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: VENTANA HER2 Dual ISH DNA Probe Cocktail

Applicant's Name and Address: Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, AZ 85755

Date of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P190031

Date of FDA Notice of Approval: July 28, 2020

II. INDICATIONS FOR USE

The VENTANA HER2 Dual ISH DNA Probe Cocktail is intended to determine *HER2* gene amplification status by enumeration of the ratio of the *HER2* gene to Chromosome 17 by light microscopy. The HER2 and Chromosome 17 probes are detected using the VENTANA Silver ISH DNP Detection Kit and the VENTANA Red ISH DIG Detection Kit by a two-color chromogenic *in situ* hybridization (ISH) in formalin-fixed, paraffin-embedded human breast carcinoma tissue specimens, following staining on a BenchMark ULTRA instrument.

The VENTANA HER2 Dual ISH DNA Probe Cocktail is indicated as an aid in the assessment of patients for whom Herceptin (trastuzumab) is being considered.

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

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

III. CONTRAINDICATIONS

There are no known contraindications associated with this test.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions are included in the VENTANA HER2 Dual ISH DNA Probe Cocktail product labeling.

V. DEVICE DESCRIPTION

The VENTANA HER2 Dual ISH DNA Probe Cocktail is formulated for use with VENTANA Silver ISH DNP Detection Kit (SISH), VENTANA Red ISH DIG Detection Kit (RISH), and accessory reagents on a BenchMark IHC/ISH instrument to be used on formalin-fixed, paraffin-embedded (FFPE) human breast cancer tissue specimens. The components of the VENTANA HER2 Dual ISH DNA Probe Cocktail are provided in Table 1 below. The ancillary reagents required for the BenchMark ULTRA system are listed in Table 2 below.

Table 1. Components of the VENTANA HER2 Dual ISH DNA Probe Cocktail

Component	Description
VENTANA HER2 Dual ISH DNA Probe Cocktail	HER2 DNP Oligo Probe Mix (0.014 mg/mL); Chr 17 DIG Oligo Probe Mix (0.00024 mg/mL); Hybrisol XX Diluent
VENTANA SILVER ISH DNP HQ	SISH DNP HQ Conjugate (0.0125 mg/mL); Avidin Diluent with B5 Blocker for Multimer
VENTANA SILVER ISH DNP HQ HRP	Mouse anti-HQ HRP Conjugate (0.025 mg/mL); Avidin Diluent with B5 Blocker for Multimer
VENTANA SILVER ISH DNP CHROMOGEN A	Silver acetate (0.0036 g/mL)
VENTANA SILVER ISH DNP CHROMOGEN B	Hydroquinone (0.0018 g/mL); Citric Acid Monohydrate (0.0136 g/mL); Sodium Citrate Trisodium Salt Dihydrate (0.0103 g/mL)
VENTANA SILVER ISH DNP CHROMOGEN C	Hydrogen peroxide 30% (0.003 mL/mL)
VENTANA RED ISH DIG NP	RISH DIG NP Conjugate (0.0075 mg/mL); Avidin Diluent with B5 Blocker for Multimer
VENTANA RED ISH DIG NP AP	RISH DIG NP AP Conjugate (0.010 mg/mL); Enhanced Avidin Diluent
VENTANA RED ISH DIG pH ENHANCER	Magnesium Chloride (2.5% w/v); Tris (0.0121 g/mL); Brij-35 30% (0.0017 g/mL); Proclin 300 (0.0005 mL/mL)
VENTANA RED ISH DIG NAPHTHOL	NAPHTHOL AS-TR Phosphate, Disodium Salt (0.007 g/mL); Tris (0.061 g/mL); Brij-35 30% (0.0017 g/mL); Proclin 300 (0.0005 mL/mL)
VENTANA RED ISH DIG FAST RED CHROMOGEN	Fast Red KL Salt (0.002 g/mL); Brij-35 30% (0.0123 g/mL); Proclin 300 (0.0005 mL/mL); Glacial Acetic Acid 99.5% (0.0006 mL/mL)

Table 2. Ancillary Reagents

Component	Description
HybReady Solution	Formamide-based buffer that is used to dilute probes and to create appropriate ISH conditions on the slide
ultraView Silver Wash II	Rinse slides between staining steps and provide stable aqueous environment for ISH
ISH Protease 3	Used to remove protein that surrounds target DNA or RNA sequences of interest for ISH
Hematoxylin II Counterstain	Stains a variety of tissue components including nuclei, mitochondria, mucin, hemoglobin, elastic fibers, and collagen
Bluing Reagent	Applied after hematoxylin to change the hue of the hematoxylin to a blue color
Sodium Chloride Sodium Citrate Buffer (SSC) (10×)	Rinse slides between staining steps and provide stable aqueous environment for ISH
EZ Prep Reagent (10×)	Remove paraffin from tissue samples during ISH
ULTRA Cell Conditioning Solution 1	Hydrolyze covalent bonds formed by formalin in tissue to increase target accessibility
ULTRA Cell Conditioning Solution 2	Hydrolyze covalent bonds formed by formalin in tissue to increase target accessibility
Ultra Liquid Coverslip	Serves as a barrier between the aqueous reagents and the air to prevent evaporation and provide a stable aqueous environment for ISH

The staining protocol (provided in Table 3 below) consists of numerous steps in which reagents are incubated for pre-determined times at specific temperatures. At the end of each incubation step, the BenchMark IHC/ISH instrument washes the sections to remove unbound material and applies a liquid coverslip which minimizes the evaporation of the aqueous reagents from the slide.

ISH signals are visualized as single copies, multiple copies and clusters. Single copies in the normal cells are used as a reference to enumerate the signals in the carcinoma nuclei. Signals should be visualized using 20×, 40×, or 60× objectives.

Assay Scoring

Once an adequate target area is identified, the reader records the counts for *HER2* and Chromosome 17 (Chr 17) copy numbers that are present in 20 representative nuclei. If the resulting *HER2*/Chr 17 ratio falls within 1.8-2.2 (inclusive), the reader is recommended to count an additional 20 nuclei, and the resulting ratio is calculated from the total 40 nuclei. *HER2* gene status is reported as non-amplified (*HER2*/Chr 17 < 2.0) or amplified (*HER2*/Chr 17 ≥ 2.0).

Device Instrument and Software

The VENTANA HER2 Dual ISH Assay is performed on the BenchMark ULTRA automated staining instrument using the Ventana system software (VSS) software version 12.3. Staining

procedure is specific for the Ventana HER2 Dual ISH Assay and operates within the VSS environment requiring that all system reagents are used together.

Specimen Preparation

Routinely processed tissues that have been fixed in 10% Neutral Buffered Formalin for 6 to 72 hours, paraffin-embedded and cut at 4 μm sections should be used. Specimens fixed < 6 hours in formalin can result in signal loss and nuclear over-digestion, as observed by pale/weak hematoxylin staining. Only fixation in 10% NBF is recommended as some fixatives produce variable staining with ISH-based assays (including Bouin's and Alcohol Formalin-Acetic Acid (AFA).

Slides should be stained immediately, as quality of nucleic acid targets in cut tissue sections may diminish over time.

Test Controls

Controls establish the validity of the test results. The following internal positive and negative controls should be assessed:

1) **Positive Tissue Controls**
   HER2 and Chr 17 sequences are present in every cell of the human body. Thus, they act as internal positive controls in every tissue specimen and must be visible (1 to 2 signals per cell) in normal (non-neoplastic) cells in and around the target carcinoma area. However, not all cells will exhibit single gene copy due to biological heterogeneity and truncation from tissue sectioning. Specific nuclear staining may be located in various cells including: stromal fibroblasts, endothelial cells, lymphocytes, and non-neoplastic breast epithelial cells. At least 50% of the normal cell nuclei should contain at least one SISH signal and at least 50% should contain at least one RISH signal for the target area to be deemed adequate.

2) **Negative Tissue Controls**
   A negative control tissue serves to demonstrate that there is no non-specific staining from any component of the assay. Normal (non-neoplastic) cell nuclei contain on average 1-2 HER2 and/or Chr 17 signals. Therefore, the normal cells also act as negative controls for non-specific staining for the assay.

Additional information about positive and negative controls are available in the product labeling.

Principle of the Procedure

The VENTANA HER2 Dual ISH DNA Probe Cocktail contains HER2 probes (labeled with the hapten dinitrophenyl or DNP) and Chromosome 17 probes (labeled with the hapten digoxigenin or DIG) formulated in a formamide-based buffer. The probes are designed to detect amplification of the *HER2* gene in invasive breast carcinoma. The HER2 DNA Probe is a mixture of oligo probes that spans approximately 300,000 base pairs along the genomic region containing the *HER2* gene (also known as ERBB2 and NEU), which is located on human Chromosome 17 (17q12). The Chromosome 17 probe is a mixture of oligo probes that target sequences within the centromeric region and serves as a reference for aneusomy. Copy numbers of both probes are enumerated in tumor nuclei and results are reported as a ratio of

HER2/Chromosome 17 to determine HER2 amplification status (*HER2*/Chromosome 17 ratio ≥ 2.0 is amplified, while a ratio < 2.0 is non-amplified).The VENTANA HER2 Dual ISH DNA Probe Cocktail is optimally formulated for use with VENTANA Silver ISH DNP Detection Kit, VENTANA Red ISH DIG Detection Kit, and accessory reagents on a BenchMark ULTRA instrument.

Patient FFPE tissue specimens are cut 4µm thick and mounted on positively-charged glass slides. These slides are placed in the Benchmark ULTRA instrument. This system first removes the paraffin wax from the tissue, and then takes the tissue through the series of hybridization and detection steps that are part of the staining protocol. During the Dual ISH staining process, DNP and DIG labeled probes are co-hybridized to their respective specific target DNA sequences within the cell nuclei. Detection of the DNP-labeled HER2 probe occurs first, using the VENTANA Silver ISH DNP Detection Kit that forms a silver precipitate in the nucleus, and a single copy of the *HER2* gene is visualized as a black dot. The DIG-labeled Chr 17 probe is then detected with the VENTANA Red ISH DIG Detection Kit that form a red precipitate. Tissues are then counterstained with Hematoxylin II for interpretation by light microscopy.

Staining Protocol

The VENTANA HER2 Dual ISH DNA Probe Cocktail is designed to be run on the BenchMark ULTRA instrument with VSS software. The staining protocol on the BenchMark ULTRA is provided in Table 3 below.

Table 3. Recommended staining conditions for VENTANA HER2 Dual ISH DNA Probe Cocktail assay on BenchMark ULTRA instrument.

Staining Condition	Condition
Baking*†	Not selected
Deparaffinization	69 °C
Cell Conditioning 1*†	16 mins
Cell Conditioning 2*†	24 mins
ISH Protease 3*†	20 mins
Denaturation	80 °C , 8 mins
Probe hybridization	44 °C , 60 mins
Stringency Wash Temperature*†	74°C
Stringency Wash Time	24 mins
Silver DNP HQ	16 mins
Silver HRP	24 mins
Silver A	8 mins
Silver B	4 mins
Silver C	8 mins
Red DIG NP	16 mins

Staining Condition	Condition
Red AP	20 mins
Red Enhancer	8 mins
Red Naphthol	4 mins
Red Fast Red	8 mins
Hematoxylin II	8 mins
Bluing Reagent	8 mins

* Only these conditions are selectable

† Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patients results.

Interpretation of HER2 Staining

The cellular staining pattern for VENTANA HER2 Dual ISH DNA Probe Cocktail assay is nuclear.

A pathologist experienced in the microscopic interpretation of anatomic pathology specimens, ISH procedures and the recognition of single and amplified *HER2* and Chromosome 17 copies should evaluate the slides. Slides should be reviewed via light microscopy using 20x, 40x, and/or 60x objectives. Controls must be evaluated before interpreting results. Use of 100x objective is not recommended.

Positive (Internal) Control Tissue Interpretation: Normal cells within, or adjacent to, the target area serves as internal positive controls of the staining. At least 50% of the normal cell nuclei should contain at least one SISH signal and at least 50% should contain at least one Red ISH signal (the SISH and Red ISH signals do not have to be in the same cells) for the target area to be deemed adequate. Failure to detect adequate signal in normal cells on any slide on the run indicates that the particular slide is inadequate for enumeration.

An additional laboratory-specific positive specimen control may be used with every staining procedure performed. Control specimens can be specimens prepared in a manner identical to patient specimens. Such controls are useful to monitor all steps of the procedure, from specimen preparation through staining. Use of a specimen prepared differently from the test specimens will provide a control for the reagents, instrument and procedures but not for fixation and specimen processing. Results with the test specimens should be analyzed on the same run. Such controls should not replace the proper evaluation of the internal controls in each patient specimen. Interpretation is the same as described above.

Patient Tissue Interpretation: The VENTANA HER2 Dual ISH DNA Probe Cocktail has been designed to enable the *HER2* gene and Chr 17 centromere to be co-hybridized and visualized via light microscopy on the same slide. In the slides stained with the VENTANA HER2 Dual ISH DNA Probe Cocktail, HER2 appears as discrete black signals (SISH) and Chr 17 as red signals (Red ISH) in nuclei of normal cells (serving as internal controls for staining adequacy) as well as in nuclei of carcinoma cells. This allows for *HER2* gene amplification status determination in the context of its chromosomal state.

HER2 gene amplification status is reported as the ratio of the number of *HER2* gene copies to the number of Chr 17 copies in nuclei of cells within an invasive breast carcinoma. Twenty nuclei, each containing red (Chr 17) and black (HER2) signals, should be enumerated. The final results for the HER2 status are reported based on the ratio calculated by dividing the sum of HER2 signals for all 20 nuclei divided by the sum of Chromosome 17 signals for all 20 nuclei. The amplification status is defined as Amplified if the *HER2*/Chr 17 ratio $\geq$ 2.0 and as Non-Amplified if the *HER2*/Chr 17 ratio < 2.0. If the *HER2*/Chr 17 ratio falls between 1.8 to 2.2, an additional 20 nuclei should be enumerated. A new ratio should then be calculated on the basis of all 40 nuclei, and the amplification status reported as described above. Refer to the VENTANA HER2 Dual ISH DNA Probe Cocktail Interpretation Guide for more information about interpretation of HER2 staining.

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.

Table 4. List of FDA approved CDx Assays for the detection of *HER2* Gene Amplification in Human Breast Cancer Tissue Specimens

Device	Company	Technology	Therapy
PathVysion HER-2 DNA Probe Kit	Abbott Molecular, Inc.	FISH	Herceptin (trastuzumab)
Pathway Anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody	Ventana Medical Systems, Inc.	IHC	Herceptin (trastuzumab) Kadcyla (ado-trastuzumab emtansine)
Insite HER-2/neu Kit	Biogenex Laboratories, Inc.	IHC	Herceptin (trastuzumab)
Spot-Light HER2 CISH Kit	Life Technologies, Inc.	CISH	Herceptin (trastuzumab)
Bond Oracle Her2 IHC System	Leica Biosystems	IHC	Herceptin (trastuzumab)
HER2 CISH pharmDx Kit	Dako Denmark A/S	CISH	Herceptin (trastuzumab)
INFORM HER2 DUAL ISH DNA Probe Cocktail	Ventana Medical Systems, Inc	Dual ISH	Herceptin (trastuzumab) Kadcyla (ado-trastuzumab emtansine)
Hercep Test	Dako Denmark A/S	IHC	Herceptin (trastuzumab) Perjeta (pertuzumab) Kadcyla (ado-trastuzumab emtansine)

Device	Company	Technology	Therapy
HER2 FISH pharmDx Kit	Dako Denmark A/S	FISH	Herceptin (trastuzumab) Perjeta (pertuzumab) Kadcyla (ado-trastuzumab emtansine)

VII. MARKETING HISTORY

A list of countries where the product is distributed/marketed is provided below; the product has not been withdrawn to date from the market in any country for reasons relating to safety and effectiveness of the device.

Countries in which the VENTANA HER2 Dual ISH DNA Probe Cocktail is currently marketed are: Australia, Austria, Belgium, Brazil, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Monaco, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Thailand, Turkey, United Kingdom.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently improper patient management decisions in breast cancer.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

All non-clinical studies were performed at Roche Diagnostics Laboratories using the VENTANA HER2 Dual ISH DNA Probe Cocktail, and all staining was performed using the staining procedure as noted in the package insert on BenchMark ULTRA instruments. These studies were performed using breast cancer specimens. Studies were conducted to characterize the assay, demonstrate the impact of pre-analytical variables on assay performance, verify precision and robustness of the assay, and establish assay stability.

1. Analytical Sensitivity and Specificity

a. Immunoreactivity

The main objective of this study was to determine the specific immunoreactivity of VENTANA HER2 Dual ISH DNA Probe Cocktail, VENTANA Silver ISH DNP Detection Kit, and VENTANA Red ISH DIG Detection Kit in FFPE neoplastic and normal tissues. One slide each from two tissue microarrays containing a range of normal tissues in the Tour of Body (TOB), neoplastic tissues in the Tour of Tumor (TOT), and additional individual tissue and tumor cases (totaling 16 types of normal tissue and 48 types of neoplastic tissues) were stained with the device.

No unexpected staining was observed with VENTANA HER2 Dual ISH DNA Probe Cocktail on normal tissue screening (where a minimum of three tissues were screened per tissue type), and neoplastic tissue screening (where a range of 1-3 tissues were screened per tumor type).

b. Sensitivity and Specificity on Metaphase Chromosomes

The objective of this study was to evaluate the sensitivity and specificity of the VENTANA HER2 Dual ISH DNA Probe Cocktail by assessing hybridization to Chr 17. Four human metaphase slides were stained with one lot of VENTANA HER2 Dual ISH DNA Probe Cocktail on one BenchMark ULTRA instrument system. The resulting images were compared to four human metaphase slides stained with a mixture of Chr 17-DIG Plasmid Probe (used in the previously approved INFORM HER2 Dual ISH DNA Probe Cocktail assay) and the VENTANA HER2 oligo probe mix. All slides were counterstained with 4% Giemsa to visualize chromosomes, and a total of 100 chromosome images were assessed.

For sequence specificity, sequences of the probes used in the VENTANA HER2 Dual ISH DNA Probe Cocktail were subjected to NCBI BLASTn searches.

Table 5. Metaphase Staining Results

Probe Mix	Co-localization of HER2 Probe and Chromosome 17	Cross-hybridization of HER2 Probe to Other Chromosome Loci	Cross-hybridization of Chromosome 17 Probe to Other Chromosome Loci
Ventana HER2/INFORM Chr 17	100% (100/100)	0% (0/100)	0% (0/100)
Ventana HER2/VENTANA Chr 17	100% (100/100)	0% (0/100)	0% (0/100)

BLASTn query results showed that both Chromosome 17 and HER2 oligo probes are specific for Chromosome 17 as only homo sapiens Chromosome 17 showed up in the list of sequences producing significant alignments and no other human chromosome was observed on the list. The study passed all acceptance criteria and demonstrated the ability of the VENTANA HER2 Dual ISH DNA Probe Cocktail to hybridize to Chr 17 with high sensitivity and specificity. The VENTANA HER2 Dual ISH DNA Probe Cocktail Assay will detect the *HER2* gene region on Chr 17 and the centromere region on Chr 17.

c. Sensitivity and Specificity on Breast Tissue

This design verification study determined that the sensitivity and specificity requirements have been met for the VENTANA HER2 Dual ISH DNA Probe Cocktail on breast tissue for the ULTRA platform. It also verified that first pass rate requirements and requirements associated with target signals, staining pattern, tissue architecture, background, and turnaround time have been met. For this study, one lot of cocktail and each detection kit were tested on two BenchMark ULTRA Instruments with three tissue sections from each of the 40 qualified breast tissue samples (15 amplified, 21 non-amplified and 4 borderline cases). One tissue section from each of the 40 qualified breast tissue samples were stained on the second BenchMark ULTRA

and stained with HybReady in lieu of the VENTANA HER2 Dual ISH Probe Cocktail to generate results that demonstrate background associated with the detection kits only.

Table 6. Summary of Results - Analytical Sensitivity and Specificity on Breast Tissue

Product Requirement	Result n/N [95% CI]
First Pass Rate (Breast)	39/40 (97.5%) [87.1, 99.6]
Non-specific Staining	40/40 (100%) [91.2, 100]
Turnaround time	Longest run - 8 h 40 min
Tissue Architecture conservation	40/40 (100%) [91.2, 100]
Silver DNP Detection Background	40/40 (100%) [91.2, 100]
Red DIG Detection Background	40/40 (100%) [91.2, 100]
Silver DNP Targets (Breast)	39/40 (97.5) [87.1, 99.6]
Red DIG Targets (Breast)	39/40 (97.5) [87.1, 99.6]
Expected Staining Pattern	39/40 (97.5) [87.1, 99.6]
Expected Staining Elements (SISH)	39/40 (97.5) [87.1, 99.6]
Expected Staining Elements (RISH)	39/40 (97.5) [87.1, 99.6]

All results passed the acceptance criteria for the study and met the first pass rate product requirements of at least 90%. Furthermore, all requirements surrounding non-specific staining, tissue architecture conservation, Silver and Red detection background, silver and red targets, and expected staining elements were met on all platforms by providing pass rates of at least 90%.

Note: The first pass rate is the percent of slides that are evaluable for clinical status on their first staining attempt which is determined by the staining quality of normal cells surrounding the tumor.

2. Robustness

a. Tissue Thickness

The objective of this study was to characterize the tissue thickness recommended for the VENTANA HER2 Dual ISH Assay on the BenchMark ULTRA instrument. Four tissue thicknesses were tested: 2, 6, 8, and 10 µm with one lot each of the VENTANA HER2 Dual ISH DNA Probe Cocktail and each detection kit, on two BenchMark ULTRA Instruments. 100% concordance was achieved for all tissue thicknesses when compared to evaluable tissue cut at 4 µm.

b. Assay Characterization

The objective of this study was to characterize the performance of the VENTANA HER2 DUAL ISH DNA Probe Cocktail on the BenchMark ULTRA under a range of protocol settings. For this study, one lot of VENTANA HER2 Dual ISH DNA Probe Cocktail was tested with one lot of each detection kit, on two BenchMark ULTRA instruments. Slides were stained under a range of test conditions that included varying cell conditioning, protease, stringency wash, baking, and

various combinations. Four breast tissue cases (2 amplified, 2 non-amplified) were tested. For each test condition, each test sample was compared to a reference sample stained under recommended conditions and deemed concordant or discordant. If any sample failed due to assay related reason, the sample was treated as discordant.

The recommended protocol conditions passed for all four cases. Protocol selections that were different from the recommended settings still generated acceptable staining except for ISH Protease 3 at 4 minutes for three of the cases, ISH Protease 3 at 8 minutes for one case, and a stringency wash temperature of 78°C for one of the cases. When the customer selectable parameters are set all at low the staining was acceptable for two of the four breast cases. When the customer selectable parameters are set all at high the staining was acceptable for all four breast cases

c. Fixation

The objective of this study was to characterize fixatives used to preserve tissues for the VENTANA HER2 Dual ISH DNA Probe Cocktail on the BenchMark ULTRA. Fixative types (and times) tested for breast cases included 10% NBF (6, 24, 48, and 72 hours); AFA (24 hours); ZF (24 hours); AlcF (24 hours). Fixative type for xenografts tested included 10% NBF, AFA, and Alco fixative. Fixation occurred for 1, 3, 6, 12, 24, and 48 hours for all xenograft conditions. For this study, one lot of VENTANA HER2 Dual ISH DNA Probe Cocktail was tested with one lot of each detection kit, on two BenchMark ULTRA instruments.

Breast cases yielded 100% concordance for all test conditions. MCF7 xenografts yielded 100% concordance for 10% NBF and Alco fixative; however, 100% concordance was not achieved for AFA. Fixation of tissues using 10% NBF for 6 to 72 hours is recommended for the assay.

3. Precision

a. Between-Lot Precision

The objective of this study was to assess between lot precision of the VENTANA HER2 Dual ISH DNA Probe Cocktail assay on ULTRA for breast tissue. Between-lot precision of production lots was assessed by testing three lots of VENTANA HER2 Dual ISH DNA Probe Cocktail, as well as three lots of each detection kit. Each probe cocktail lot was paired with a combination of the RISH and SISH detection kits (each detection kit was treated as a pair).

Test cases were distributed as shown in the table below.

Table 7: Summary of Tissue Cases – Precision Studies

Number of Breast Cases	Description	*HER2*/Chr 17 Ratio
12	Non-amplified (Negative)	<1.5
2	Non-amplified (Negative) borderline	1.5 to < 2*
2	Amplified (Positive) borderline	≥2 to 2.5*
12	Amplified (Positive)	>2.5

Number of Breast Cases	Description	*HER2*/Chr 17 Ratio

*The only clinical results for the VENTANA HER2 Dual ISH DNA Probe Cocktail assay are amplified (*HER2*/Chr 17 ratio ≥2.0) or non-amplified (*HER2*/Chr 17 ratio <2.0). Borderline is not a clinical result for the assays. The 1.5-2.5 range was used only to ensure an adequate number of samples near the 2.0 cut-off.

For between-lot precision, a 2-sided 95% confidence interval (CI) for PPA, NPA and OPA were calculated using percentile bootstrap method; in instances where the point estimate was 100%, Wilson Score method was used. Results are provided in Table 8 below.

Table 8: Between-Lot Precision

Study	Analysis	Point Estimate (95% CI)	n/N
Between-lot reproducibility	PPA	100% (96.9, 100.0)	121/121
	NPA	100% (97.0, 100.0)	123/123
	OPA	100% (98.5, 100.0)	244/244

PPA: Positive Percent agreement; NPA: Negative Percent Agreement; OPA: Overall Percent Agreement

b. Run, Platform and Day Precision

The objective of this study was to assess the within-run, within-instrument, and between-day reproducibility of the VENTANA HER2 Dual ISH DNA Probe Cocktail on BenchMark ULTRA for breast tissue.

For within-run, one lot of VENTANA HER2 Dual ISH DNA Probe Cocktail was used with one lot of each detection kit, and one BenchMark ULTRA Instrument. Samples were stained in duplicate across multiple runs on 28 cases (28 cases × 2 slides × 5 runs = 280 slides for analysis).

For between-instrument, one lot of VENTANA HER2 Dual ISH DNA Probe Cocktail was used with one lot of each detection kit, and three BenchMark ULTRA Instruments. Samples were stained in duplicate across three instruments for 28 cases (28 cases × 2 slides × 3 instruments = 168 slides for analysis).

For between-day, one lot of VENTANA HER2 Dual ISH DNA Probe Cocktail was used with one lot of each detection kit, and one BenchMark ULTRA Instrument. Samples were stained in duplicate across multiple days for 28 cases (28 cases x 2 slides x 6 days = 336 slides for analysis). For each set of 28 cases, 12 were amplified, 2 were borderline amplified, 2 were borderline non-amplified and 12 were non-amplified.

For within-run precision, the two replicates were compared to each other for amplified/non-amplified status. For between-instrument and between-day precision, the case modal status was used as reference. The agreement rates for all studies for all cases are provided in Table 9 below.

Table 9: Summary of Results – Within-Run, Between-Instrument, and Between-Day Precision

Repeatability/Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-run	Amplified	APA	194/194	100	(98.1, 100)
	Non-Amplified	ANA	186/186	100	(98.0, 100)
	Total	OPA	190/190	100	(98.0, 100)
Between-instrument	Amplified	PPA	84/84	100	(95.6, 100)
	Non-Amplified	NPA	84/84	100	(95.6, 100)
	Total	OPA	168/168	100	(97.8, 100)
Between-day	Amplified	PPA	139/139	100	(97.3, 100)
	Non-Amplified	NPA	135/135	100	(97.2, 100)
	Total	OPA	274/274	100	(98.6, 100)

4. Reader Precision/Within/Between-Reader

The objective of this study was to assess the within-reader and between-reader reproducibility of the VENTANA HER2 Dual ISH DNA Probe Cocktail on breast tissue. For this study, one lot of VENTANA HER2 Dual ISH DNA Probe Cocktail was used with one lot of each detection kit, and one BenchMark ULTRA Instrument. Slides were evaluated by three pathologists who had not seen slides from these cases previously.

For the between-reader precision, three readers evaluated the same 60 breast carcinoma specimens. For within-reader precision, the set of 60 slides were read twice after a minimum of two weeks between reads.

Table 10: Summary of Tissue Cases – Within/Between-Reader Reproducibility

Number of Breast Cases	Description	*HER2*/Chr 17 Ratio
27	Non-amplified (Negative)	<1.5
3	Non-amplified (Negative) borderline	1.5 to < 2*
3	Amplified (Positive) borderline	≥2 to 2.5*
27	Amplified (Positive)	>2.5

*The only clinical results for the VENTANA HER2 Dual ISH DNA Probe Cocktail assay are amplified (*HER2*/Chr 17 ratio ≥2.0) or non-amplified (*HER2*/Chr 17 ratio <2.0). Borderline is not a clinical result for the assays. The 1.5-2.5 range was used only to ensure an adequate number of samples near the 2.0 cut-off.

For within-reader precision, the two reads (two-week washout in between) were compared to each other for amplified/non-amplified status. For between-reader precision, the pairwise comparison was conducted between each pair of readers for the two reads. The following agreement rates for both studies are provided in Table 11 below.

Table 11: Summary of Results – Within/Between-Reader Reproducibility

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-Reader	Amplified	APA	178/181	98.3	(96.3, 100.0)
	Non-Amplified	ANA	174/177	98.3	(96.1, 100.0)
	Total	OPA	176/179	98.3	(96.1, 100.0)
Between Reader	Amplified	APA	350/362	96.7	(93.2, 99.4)
	Non-Amplified	ANA	342/354	96.6	(92.8, 99.4)
	Total	OPA	346/358	96.6	(92.8, 99.4)

Note: 95% CIs were calculated using the percentile bootstrap method. Six cases with ISH ratios between 1.5 to 2.5, inclusive, were included in the study.

5. External Reproducibility

An inter-laboratory reproducibility (ILR) study was conducted to demonstrate reproducibility of the VENTANA HER2 Dual ISH DNA Probe Cocktail assay, in conjunction with VENTANA Silver ISH DNP Detection Kit and VENTANA Red ISH DIG Detection Kit as used on the BenchMark ULTRA instrument in determining *HER2* gene status (amplified or non-amplified) in invasive breast carcinoma tissue specimens.

Twenty-eight unique, de-identified, archival, formalin-fixed, paraffin-embedded (FFPE) invasive breast carcinoma tissue specimens representing 14 HER2-amplified (*HER2*:Chromosome 17, Chr 17 ratio ≥ 2.0) and 14 HER2 non-amplified (*HER2*:Chr 17 ratio < 2.0) cases, including four borderline cases (*HER2*:Chr 17 ratio 1.5-2.5) were stained at 3 external laboratories on each of 5 non-consecutive days over a period of at least 20 days. The sample set contained a total of 420 slides (28 cases × 3 sites × 5 days), with 140 case slides being stained at each site. Prior to reading, slides were blinded and randomized. Two qualified pathologists (readers) at each site independently evaluated each reading set to assign a *HER2* gene status (amplified or non-amplified) to each case.

The assay reproducibility was determined by PPA and NPA rates across all evaluable observations. For each case, all evaluable observations (amplified or non-amplified) were compared against the modal result for each case. These comparisons were pooled across sites, readers, and days. The results of the study are summarized in Table 12 below.

Table 12: ILR - Agreement Rates on the BenchMark ULTRA instrument for Breast Carcinoma.

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	PPA	401/410	97.8	(94.6, 100.0)

Inter-Laboratory Reproducibility		Agreement			
		Type	n/N	%	95% CI
		NPA	416/417	99.8	(99.3, 100.0)
		OPA	817/827	98.8	(97.0, 100.0)
Within-Site (3 sites)	Site A	PPA	137/138	99.3	(98.0, 100.0)
		NPA	137/138	99.3	(98.0, 100.0)
		OPA	274/276	99.3	(98.6, 100.0)
	Site B	PPA	137/138	99.3	(98.0, 100.0)
		NPA	140/140	100.0	(97.3, 100.0)
		OPA	277/278	99.6	(98.9, 100.0)
	Site C	PPA	124/124	100.0	(97.0, 100.0)
		NPA	146/149	98.0	(94.3, 100.0)
		OPA	270/273	98.9	(96.7, 100.0)
Between-Reader (6 readers, 2 pathologists per site)		APA	392/398	98.5	(97.0, 99.7)
		ANA	418/424	98.6	(96.8, 99.8)
		OPA	405/411	98.5	(96.9, 99.8)

6. Stability Studies

a. Real Time and Ship Stress Stability

The purpose of this study was to determine the stability (shelf-life and in-use) and shipping category of the VENTANA HER2 Dual ISH DNA Probe Cocktail. Stability was assessed by evaluating the Assay's performance on both non-amplified and amplified FFPE breast carcinoma tissue. Three production lots each of VENTANA HER2 Dual ISH DNA Probe Cocktail, VENTANA Silver ISH DNP Detection Kit, and VENTANA Red ISH DIG Detection Kit were subjected to the ship stress conditions and tested at specified intervals until 26 months or failure, whichever occurred first. To satisfy regional stability testing requirements of designated countries, stability testing was conducted using three Stability Master Lots (SML). Each SML configuration was assembled using a single lot of VENTANA HER2 Dual ISH DNA Probe Cocktail, VENTANA Silver ISH DNP Detection Kit, and VENTANA Red ISH DIG Detection Kit. All components from the SMLs are being monitored for stability dating (i.e. product under test).

Each SML of Ventana HER2 Dual ISH DNA Probe Cocktail was assigned a sample set of breast carcinoma tissues containing amplified and non-amplified cases. The ratio of *HER2* to Chr 17 signal in nuclei of carcinoma cells determines the *HER2* gene status (amplified or not amplified). *HER2*/Chr 17 ratio ≥2.0 is amplified, while a ratio <2.0 is non-amplified.

All conditions for all three production lots of all components of this assay passed the acceptance criteria to date. All lots passed for all conditions to support a 12-month stability claim under intended storage condition (2°C-8°C). The study will continue through the 26-month time point.

Table 13: Summary of Interim Results – Real Time Stability and Ship Stress

Storage Condition	Time Point (Month)	SML 1 Results (Pass/Fail/Invalid)	SML 2 Results (Pass/Fail/Invalid)	SML 3 Results (Pass/Fail/Invalid)
Real Time Stability at Intended Storage (5 ± 3 °C)	Day 0	Pass	Pass	Pass
	6	Pass	Pass	Pass
	8	Pass	Pass	Pass
	14	Pass	Pass	Pass
Hot Ship Stress - Cat A (33 ± 3 °C for 192 hours)	Month 14	Pass	Pass	Pass
Hot Ship Stress - Cat B +(18 ± 3 °C for 192 hours)	Month 14	Pass	Pass	Pass
Cold Ship Stress - Freeze/Thaw (-20 ± 5 °C for 192 hours)	Month 14	Pass	Pass	Pass

b. Cut Slide Stability

The objective of this protocol was to demonstrate stability of cut, unstained sections from FFPE breast carcinoma tissue. This data is used to support cut slide stability listed in the package insert of 12 months when stored between 2 to 8°C. The study tested two conditions; 2-8ºC and 15-30ºC, at a number of time points ranging through 12 months. At each time point, three slides from each case were tested.

One lot of VENTANA HER2 Dual ISH DNA Probe Cocktail was used with one lot of each detection kit, and one BenchMark ULTRA Instrument. Two breast tissue cases were used (one amplified and one non-amplified).

All cases showed 100% amplification status concordance to the Day 0 amplification status. The stability of the cut breast slides is at least 12 months at 2 to 8°C and 15 to 30°C.

X. SUMMARY OF PRIMARY CLINICAL STUDY

A concordance study comparing the VENTANA HER2 Dual ISH Assay and the PathVysion HER-2 DNA Probe Kit (FISH assay) was designed to evaluate the concordance of the two assays in the determination of *HER2* gene status in invasive breast carcinoma, Three clinical laboratories participated for the VENTANA HER2 Dual ISH DNA Probe Cocktail testing, and one central laboratory performed the PathVysion HER-2 FISH testing.

A. Concordance Study with PathVysion

1. Study Objective

The primary objective of this study was to determine the PPA and NPA rates between the VENTANA HER2 Dual ISH Assay and PathVysion HER-2 FISH assay in determining *HER2* gene status (amplified or non-amplified) in invasive breast carcinoma specimens.

2. Methodology

Matched samples were analyzed by both the VENTANA HER2 Dual ISH Assay and PathVysion HER-2 FISH assay. Additionally, HER2/neu protein status was also determined for enrolled cases using the PATHWAY HER-2/*neu* (4B5) immunohistochemistry (IHC) assay. The study included 605 samples (281 amplified, 266 non-amplified, and 58 borderline).

Three laboratories performed testing by the VENTANA HER2 Dual ISH Assay on approximately 200 cases each using one lot of VENTANA HER2 Dual ISH DNA Probe Cocktail, one lot of VENTANA Silver ISH DNP Detection Kit and one lot of VENTANA Red ISH DIG Detection Kit. Each site utilized one BenchMark ULTRA stainer (three instruments total) and samples were read by one reader at each site (for a total of three readers). All samples were analyzed to determine *HER2* gene status using PathVysion HER-2 FISH assay at one central laboratory. One central laboratory, different from VENTANA HER2 Dual ISH Assay and PathVysion testing sites, conducted staining and evaluation of the tissue sections using the PATHWAY HER-2/*neu* (4B5) Rabbit Monoclonal Primary Antibody to determine HER-2/neu IHC status. The clinical sites that evaluated the VENTANA HER2 Dual ISH Assay and the central laboratories that evaluated the PathVysion and IHC assays were blinded to case status and original case identifier to prevent any bias in evaluation of the specimens.

The PPA and NPA between the VENTANA Dual ISH assay and the PathVysion assay were calculated based on a stratified analysis by borderline, amplified, and non-amplified cases.

3. Requirements & Acceptance Criteria

When comparing breast samples stained with the VENTANA HER2 Dual ISH Assay to the on-market PathVysion HER-2 DNA Probe Kit, the VENTANA HER2 Dual ISH Assay shall produce staining results with the lower bounds of 2-sided 95% confidence intervals for PPA and NPA of at least 85%. The secondary endpoint for this study was the First Pass Rate for the VENTANA Dual ISH assay.

The results of the concordance study are summarized in Table 14 below.

Table 14: Agreement between VENTANA HER2 Dual ISH DNA Probe Cocktail and Abbott/Vysis PathVysion HER-2 DNA Probe Kit in a cohort of human breast carcinoma specimens.

	PathVysion HER-2 FISH Result

VENTANA HER2 Dual ISH DNA Probe Cocktail Result	Amplified	Non-Amplified	Total
Amplified	270	12	282
Non-Amplified	32	291	323
Total	302	303	605
	n/N	% (95% Score CI)	
Positive Percent Agreement	270/302	89.4 (85.4, 92.4)	
Negative Percent Agreement	291/303	96.0 (93.2, 97.7)	

4. Conclusion

Equivalent performance between the VENTANA HER2 Dual ISH Assay and the PathVysion HER-2 FISH assay was demonstrated. The primary acceptance criteria for establishing equivalent performance between the VENTANA Dual ISH and PathVysion assays required lower bounds of the 2-sided 95% confidence intervals of both PPA and NPA to be 85% or higher. Therefore, the study met its primary acceptance criteria by demonstrating equivalent performance.

The first pass rate was determined for three sites, Site A, Site B and Site C. Higher failure of first pass rate was mainly associated with cases obtained from Site A (39%), whereas first pass failure rates for cases obtained from Sites B and C were 5.7% and 4.6%, respectively. Furthermore, similar failure rates were obtained from the PathVysion assay for samples obtained from these sites (A = 39%, B = 3.8%, C = 7.2%), and may be a result of median block age (A = 850.5 days, B = 285 days, C = 301 days).

5. Pediatric Extrapolation

In this premarket application, existing clinical study data was not leveraged to support approval of a pediatric patient population.

B. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The clinical study included 6 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

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

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Hematology and Pathology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XI. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

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

A. Safety Conclusions

The VENTANA HER2 Dual ISH DNA Probe Cocktail is an *in vitro* diagnostic device, which involves tumor specimens collected from patients with breast cancer. The risks of the device are based on data collected in the nonclinical laboratory studies and the concordance study conducted to support PMA approval as described above, as well as the known toxicity profile of trastuzumab. Risks of the VENTANA HER2 Dual ISH DNA Probe Cocktail are associated with failure of the device to perform as expected or failure to correctly interpret test results. As VENTANA HER2 Dual ISH DNA Probe Cocktail is intended for use to identify patients for Herceptin (trastuzumab) therapy, if incorrect, or false results are reported, then breast cancer patients may not receive the proper treatment. Patients with false positive results may undergo treatment with Herceptin (trastuzumab) without much clinical benefit and may experience adverse reactions associated with Herceptin (trastuzumab) therapy. Patients with false negative results may not be considered for treatment with Herceptin (trastuzumab), and therefore, may receive other treatment options. There is also a risk of delayed results, which may lead to a delay in treatment with Herceptin (trastuzumab).

B. Effectiveness Conclusions

The effectiveness of the VENTANA HER2 Dual ISH DNA Probe Cocktail in determining *HER2* gene status (amplified or non-amplified) in invasive breast carcinoma specimens was demonstrated through a concordance study comparing this assay to the FDA approved PathVysion FISH assay. These data, as a surrogate for clinical outcomes data from a clinical trial using patients in a comparable intended use population, support the performance of this device in identifying breast cancer patients who will benefit from the therapeutic when used in accordance with the instructions for use.

The performance of the VENTANA HER2 Dual ISH DNA Probe Cocktail was also supported by the analytical validation studies.

C. Risk/Benefit Analysis

The benefits expected for patients with breast cancer who have been classified as HER2-positive as per the Intended Use of this device, and treated with trastuzumab according to the Herceptin label, which includes studies based on both IHC and FISH, are as follows:

Improved disease-free survival and overall survival with adjuvant treatment; improved time to progression in treatment of first-line metastatic breast cancer; and an objective response rate of 14% in previously treated metastatic breast cancer.

The risks of the use of this device in patients with breast cancer relate to forgoing potentially beneficial therapy due to a false negative test result and occurrence of toxicity and adverse events due to Herceptin without the likelihood of benefit (false positive test).

The benefits expected to accrue to patients with invasive breast cancer classified as HER2-positive according to the Intended Use of this device, and treated with trastuzumab according to the FDA-approved label, are clinically significant, with a meaningful improvement in disease-free survival and overall survival in the adjuvant setting, and an improvement in time to progression and a clinically meaningful response rate in the metastatic setting. The risks of a false positive test include trastuzumab toxicity, which can at times be severe, while receiving therapy which may not be effective. The risks of a false negative include forgoing effective therapy. On balance, there is a reasonable assurance that the benefits exceed the risks when this device and the referent drug are used according to the FDA-approved intended use statements.

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use.

XII. CDRH DECISION

CDRH issued an approval order on July 28, 2020.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIII. 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.