing results for each case are listed here as HER2/Chr 17 ratio and amplification status: Case 005 (1.27: Non-amp); Case 002 (1.14: Non-amp); Case 006 (2.31 Amp); Case 009 (3.89 Amp); Case 004 (3.68 Amp); Case 001 (5.00 Amp).

Table 23 The HER2/Ch17 ratios for each case, SD, and subsequent amplification status for each case.

	HER2/CH17 Ratio from all reads				Amplification Status	
HER2 Screening Status	**Case #**	**N**	**Mean**	**SD**	**Number of slides scored as Amplified (% of slides scored Amplified)**	**Number of slides scored as Non-Amplified (% of slides scored as Non-Amplified)**
Non-Amp	005	57	1.06	0.10	0	57 (100.0)
Non-Amp	002	42	1.16	0.14	0	42 (100.0)
Low Amp	006	60	2.09	0.45	29 (48.3)	31 (51.7)
Low Amp	009	60	4.19	1.01	60 (100.0)	0
Amp	004	57	6.73	2.10	57 (100.0)	0
Amp	001	58	7.18	2.41	57 (98.3)	1 (1.7)

The results indicate that cases 005 and 002, both with mean ratios ~1.0, were classified as non-amplified 100% of the time. Cases 009, 004, and 001 (clustered for HER2) were classified as amplified in all but one read for one case. Case 006, whose HER2/Chr 17 ratio falls essentially at the amplification cut-off (mean of 2.09) was evaluated as non-amplified 48.3% of the time and amplified as 51.7% of the time. This result is expected, since cases at a decision threshold will be classified randomly to either side of the threshold approximately 50% of the time ("Assay Migration Studies for In Vitro Diagnostic Devices", issued January 5, 2009). These data confirm that cases within the 1.8-2.2 ratio range (known to represent ~1.4% of all cases: References 7, 8) should be interpreted with caution (see **Table 24**).

Table 24 Shows that the discordances seen for case 006, which falls at the amplification cut-off, were distributed essentially randomly among reader, site, replicate and day.							
Site	Replicate	Reader	Day				
			1	2	3	4	5
A	1	1	Non-Amp	Non-Amp	Non-Amp	Non-Amp	Non-Amp
		2	Amp	Non-Amp	Amp	Non-Amp	Amp
	2	1	Non-Amp	Non-Amp	Amp	Non-Amp	Non-Amp
		2	Amp	Amp	Amp	Non-Amp	Amp
B	1	1	Amp	Amp	Amp	Amp	Amp
		2	Non-Amp	Amp	Non-Amp	Non-Amp	Non-Amp
	2	1	Non-Amp	Amp	Amp	Amp	Non-Amp
		2	Amp	Amp	Non-Amp	Amp	Non-Amp
C	1	1	Non-Amp	Amp	Non-Amp	Non-Amp	Non-Amp
		2	Amp	Non-Amp	Amp	Non-Amp	Non-Amp
	2	1	Non-Amp	Non-Amp	Amp	Amp	Non-Amp
		2	Amp	Amp	Amp	Amp	Non-Amp

Table below outlines agreement rates between 2 readers at each site in HER2 status from the Dual ISH assay, among all six cases. This table includes all slides where a HER2 amplification status was derived for both readers at a site. The counts indicate number of slides, not number of unique cases. APA and ANA were calculated as 2 times the number of slides in agreement, divided by the sum of 2 times the number of slides in agreement and total number of slides in disagreement. These average values are used so that results provided by one reader are not weighted more than results provided by the other reader.

Table 25 Agreement Between Readers at Each Site for HER2 Amplification Status.				
Site A		Reader 1		
		Amplified	Non-Amplified	Total
Reader 2	Amplified	31	6	37
	Non-Amplified	0	19	19
	Total	31	25	56
		n/N	%	
Average Positive Agreement (APA)		62/68	91.2	
Average Negative Agreement (ANA)		38/44	86.4	
Site B		Reader 1		
		Amplified	Non-Amplified	Total
Reader 2	Amplified	31	1	32
	Non-Amplified	5	19	24
	Total	36	20	56
		n/N	%	
Average Positive Agreement (APA)		62/68	91.2	
Average Negative Agreement (ANA)		38/44	86.4	
Site C		Reader 1		
		Amplified	Non-Amplified	Total

Table 25 Agreement Between Readers at Each Site for HER2 Amplification Status.				
Reader 2	Amplified	29	5	34
	Non-Amplified	1	17	18
	Total	30	22	52

	n/N	%
Average Positive Agreement (APA)	58/64	90.6
Average Negative Agreement (ANA)	34/40	85.0

C. Agreement between INFORM® HER2 Dual ISH DNA Probe Cocktail and PathVysion HER-2 FISH: Diagnostic Sensitivity and Specificity

To evaluate the agreement rate in HER2 gene status between PathVysion HER-2 FISH and the INFORM® HER2 Dual ISH DNA Probe Cocktail assay in a pilot study, approximately 90 formalin-fixed, paraffin embedded invasive breast carcinoma cases were stained with both assays. The specimen cohort was characterized for HER2 protein status by immunohistochemistry using the Ventana PATHWAY HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody assay. To ensure that the FISH and SISH reads were performed within the same target area, the specimens were evaluated at Ventana for the presence of invasive breast carcinoma and the area was circled on the hematoxylin and eosin (H&E) slide. One H&E slide with the invasive carcinoma region circled and two unstained slides were sent to a central FISH testing lab. Each case was stained using the recommended staining protocol for the INFORM® HER2 Dual ISH DNA Probe Cocktail assay provided in the package insert at Ventana and evaluated by two qualified readers using the developed scoring algorithm. The readers noted if the area was deemed to be inadequate for enumeration. At least 20 nuclei were enumerated for HER2 and Chr 17 signals in that target area. An additional 20 nuclei were counted for those cases with a HER2/Chr 17 ratio between 1.8 and 2.2. Amplified cases were defined as HER2/Chr 17 ratios greater than or equal to 2.0, and non-amplified cases as HER2/Chr 17 ratios less than 2.0. If a stained specimen slide was inadequate and another slide for that specimen was available, the second slide was stained with a modified staining protocol (based on the troubleshooting guide in the draft package insert) and evaluated.

The results from the INFORM HER2 Dual ISH assay were compared with the HER2 gene status obtained from Abbott/Vysis FISH testing at a central lab(see **Table**, **Table**), and **Table**). The results indicate > 89.5% negative agreement rates and >95% positive agreement rates between the FISH and Dual ISH assays, for both readers. Overall agreement rates between the two assays were 95% for both readers. It should be noted that both readers read the same number of slides in this study, and the results are reported for the cases that had enumerable Dual ISH for both readers, as well as valid Vysis FISH results.

The Dual ISH results from both readers were compared and are presented as percent positive agreement and percent negative agreement (reader A to Reader B) and as an average positive agreement (APA) and average negative

agreement (APA). APA and ANA were calculated as 2 times the number of slides in agreement, divided by the sum of 2 times the number of slides in agreement and the total number of slides in disagreement.

Table 26 Agreement between Vysis FISH results and Reader A's Dual ISH result for HER2 gene status.

Reader A		Vysis FISH Result		
		Amplified	Non-Amplified	Total
INFORM HER2 Dual ISH Result	Amplified	18	2	20
	Non-Amplified	1	40	41
	Total	19	42	61

	n/N	%(95% Score CI)
Percent Positive Agreement	18/19	94.7 (75.4-99.1)
Percent Negative Agreement	40/42	95.2 (84.2-98.7)
Percent Overall Agreement	58/61	95.1 (86.5-98.3)

Table 27 Agreement between Vysis FISH result and Reader B's Dual ISH result for HER2 gene status.

Reader B		Vysis FISH Result		
		Amplified	Non-Amplified	Total
INFORM HER2 Dual ISH Result	Amplified	17	1	18
	Non-Amplified	2	41	43
	Total	19	42	61

	n/N	%(95% Score CI)
Percent Positive Agreement	17/19	89.5 (68.6-97.1)
Percent Negative Agreement	41/42	97.6 (87.7-99.6)
Percent Overall Agreement	58/61	95.1 (86.5-98.3)

Table 28 Agreement of Reader A and Reader B for HER2 Dual ISH results on human breast carcinoma cases.				
		Reader B		
		Amplified	Non-Amplified	Total
Reader A	Amplified	18	2	20
	Non-Amplified	0	41	41
	Total	18	43	61

	n/N	%
Percent Positive Agreement	18/18	100
Percent Negative Agreement	41/43	95.3
Percent Overall Agreement	59/61	96.7

	n/N	%
Average Positive Agreement (APA)	36/38	94.7
Average Negative Agreement (ANA)	82/84	97.6

D. Summary of Clinical Results

1. Method Comparison

A method comparison study testing the ability of the INFORM® HER2 Dual ISH DNA Probe Cocktail assay to produce assessments of Her2 gene amplification that agreed with the PathVysion HER-2 FISH (Abbott/Vysis) was conducted at three sites as the pivotal study. The positive and negative agreement rates (with score 95% confidence intervals) were 96% (92.6-97.9) and 92.3% (88.6-94.8) from the pooled sites, respectively. The results of this study established that the INFORM® HER2 Dual ISH DNA Probe Cocktail assay has equivalent performance to the approved PathVysion HER-2 DNA Probe Kit.

2. Inter-laboratory Reproducibility

An inter-laboratory reproducibility study was conducted at the same three sites to demonstrate that the performance of the INFORM® HER2 Dual ISH DNA Probe Cocktail assay is reproducible when performed at different laboratories. The results of this study establish that there is very low variability in the estimation of the HER2/Chr 17 ratio across sites, days, readers and within run.

3. Inter-Observer Study

An inter-observer agreement study was conducted to demonstrate that the performance of the INFORM® HER2 Dual ISH DNA Probe Cocktail assay is reproducible when results are interpreted by different readers. The results

of this study establish that there is agreement of diagnosis of HER2 gene status as determined by INFORM® HER2 Dual ISH DNA Probe Cocktail with PathVysion FISH. Inter-observer agreement in determination of HER2 gene status is reproducible.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

A. Analysis of Borderline Cases

For this analysis, PathVysion HER-2 FISH assay and INFORM HER2 Dual ISH assay non-amplified status was defined as a HER2/Chr 17 ratio of <1.8; amplified status was defined as a HER2/Chr 17 ratio of >2.2; equivocal status was defined as a HER2/Chr 17 ratio between 1.8 and 2.2, inclusive; PATHWAY HER2/neu (4B5) IHC assay negative status was defined as a score of 0 and 1+; positive status was defined as a score of 3+; equivocal status was defined as a score of 2+. Two-sided, score 95% CI were calculated. Data from all clinical sites were pooled for this analysis (See **Table 29** and **Table 30**).

Table 29 IHC vs. FISH Comparison of Borderline Cases

		PathVysion HER-2 FISH Result			
		Amplified (>2.2)	Equivocal (1.8-2.2)	Non-Amplified (<1.8)	Total
PATHWAY HER2/neu (4B5) Results	Positive (3+)	211	7	22	240
	Equivocal (2+)	7	2	84	93
	Negative (0/1+)	2	3	172	177
	Total	220	12	278	510
		n/N	% (score 95% CI)		
Percent Overall Agreement		385/510	75.5 (71.6-79.0)		

Table 30 IHC vs. Dual ISH Comparison of Borderline Cases

		INFORM HER2 Dual ISH Results			
		Amplified (>2.2)	Equivocal (1.8-2.2)	Non-Amplified (<1.8)	Total
PATHWAY HER2/neu (4B5) Results	Positive (3+)	219	2	19	240
	Equivocal (2+)	12	3	78	93
	Negative (0/1+)	2	3	172	177
	Total	233	8	269	510
		n/N	% (score 95% CI)		
Percent Overall Agreement		394/510	77.3 (73.4-80.7)		

Positive agreement was 94.0 %, negative agreement was 63.9%, and total agreement was 77.3%. When equivocals are considered to be correct calls, regardless of whether they are scored as amplified or non-amplified, the

positive agreement increases to 99.1% and negative agreement increase to 92.9%. All of these numbers compare favorably to FISH which demonstrated 95.9% positive agreement and 61.9% negative agreement. With equivocal samples included, these increase to 99.1% and 92.1% respectively.

B. Bridging Data

The Clinical Method Comparison study was performed between the Abbott/Vysis PathVysion HER2 DNA Probe Kit and Ventana's INFORM® HER2 Dual ISH DNA Probe Cocktail assay to demonstrate equivalent performance in determination of HER2 gene status in formalin-fixed, paraffin embedded human breast carcinoma specimens. In that study, the central testing laboratory, utilized the VP 2000 Tissue Processor for pretreatment steps for the PathVysion HER2 fluorescent in situ hybridization (FISH) testing. Because the Vysis device was approved with instructions for a manual technique only, the Sponsor, through the central testing laboratory, conducted a bridging study to demonstrate that the FISH results generated with the automated system were comparable to those generated with the fully manual Vysis method.

The new study was performed on 164 invasive breast cancer specimens. This study included all 14 cases that were equivocal by either FISH or DDISH in the Method Comparison Study so that nearly 9% of the cases in the cohort were equivocal, which is significantly higher than the 1.4% seen in the clinical population. Results also are compared with HER2 gene status obtained from the INFORM HER12 Dual ISH DNA Probe Cocktail assay. The data are presented with equivocal zone cases included in the analyses. The results indicate that the HER2 gene status results for cases (including equivocal zone) stained with FISH using the VP 2000 Tissue Processor for pretreatment steps are equivalent to those obtained when performing the fully manual FISH method, with 94.4 (68/72) for positive percent agreement, 88.2 (67/76) for negative percent agreement rate and 91.2 (135/148) for overall percent agreement rate. The data are summarized in Table 31 below:

Table 31 - VP 2000 FISH to Manual FISH All Sites Including Equivocal Zone Cohort

	Manual FISH Result		
VP2000 FISH Result	Amplified (HER2/Chr 17 Ratio ≥2.0)	Non-Amplified (HER2/Chr 17 Ratio <2.0)	Total
Amplified (HER2/Chr 17 Ratio ≥2.0)	68	9	77
Non-Amplified (HER2/Chr 17 Ratio <2.0)	4	67	71
Total	72	76	148
Agreement	n/N	% Agreement Rate	95% Score CI

Table 31 - VP 2000 FISH to Manual FISH All Sites Including Equivocal Zone Cohort			
Positive Percent Agreement (PPA)	68/72	94.4	86.6-97.8
Negative Percent Agreement (NPA)	67/76	88.2	79.0-93.6
Overall Percent Agreement (OPA)	135/148	91.2	85.6-94.8

XII. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Safety Conclusions

The INFORM® HER2 Dual ISH DNA Probe Cocktail assay is an *in vitro* diagnostic test which is to be used in a laboratory setting by qualified professionals. The instrument Operator's Manual, INFORM® HER2 Dual ISH DNA Probe Cocktail assay Package Inserts, Material Safety Data Sheets, and Interpretative Guide contain appropriate precautions and warnings. The information provided to the reagent user is appropriate and sufficient to utilize the product in a safe manner when instructions are followed.

The adverse effects of the device are based on data collected in a clinical studies conducted to support PMA approval as described above. A study using patient samples with unknown Her2 gene amplification status was not conducted. Instead, results generated using this device were compared favorably to an additional device for which prospective clinical data was available and for which reasonably safe and effective use was demonstrated.

Further, the device was shown to have reasonable reproducibility that is essential to prolonged safety of the device. However, potential institutional differences should be recognized and individual proficiency should be assessed prior to implementation of this test

B. Effectiveness Conclusions

The information demonstrating the potential of the device to be used effectively is based on data collected in clinical studies conducted to support PMA approval as described above. The use of unknown patient samples was not conducted, but instead results generated using this device were compared

favorably to an additional device for which prospective clinical data was available.

C. Overall Conclusions

The results from the non-clinical and clinical studies presented in this original PMA application submission establish reasonable assurance that the INFORM® HER2 Dual ISH DNA Probe Cocktail assay is safe and effective for its intended use when used in accordance with product labeling.

Benefit/Risk:

The Ventana Medical Systems, Inc. (Ventana) INFORM® HER2 Dual ISH DNA Probe Cocktail is designed to quantitatively detect amplification of the HER2 gene and the INFORM® HER2 Dual ISH DNA Probe Cocktail is indicated as an aid in the assessment of patients for whom HERCEPTIN (trastuzumab) treatment is being considered. Results from the INFORM *HER2* DNA Probe are intended for use as an adjunct to existing clinical and pathologic information currently used for estimating prognosis in patients with invasive breast cancer.

In many clinical studies amplification and/or over-expression of HER2 has been shown to be associated with a poor clinical outcome for women with invasive breast cancer, and correlated with several negative prognostic variables, including estrogen receptor (ER) negative status, high S-phase fraction, positive nodal status, mutated p53, and high nuclear grade. Over-expression of the HER2 protein, amplification of the HER2 gene, or both occurs in approximately 15 to 25 percent of breast cancers, and are associated with aggressive tumor behavior. In several studies, invasive breast cancer patients (both node positive and node negative) with an amplified HER2 gene displayed decreased overall survival and a higher frequency of recurrence. Results from clinical studies measuring HER2 protein over-expression by immunohistochemistry were similar to those obtained by HER2 gene amplification.

Trastuzumab (HERCEPTIN) is a humanized monoclonal antibody against the extra-cellular domain of HER2 and has been shown to benefit patients with HER2-positive breast cancer (1-6). Clinical studies have shown that breast cancer patients with high HER2 protein over-expression and/or gene amplification benefit most from trastuzumab. HERCEPTIN has been shown to arrest and in some cases reverse the growth of breast cancer for some patients. Determination of HER2 protein over-expression and/or gene amplification is essential for selecting invasive breast cancer patients for whom trastuzumab therapy is being considered and clinically indicated.

The known risks of HERCEPTIN treatment include infusion toxicity (chills, fever, pain, pain at the tumor site, asthenia, nausea, vomiting and headache), and cardiotoxicity. Other unknown adverse events may also occur for patients exposed to these therapies.

A false positive scoring result may lead to patients being treated with HERCEPTIN alone or HERCEPTIN in combination with other therapies with the possibility of experiencing adverse events as a result of receiving these therapies.

A false negative scoring result may deny patients that might benefit from HERCEPTIN alone or HERCEPTIN in combination with other therapies from receiving these therapies.

Therefore, there is a benefit in knowing *HER2* gene and/or protein status in invasive breast cancer patients so that clinicians can make more informed decisions to improve the overall management of their breast cancer patients.

XIV. CDRH DECISION

CDRH issued an approval order on June 14, 2011. The final conditions of approval are cited in the approval order.

The applicant's manufacturing facility was inspected on May 19, 2011 and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

1. Barlund M, Tirkkonene M, Forozan F, Tanner MM, Kallioniemi O, et al. Increased copy number at 17q22-q24 by CGH in breast cancer is due to high-level amplification of two separate regions. Genes Chrom Cancer 1997; 20: 372-376.
2. Baselga J, Carbonell X, Castaneda-Soto NJ, et al. Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule. J Clin Oncol 2005;23:2162-2171.
3. Bianchi S, Paglierani M, Zampi G, et al. Prognostic significance of c-erbB-2 expression in node negative breast cancer. Br J Cancer 1993;67:625-629.
4. Bilous M et al. Current perspectives on HER2 Testing: A review of national testing guidelines. Mod Pathol 2003:173-182.

5. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clinical Oncol 1999;17:2639-2648.

6. Coussens L, Yang-Feng TL, Liao Y-C, Chen E, Gray A, McGrath J, et al. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. Science 1985;230:1132-1139.

7. Press MF, Bernstein L, Thomas PA, Meisner LF, et al. HER-2/neu gene amplification characterized by fluorescence in situ hybridization: Poor prognosis in node-negative breast carcinomas. J Clin Oncol 1997;15:2894-2904.

8. Wolff, AC, Hammond, MEH, Schwartz, JN, et al. American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer. Arch Pathol Lab Med 2007;131:18-43.