

Bond™ Oracle™ HER2 IHC System

Instructions For Use

For use on Leica Biosystems' BOND fully automated, advanced staining system.

Product Code TA9145 is designed to stain 60 tests (150 slides):

60 test slides with HER2 Primary Antibody

60 test slides with HER2 Negative Control

15 HER2 Control Slides with HER2 Primary Antibody

15 positive in-house tissue controls with HER2 Primary Antibody



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Intended Use

For in vitro diagnostic use

Bond Oracle HER2 IHC System is a semi-quantitative immunohistochemical (IHC) assay to determine HER2 (Human Epidermal Growth Factor Receptor 2) oncoprotein status in breast cancer tissue and adenocarcinomas of the stomach (including gastroesophageal junction) processed for histological evaluation. The Bond Oracle HER2 IHC System is indicated as an aid in the assessment of patients for whom Herceptin® (trastuzumab) treatment is being considered (see Herceptin® package insert).

Note: All of the patients in the Herceptin® clinical trials were selected using an investigational immunocytochemical Clinical Trial Assay (CTA). None of the patients in those trials were selected using the Bond Oracle HER2 IHC System. The Bond Oracle HER2 IHC System has been compared to the Dako HercepTest™ on an independent set of samples and found to provide acceptably concordant results, as indicated in the Clinical Concordance Summary. The actual correlation of the Bond Oracle HER2 IHC System to clinical outcome has not been established.

All of the patients in the Herceptin® advanced gastric cancer (ToGA) clinical trials were selected using the Dako HercepTest. None of the patients in those trials were selected using the Bond Oracle HER2 IHC System. The Bond Oracle HER2 IHC System has been compared to the Ventana Medical Systems Inc. PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody on an independent set of samples and found to provide acceptably concordant results, as indicated in the Clinical Concordance Summary (Gastric). The actual correlation of the Bond Oracle HER2 IHC System to clinical outcome has not been established.

Summary and Explanation

Background

The Bond Oracle HER2 IHC System contains the mouse monoclonal anti-HER2 antibody (clone CB11). Clone CB11, originally developed by Corbett et al (1) and manufactured by Novocastra Laboratories Ltd (now Leica Biosystems Newcastle Ltd), is directed against the internal domain of the HER2 oncoprotein.

In a proportion of breast and gastric cancer patients, the HER2 oncoprotein is overexpressed as part of the process of malignant transformation and tumor progression (2). HER2 status has also been shown to have important implications for the treatment of gastric cancer (3). Overexpression of the HER2 oncoprotein found in breast cancer cells suggests HER2 as a target for an antibody-based therapy, whilst results from the ToGA trial clearly indicate that the use of Herceptin® in gastric cancer along with chemotherapy is an effective treatment which improves overall survival in HER2 positive gastric cancers (4). Herceptin® is a humanized monoclonal antibody (5) that binds with high affinity to the HER2 oncoprotein and has been shown to inhibit the proliferation of human tumor cells that overexpress HER2 oncoprotein both in vitro and in vivo (6–8).

Since the first immunoperoxidase technique, reported by Nakane and Pierce (9), many developments have occurred within the field of immunohistochemistry, resulting in increased sensitivity. A recent development has been the use of polymeric labeling. This technology has been applied to both primary antibodies and immunohistochemical detection systems (10). The Compact Polymer™ detection system utilized by the Bond Oracle HER2 IHC System is part of a family of novel, controlled polymerization technologies that have been specifically developed to prepare polymeric HRP-linked antibody conjugates. As this polymer technology is utilized in the Bond Oracle HER2 IHC System, the problem of nonspecific endogenous biotin staining, which may be seen with streptavidin/biotin detection systems, does not occur.

Expression of HER2

The HER2 oncoprotein is expressed at levels detectable by immunohistochemistry in up to 20% of adenocarcinomas from various sites. Between 10% and 20% of invasive ductal carcinomas of the breast (11) and 20% of gastric cancers (12-14) are positive for HER2 oncoprotein. 90% of cases of ductal carcinoma in situ (DCIS) of comedo type are positive (15), together with almost all cases of Paget's disease of the breast (16).

Clinical Concordance Summary

The Bond Oracle HER2 IHC System was developed to provide an alternative to the investigational Clinical Trial Assay (CTA) used in the Herceptin® clinical studies. The performance of the Bond Oracle HER2 IHC System for determination of HER2 oncoprotein overexpression on breast tissue was evaluated in an independent study comparing the results of the Bond Oracle HER2 IHC System to the Dako HercepTest on 431 breast tumor specimens, of US origin. None of these tumor specimens were obtained from patients in the Herceptin® clinical trials. The results indicated a 92.34% concordance in a 2x2 analysis (95% confidence intervals of 89.42% to 94.67%) and 86.54% in a 3x3 analysis (95% confidence intervals of 82.95% to 89.62%) between the results from the two assays.

The performance of the Bond Oracle HER2 IHC System for determination of HER2 oncoprotein overexpression on adenocarcinomas of the stomach (including gastroesophageal junction) was evaluated in an independent study comparing the results of the Bond Oracle HER2 IHC System to Ventana Medical Systems Inc. PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody on 287 gastric tumor specimens, of Chinese origin. None of these tumor specimens were obtained from patients in the Herceptin® clinical trials. The results indicated a 95.12% concordance in a 2x2 analysis (95% confidence intervals of 91.95% to 97.31%) and 89.90% in a 3x3 analysis (95% confidence intervals of 85.81% to 93.13%) between the results from the Bond Oracle HER2 IHC System to the Ventana PATHWAY anti-HER-2/neu (4B5) respectively.

Principle of Procedure

The Bond Oracle HER2 IHC System contains components required to complete an immunohistochemical staining procedure for formalin-fixed, paraffin-embedded tissues. Following incubation with the ready-to-use HER2 Primary Antibody (clone CB11), this system employs ready-to-use Compact Polymer technology. The enzymatic conversion of the subsequently added chromogen results in the formation of a visible reaction product at the antigenic site. The tissue sections may then be counterstained, dehydrated, cleared and mounted. Results are interpreted using light microscopy. Control slides with four formalin-fixed, paraffin-embedded human breast cancer cell lines are provided to validate staining runs. The four cell lines demonstrate HER2 oncoprotein expression at 0, 1+, 2+ and 3+ intensities. The staining intensity of these cell lines correlates to both HER2 oncoprotein receptor load per cell and HER2 gene amplification status.

The Bond Oracle HER2 IHC System (product code TA9145) is for use on the Leica Biosystems' BOND fully automated, advanced staining system.

Components Provided

The materials listed below (Table 1) are sufficient to stain 150 slides (60 test slides incubated with HER2 Primary Antibody, 60 corresponding test slides incubated with HER2 Negative Control, 15 HER2 Control Slides incubated with HER2 Primary Antibody and 15 in-house positive tissue controls incubated with HER2 Primary Antibody). The number of tests is based on the use of a 150 µL automated dispense per slide. The kit provides materials sufficient for a maximum of 15 individual BOND staining runs.

HER2 Control Slides, (x15)	Sections of formalin-fixed, paraffin-embedded, human breast cancer cell lines that demonstrate HER2 oncoprotein expression at 0, 1+, 2+ and 3+ staining intensities when stained in accordance with the protocol provided. These sections are fully adhered and do not require further baking.
HER2 Primary Antibody, 13.5 mL	Contains ready-to-use, affinity-purified, mouse monoclonal IgG antibody, clone CB11 and 0.035% 2-methylisothiazol-3(2H)-one.
HER2 Negative Control, 9 mL	Contains ready-to-use mouse IgG at an equivalent concentration to the HER2 Primary Antibody and 0.035% 2-methylisothiazol-3(2H)-one.
Peroxide Block, 22.5 mL	Contains 3-4% hydrogen peroxide.
Post Primary, 22.5 mL	Rabbit anti-mouse IgG (<10 µg/mL) in Tris-buffered saline containing 10% (v/v) animal serum and 0.01% 2-methylisothiazol-3(2H)-one.
Polymer, 22.5 mL	Poly-HRP goat anti-rabbit IgG (<25 µg/mL) in Tris-buffered saline containing 10% (v/v) animal serum and 0.01% 2-methylisothiazol-3(2H)-one.
DAB Part 1, 2.25 mL	Contains 66 mM 3,3'-diaminobenzidine tetrahydrochloride, in a stabilizer solution.
DAB Part B (x2), 22.5 mL	Contains ≤0.1% (v/v) hydrogen peroxide.
Hematoxylin, 22.5 mL	Contains <0.1% hematoxylin.

Table 1. Bond Oracle HER2 IHC System components

Directions on Use

All reagents supplied are formulated specifically for use with this assay and lot numbers are specific for each Bond Oracle HER2 IHC System. For the assay to be valid, no substitutions should be made.

Storage and Stability

Store at 2–8 °C. Do not freeze. Return to 2–8 °C immediately after use. Any deviation from these conditions will invalidate the assay. Ensure the Bond Oracle HER2 IHC System used is within its designated expiry date. The signs indicating contamination and/or instability of the Bond Oracle HER2 IHC System are: turbidity of the solutions, odor development, and presence of precipitate. Storage conditions other than those specified above must be verified by the user.

Specimen Preparation

All specimens must be prepared to preserve the tissue for immunohistochemical staining. Standard methods of tissue processing should be used for all specimens (17).

It is recommended that tissues are prepared in formalin-based fixatives and are routinely processed and paraffin-embedded. For example, resection specimens should be blocked into a thickness of 3–4 mm and fixed for 18–24 hours in 10% neutral-buffered formalin. The tissues should then be dehydrated in a series of alcohols and cleared through xylene, followed by impregnation with molten paraffin wax, held at no more than 60 °C. Tissue specimens should be sectioned between 3–5 µm.

The slides required for HER2 oncoprotein evaluation and tumor verification should be prepared at the same time. To preserve antigenicity, tissue sections mounted on slides (Leica BOND

Plus Slides – product code S21.2113 or Apex BOND Slides - product code 3800040) should be stained within 4–6 weeks of sectioning when held at room temperature (18–24 °C). Following sectioning, it is recommended that slides are incubated for 12–18 hours (overnight) at 37 °C. Sections which require additional adherence may be incubated at 60 °C for a further hour.

In the USA, the Clinical Laboratory Improvement Act of 1988 requires in 42 CFR 493.1259(b) that “The laboratory must retain stained slides for at least ten years from the date of examination and retain specimen blocks at least two years from the date of examination”.

Warnings and Precautions

For professional users only.

One or more components in the product are hazardous.

As a rule, persons under 18 years of age are not allowed to work with this product. Users must be carefully instructed in the proper work procedure, the hazardous properties of the product and the necessary safety instructions.

Symptoms of overexposure to ProClin™ 950, the preservative used in the Oracle reagents, may include skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of ProClin™ 950 in this product is up to a maximum of 0.35%. These solutions do not meet the OSHA criteria for a hazardous substance. A Material Safety Data Sheet is available upon request or from www.LeicaBiosystems.com.

Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions.

Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. Seek medical advice. Consult federal, state or local regulations for disposal of any potentially toxic components.

Minimize microbial contamination of reagents or an increase in nonspecific staining may occur.

Procedure

A. Reagents required but not supplied

- BOND Dewax Solution (product code AR9222)
- BOND Epitope Retrieval Solution 1 (product code AR9961)
- BOND Wash Solution x10 Concentrate (product code AR9590)
- Standard solvents used in immunohistochemistry (e.g. ethanol, absolute and graded)
- Xylene (or xylene substitutes)
- Mounting medium
- Distilled or de-ionized water

B. Equipment required but not supplied

- Leica Biosystems' BOND-MAX and BOND-III fully automated, advanced staining system(s)
- BOND Universal Covertiles™ (product code S21.2001, S21.4583 or S21.4611)
- BOND Mixing Stations (product code S21.1971)
- Drying oven, capable of maintaining 60 °C
- Light microscope (4–40x objective magnification)
- Slides (Leica BOND Plus Slides – product code S21.2113 or Apex BOND Slides - product code 3800040)
- Coverslips
- BOND Slide Label & Print Ribbon (product code S21.4564)
- BOND Aspirating Probe Cleaning System (product code CS9100)

C. Methodology

- Prior to undertaking this methodology, users must be trained in BOND fully automated immunohistochemical techniques.

- Each test section to be stained with the HER2 Primary Antibody will require an identical section for staining with the HER2 Negative Control. The negative control section allows differentiation between specific and nonspecific staining at the antigen site. Each BOND staining run should include a HER2 Control Slide. At the end of the staining protocol, if the cell lines do not demonstrate the correct staining patterns (refer to Bond Oracle HER2 IHC System Interpretation Guide), the run should be regarded as invalid.

D. Slide Layout

A new BOND Universal Covertile (product code S21.2001, S21.4583 or S21.4611) should be used with each slide. The use of BOND Universal Covertiles which have been previously utilized for either immunohistochemical or in situ hybridization staining has not been validated with this test.

The slide tray layout (Table 2) enables optimal performance of the Bond Oracle HER2 IHC System and the full 60 tests to be obtained.








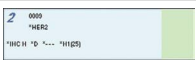


Slide Position	Slide Description	Reagent	Tissue Type	Slide Icon
1	Case 1	*HER2 Negative Control	Test	
2	Case 2	*HER2 Negative Control	Test	
3	Case 3	*HER2 Negative Control	Test	
4	Case 4	*HER2 Negative Control	Test	
5	Case 1	*HER2PrimaryAntibody	Test	
6	Case 2	*HER2PrimaryAntibody	Test	
7	Case 3	*HER2PrimaryAntibody	Test	
8	Case 4	*HER2PrimaryAntibody	Test	
9	HER2 Control Slide	*HER2PrimaryAntibody	Positive	
10	In-house Tissue Control	*HER2PrimaryAntibody	Positive	

Table 2. Slide tray layout, showing tissue type and reagent

E. Procedure Steps

Follow the steps below to set up a slide tray with the layout described in Table 2. These instructions should be read in conjunction with the BOND System User Manual.

- On the BOND instrument, ensure the bulk and hazardous waste containers have enough capacity to perform the required staining runs.
- Ensure there is adequate alcohol, distilled or de-ionized water, BOND Dewax Solution (supplied as ready-to-use), BOND Epitope Retrieval Solution 1 (supplied as ready-to-use) and BOND Wash Solution (supplied as x10 concentrate) in the bulk reagent containers to perform the required staining runs.

3. Ensure that a clean BOND Mixing Station is installed.
4. Turn on the BOND fully automated, advanced staining system.
5. Turn on the BOND Controller attached to the BOND fully automated, advanced staining system.
6. Open the BOND software.
7. For a new Bond Oracle HER2 IHC System, scan the reagent tray barcodes with the handheld scanner to enter the system into the BOND reagent inventory.
8. Go to the Slide setup screen and click **Add case**.
9. Enter details for the first case. Ensure the dispense volume is set to **150 µL** and the preparation protocol is ***Dewax**. Click OK.
10. With the case highlighted in the Slide setup screen click **Add slide**.
11. First, add patient test slides. Ensure tissue type is set to **Test tissue**.
12. Confirm the dispense volume is **150 µL** and the preparation protocol is ***Dewax**.
13. Select staining mode values **Single** and **Oracle** (do not click **Oracle control**).
14. Select process **IHC**.
15. Select ***HER2 Negative Control** from the marker list. The Protocols tab defaults to the correct staining protocol (***IHC Protocol H**) and HIER protocol (***HIER 25 min with ER1 (97)**).
16. Click **Add slide**. The negative control reagent slide is created.
17. Still in the Add slide dialog, select ***HER2 Primary Antibody** from the marker list. Default protocols and all other settings remain unchanged.
18. Click **Add slide**. The test slide is created.
19. Repeat steps 8 to 18 until all cases and patient test slides have been created.
20. Next, create the HER2 Control Slide. Add it to the last case or create a new case for control slides, depending on your standard laboratory practices.

Important note: It is a requirement of the Bond Oracle HER2 IHC System that a HER2 Control Slide is included in each run (ie slide tray) in order to validate the assay.
21. In the Add slide dialog set tissue type to **Positive tissue**.
22. Click **Oracle control**.
23. Select the lot number of the HER2 Control Slide in the **Lot No** list. The lot number is inscribed on the label area of the slide.

Important note: The HER2 Control Slide must come from the same Bond Oracle HER2 IHC System that will be used.
24. Select ***HER2 Primary Antibody** from the marker list. Retain dispense volume, staining mode, process and protocol settings.
25. Click **Add slide** to add the HER2 Control Slide.
26. Finally, add a positive in-house tissue control slide.
27. Deselect **Oracle control**.
28. Select ***HER2 Primary Antibody** from the marker list. Retain dispense volume, staining mode, and process and protocol settings. Tissue type remains **Positive tissue**.
29. Click **Add slide**. This completes slide creation.

30. Print slide labels. All Oracle slide labels have "OC" printed on them. The label for the HER2 Control Slide also includes the Bond Oracle HER2 IHC System lot number.
31. Label slides appropriately.
32. Open the lids of all Bond Oracle HER2 IHC System containers and load the reagent tray onto the BOND.
33. Place slides onto the slide tray in the order indicated in section D, Table 2. Apply new Covertiles.
34. Load the slide tray onto the BOND and press the **Load/Unload** button.
35. Confirm that the slides have been scanned and click the **Run (Play)** button on the System status screen.
36. Ensure that the tray indicator field displays **Proc (OK)** and batch number and finish time are displayed.
37. When the run is completed press the **Load/Unload** button and remove the slide trays from the BOND.
38. Remove Covertiles and rinse the slides in de-ionized water.
39. Dehydrate, clear and mount sections.

Quality Control

Differences in tissue fixation, processing and embedding in the user's laboratory may produce significant variability in results, necessitating regular performance of in-house controls in addition to the HER2 Control Slides supplied by Leica Biosystems in the Bond Oracle HER2 IHC System. Consult the quality control guidelines of the College of American Pathologists (CAP) Certification Program for Immunohistochemistry; see also CLSI (formerly NCCLS) Quality Assurance for Immunocytochemistry, Approved Guideline (17) and Special Report: Quality Control in Immunohistochemistry (18). In addition, refer to Table 3 below for the types of immunohistochemical quality controls and their purposes.

Sample*	Description	HER2 Primary Antibody Staining	HER2 Negative Control Staining
HER2 Control Slide	As supplied in the Bond Oracle HER2 IHC System.	Controls staining procedure, and indicates the validity of the reagent performance.	Detection of nonspecific background staining
In-house Positive Control Tissue	Tissue containing target antigen. The ideal control is weakly positive staining tissue so as to define subtle changes in primary antibody sensitivity.	Controls all steps of the analysis. Validates tissue preparation and Bond Oracle HER2 IHC System staining performance.	
In-house Negative Control Tissue Component	Tissues or cells expected to be negative (could be located in patient tissue or positive/negative control tissue components).	Detection of nonspecific antibody cross-reactivity with cells/cellular components.	

*Fixed and processed as per patient sample

Table 3. Immunohistochemical quality controls and their purpose

Control tissue should be biopsy or surgical specimens, formalin-fixed, processed and paraffin-embedded as soon as possible, and in the same manner as the patient sample(s). Specimens must be handled appropriately to preserve the tissue antigenicity for immunohistochemical staining. Standard methods of tissue processing should be employed for all specimens (17).

HER2 Control Slide – HER2 Primary Antibody

Each of the supplied HER2 Control Slides contains four formalin-fixed, paraffin-embedded human breast cancer cell line cores with staining intensity scores of 0, 1+, 2+ and 3+. One slide must be included in each test run (ie slide tray). The correct evaluation of the HER2 Control Slide supplied by Leica Biosystems indicates the validity of the test (refer to Bond Oracle HER2 IHC System Interpretation Guide). The HER2 Control Slides supplied with this system validate reagent performance only and do not verify tissue preparation.

In-house Positive Control Tissue – HER2 Primary Antibody

If in-house positive control tissue components are used, they should be biopsy or surgical specimens fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). Positive tissue controls are indicative of correctly prepared tissues and valid staining techniques. At least one positive control component for each test run should be included. The positive control section should demonstrate weak positive staining so as to define subtle changes in primary antibody sensitivity.

Note: Known positive control tissue components should only be utilized for monitoring the correct performance of processed tissues together with test reagents, NOT as an aid in formulating a specific interpretation of patient samples. If the positive control tissue fails to demonstrate appropriate positive staining, results obtained with patient specimens should be considered invalid.

A multi tissue control block containing tumors representing all 4 HER2 grades may also be effectively utilized as appropriate in-house control material.

In-house Negative Control Tissue Component – HER2 Primary Antibody

If in-house negative control components are used, they should be fresh biopsy or surgical specimens fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). Use of control tissue, known to be HER2 oncoprotein negative, with each staining run verifies the specificity of the primary antibody and provides an indication of any nonspecific background staining. The variety of different cell types present in most tissue sections offers internal negative control sites (this should be verified by the user). Normal breast ducts unassociated with tumor may provide a reference to the validity of the assay. If specific staining occurs in the internal negative control tissue, results with the patient specimens should be considered invalid.

The use of multi-tissue control block representing all four HER2 grades may be utilized for the purposes of negative and positive control tissues.

Patient Tissue – HER2 Negative Control

Use the supplied HER2 Negative Control in place of the HER2 Primary Antibody on a corresponding section for each patient test to evaluate nonspecific staining and allow accurate interpretation of specific HER2 oncoprotein staining at the antigenic site.

Patient Tissue – HER2 Primary Antibody

Positive staining intensity should be assessed within the context of any nonspecific background staining with the HER2 Negative Control. As with any immunohistochemical test, a negative result means that the antigen was not detected, not that the antigen was absent in the cells/tissue assayed. Refer to Slide Screening Order Rationale, Limitations, Performance Evaluation and Immunoreactivity for specific information regarding Bond Oracle HER2 IHC System immunoreactivity.

Assay Verification

Prior to the initial use of any antibody or staining system in a diagnostic procedure, the user should verify the antibody's specificity by testing it on a series of in-house tissues with known immunohistochemical positive and negative profiles. Refer to Quality Control as previously outlined and the quality control requirements of the CAP Certification Program for Immunohistochemistry and/or CLSI (formerly NCCLS) Quality Assurance for Immunocytochemistry, Approved Guideline (17). These quality control procedures should be repeated for each new antibody lot, or whenever there is a change in assay parameters. Human invasive (infiltrating) ductal breast carcinoma with known HER2 oncoprotein staining intensities from 0 to 3+ and other suitably negative tissues are appropriate for assay verification.

Interpretation of Staining - Breast

For the determination of HER2 oncoprotein expression, only membrane staining pattern and intensity should be evaluated using the scale presented in Table 4. A pathologist using a bright-field microscope should perform slide evaluation. For evaluation of the immunohistochemical staining and scoring, an objective of 10x magnification is appropriate. The use of 20–40x objective magnification should be used in the confirmation of the score. Cytoplasmic staining should be considered as nonspecific staining and is not to be included in the assessment of membrane staining intensity (19). To aid in the differentiation of 0, 1+, 2+, and 3+ staining, refer to the Bond Oracle HER2 IHC System Interpretation Guide for representative images of the staining intensities. Only specimens from patients with invasive breast carcinoma should be scored. In cases with carcinoma *in situ* and invasive carcinoma in the same specimen, only the invasive component should be scored.

Immunohistochemical Staining Pattern	Score	Assessment
No staining is observed or membrane staining is observed in less than 10% of the tumor cells.	0	Negative
Faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.	1+	Negative
Weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.	2+	Equivocal (Weakly Positive)
Strong complete membrane staining is observed in more than 10% of the tumor cells.	3+	Strongly Positive

Table 4. Interpretation of HER2 staining for Breast tissue specimens

Bond Oracle HER2 IHC System staining results are interpreted as negative for HER2 oncoprotein expression with scores of 0 and 1+ staining intensity, equivocal (weakly positive) with a score of 2+ staining intensity, and strongly positive with a score of 3+ staining intensity. Bond Oracle HER2 IHC System is not intended to provide prognostic information to the patient and/or physician and has not been validated for that purpose. For each staining assessment, slides should be examined in the order presented below to determine the validity of the staining run and enable semi-quantitative assessment of the staining intensity of the sample tissue.

Interpretation of Staining - Gastric

For the determination of HER2 oncoprotein expression, only membrane staining pattern and intensity should be evaluated using the scale presented in Tables 5 and 6. A pathologist using a brightfield microscope should perform the slide evaluation. For evaluation of the immunohistochemical staining and scoring, an objective of 10x magnification is appropriate. The use of 20–40x objective magnification should be used in the confirmation of the score. Cytoplasmic staining should be considered as non-specific staining and is not to be included in the assessment of membrane staining intensity (15). To aid in the differentiation of 0, 1+, 2+, and 3+ staining, refer to the Bond Oracle HER2 IHC System Gastric Interpretation Guide for representative images of the staining intensities. Only specimens from patients with stomach or gastroesophageal junction adenocarcinoma should be scored.

	Immunohistochemical Staining Pattern	Score	Assessment
Surgical Specimens	No staining is observed or membrane staining is observed in less than 10% of the tumor cells.	0	Negative
	Faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.	1+	Negative
	Weak to moderate complete, basolateral or lateral membrane staining is observed in equal to or more than 10% of the tumor cells.	2+	Equivocal (Weakly Positive)
	Strong complete, basolateral or lateral membrane staining is observed in more than 10% of the tumor cells.	3+	Strongly Positive

Table 5. Interpretation of HER2 staining in gastric cancer surgical specimens

	Immunohistochemical Staining Pattern	Score	Assessment
Biopsy Specimens	No staining is observed in any tumor cell	0	Negative
	Tumor cell cluster with faint/barely perceptible membrane staining is observed, irrespective of percentage of cells stained	1+	Negative
	Tumor cell cluster with weak to moderate complete, basolateral or lateral membrane staining is observed, irrespective of percentage of cells stained	2+	Equivocal (Weakly Positive)
	Tumor cell cluster with strong complete, basolateral or lateral membrane staining observed, irrespective of percentage of cells stained	3+	Strongly Positive

Table 6. Interpretation of HER2 staining in gastric cancer biopsy specimens

For interpretation of Bond Oracle HER2 IHC System stained biopsies a cluster of at least five tumor cells is recommended.

Slide Screening Order Rationale

Slides should be screened in the following order:

1. HER2 Control Slide – HER2 Primary Antibody

A valid assay with the Oracle HER2 Control Slide shows the following:

- Presence of strong brown, complete cell membrane staining in the 3+ Control Cell Line SK-BR-3.
- Presence of weak to moderate brown, complete cell membrane staining in the 2+ Control Cell Line, MDA-MB-453.
- Presence of faint/barely perceptible brown, incomplete cell membrane staining in the 1+ Control Cell Line, MDA-MB-175.
- No staining in the 0 Control Cell Line MDA-MB-231.

Important note: A feature of the MDA-MB-175 1+ control cell line is a distinct growth pattern in which the cells form clusters. These clusters give rise to a continuous luminal brush border region across the cell cluster. This brush border staining will be stronger than that of the rest of the cell membrane. It is the faint/barely perceptible incomplete cell membrane staining that is the correct HER2 oncoprotein 1+ staining pattern. Dot-like immunostaining of the Golgi region in the cytoplasm may also be observed in this cell line.

2. In-house Positive Control Tissue – HER2 Primary Antibody

The PRESENCE of brown membrane staining should be observed corresponding to the known HER2 oncoprotein status of the chosen positive control.

3. In-house Negative Control Tissue Component – HER2 Positive Control

The ABSENCE of membrane staining should be observed. A negative control tissue component confirms the lack of detection system cross-reactivity to specifically targeted cells/cellular components. If membrane staining occurs in a negative control tissue component, results with the patient specimen should be considered invalid.

4. Patient Tissue – stained using the HER2 Negative Control

The ABSENCE of membrane staining verifies the specific labeling of the target antigen by the primary antibody. Other brown staining occurring in the cytoplasm of the specimen treated with the HER2 Negative Control, such as in connective tissue, leukocytes, erythrocytes, or necrotic tissue, should be considered nonspecific background staining and should be noted.

5. Patient Tissue – stained using the HER2 Primary Antibody

HER2 oncoprotein expression levels are determined by the criteria defined in both Tables 4, 5 and 6 and in the Bond Oracle HER2 IHC System Interpretation Guides.

Limitations

A. General Limitations

Immunohistochemistry is a laboratory based, multi-step technique, used to aid in the interpretation and determination of histopathological characteristics. It is a technique which requires specialized training in all aspects of procedure (including the selection of appropriate reagents, tissue, fixation, processing and IHC slide preparation) and interpretation.

Immunohistochemical staining of tissue is dependent on the handling, fixation and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifact, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation, embedding methods, or to inherent irregularities within the tissue (21). Excessive or incomplete counterstaining may also compromise correct interpretation of the results.

Nonspecific staining, if present, usually has a diffuse appearance. Sporadic staining of connective tissue may also be observed in sections from excessively formalin-fixed tissues. Use intact cells for interpretation of staining results. Necrotic or degenerated cells often stain

nonspecifically (22). False-positive results may be seen due to non-immunological binding of proteins or substrate reaction products. They may also be caused by endogenous enzymes such as pseudoperoxidase (erythrocytes) or endogenous peroxidase (cytochrome C), depending on the type of immunohistochemical stain used.

Tissues from patients infected with Hepatitis B virus and containing Hepatitis B virus surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase (23).

Unexpected immunohistochemical staining, or variations in the staining, may be as a result of alterations in the expression levels of the encoding genes or antigens. Any change in expected staining patterns should be interpreted in association with all other diagnostic investigations.

The interpretation of immunohistochemical staining should be complemented by morphological studies and the use of suitable control material, and should be evaluated within the context of the patient's clinical history and other any diagnostic tests by a qualified pathologist.

The performance of the assay (ie assessments of adequacy of both positive and negative controls) and the interpretation of any immunohistochemical staining or its absence must be carried out in an appropriately accredited/licensed laboratory under the supervision of a suitably qualified and experienced pathologist, who is responsible for the overall assessment of the immunohistochemical assay and its interpretation.

B. Product Specific Limitations

This product is not intended for use in flow cytometry. Performance characteristics have not been determined for flow cytometry.

False negative results may be seen as a result of the degradation of antigens in the tissue section. Slides required for HER2 oncoprotein evaluation and tumor verification should be prepared at the same time. To preserve antigenicity, tissue sections mounted on slides (Leica BOND Plus Slides – product code S21.2113 or Apex BOND Slides - product code 3800040) should be stained within 4–6 weeks of sectioning when held at room temperature (18–24 °C). Following sectioning, slides are recommended to be incubated for 12–18 hours at 37 °C. Sections which require further adherence may be incubated at 60 °C for a further hour.

Minimal natural variation of immunohistochemical profile will be seen between growth batches of cell lines utilized within the Bond Oracle HER2 IHC System. This natural variation is well within acceptable tolerance levels of a biological entity and does not affect the interpretation or performance of the system.

Characterization of the cell lines using both flow cytometry and in situ hybridization as presented in Table 7 are also subject to natural biological variation. Technical and interpretational variation of control cell lines as assessed by fluorescent in situ hybridization is also reported (24).

Assessment of the HER2 Control Slides should take into account all relevant expiry dates. Store the Bond Oracle HER2 IHC System at 2–8 °C. Do not freeze. Return to 2–8 °C immediately after use. Any deviations from these conditions will invalidate the assay.

Do not replace Bond Oracle HER2 IHC System reagents with any other components either supplied by Leica Biosystems or by other manufacturers. To do so will invalidate the assay.

It is essential that all of the steps outlined in sections C to E (Procedure) are performed in the prescribed order. Any deviation from this order will invalidate the assay.

It is essential that tissues fixed only in formalin-based fixatives be used in the assay. The use of any other type of fixative will invalidate the assay.

Tissue sections cut outside of the recommended thickness range have not been validated. The use of any other section thickness may invalidate the assay.

Cell Line Data

Cell Line	Bond Oracle HER2 IHC System Profile	HER2 Receptor Load per Cell*	HER2 Gene Amplification Status [†]	
			HER2 Copy Number	HER2:Chr17 Gene Ratio
SK-BR-3	3+	4.3x10 ⁵	13.35	3.55
MDA-MB-453	2+	1.4x10 ⁵	5.73	2.05
MDA-MB-175	1+	6.3x10 ⁴	3.33	1.20
MDA-MB-231	0	9.3x10 ³	3.15	1.13

*HER2 receptor load analysis as assessed by flow cytometry. †HER2 Gene Amplification Status as assessed by dual probe (HER2:Chromosome 17) FISH.

Table 7. HER2 Control Slide profile

Clinical Concordance of Bond Oracle HER2 IHC System v Dako HercepTest - Breast

Part one of the study examined the suitability of the Bond Oracle HER2 IHC System for use as an aid in determination of treatment with Herceptin® (trastuzumab) therapy. The study was designed to examine the concordance between the Bond Oracle HER2 IHC System and the Dako HercepTest, considered as the 'gold standard' for this assay. The acceptance criterion was defined as greater than 75% overall concordance between the two tests with a 95% confidence interval (CI).

The study was conducted as a two-site, US based, blinded evaluation. Each investigational site was supplied with formalin-fixed, paraffin-embedded breast cancer samples of known HER2 status. Cases were selected in reverse consecutive order from the clinical archives, representing the consecutive flow of cases into a histopathology department for clinical testing, and tested independently of other prognostic and/or predictive factors, with no bias introduced to the cohort. Cohorts of 160 and 292 specimens were tested at Site 1 and Site 2 respectively. Each cohort had an equal representation of equivocal/positive (2+, 3+) and negative (0, 1+) cases, based on previously assigned HER2 IHC scores, resulting in a total study population of 452 samples. Twelve samples were considered unsuitable, due to lack of sufficient invasive tumor and were removed from the study. A further nine samples could not be scored as a result of tissue lifting from the slide surface, resulting in a final study population of 431 samples.

All cases were stained with the HercepTest according to the manufacturer's instructions as specified in the package insert. Sequential sections from each case were stained with the Bond Oracle HER2 IHC System on board an automated Leica Biosystems BOND fully automated, advanced staining system. All cases were de-linked from unique patient identifying information and were accompanied by clinical data relating to tumor size, tumor stage, tumor grade and estrogen receptor status.

All stained slides were masked and scored in a randomized fashion by trained observers at two sites. For 2x2 concordance analysis, scores were interpreted as negative if the staining intensity was 0 or 1+, and positive for scores of 2+ or 3+. For 3x3 concordance analysis, scores were interpreted as negative if the staining was 0 or 1+, equivocal for scores of 2+ and positive for scores of 3+. Data was then analyzed for positive staining agreement and negative staining agreement.

2x2 Concordance Results

In this primary analysis the test results from the two tests (Bond Oracle HER2 IHC System and Dako HercepTest) are categorized as negative (0, 1+) or positive (2+, 3+). The frequencies of four possible combinations are displayed in a 2x2 table format (see Table 8). Then, the overall concordance rate based on this 2x2 table was calculated accompanied by a 95% exact confidence interval (based on the binomial distribution).

The null hypothesis (H_0), which the success criteria are set against, is that concordance is no greater than 75%.

The observed agreement for 431 samples between the two tests in a 2x2 analysis show a concordance of 92.34% (398/431) with a 95% CI of 89.42% - 94.67%. This data supports rejection of the null hypothesis (H_0) that agreement was no greater than 75% with a p-value < 0.0001.

The percentage Positive Agreement (sensitivity) or the ability of Bond Oracle HER2 IHC System to correctly identify HercepTest positive cases (the percentage of specimens scored positive by both Bond Oracle HER2 IHC System and HercepTest out of all the HercepTest positive cases) was 84.87% (129/152) with a 95% CI of 78.17%-90.16%. The percentage Negative Agreement (specificity) or the ability of the test to correctly identify HercepTest negative cases (the percentage of specimens scored negative by both Bond Oracle HER2 IHC System and HercepTest out of all the HercepTest negative cases) was 96.42% (269/279) with a 95% CI of 93.51%-98.27%.

		HercepTest		
		Negative	Positive	Totals
Bond Oracle HER2 IHC System	Negative	269	23	292
	Positive	10	129	139
	Totals	279	152	431

2x2 Concordance (95% CI) = 92.34% (89.42 to 94.67%); p < 0.0001

Table 8. 2x2 concordance of Bond Oracle HER2 IHC System with HercepTest

3x3 Concordance Results

Data was grouped as negative (0 or 1+), equivocal (2+) or positive (3+) for 3x3 analysis and showed a concordance of 86.54% (373/431) with a 95% CI of 82.95% to 89.62%. Therefore, the null hypothesis (H_0) that agreement was no greater than 75% was rejected with a p-value < 0.0001.

The percentage Positive Agreement for 3+ (the percentage of specimens scored 3+ positive by both Bond Oracle HER2 IHC System and HercepTest out of all the 3+ HercepTest positive cases) in this study was 73.33% (66/90) with a 95% CI of 62.97% to 82.11%. The percentage Negative Agreement was 96.42% (269/279) with a 95% CI of 93.51% to 98.27. See Table 9.

		HercepTest			Totals
		Negative (0 or 1+)	2+	3+	
Bond Oracle HER2 IHC System	Negative (0 or 1+)	269	23	0	292
	2+	10	38	24	72
	3+	0	1	66	67
	Totals	279	62	90	431

3x3 Concordance (95% CI) = 86.54% (82.95% to 89.62%); p < 0.0001

Table 9. 3x3 concordance of Bond Oracle HER2 IHC System with HercepTest

In conclusion, the data generated in this study demonstrates that the Bond Oracle HER2 IHC System can be used as an aid in determination of treatment for Herceptin® (trastuzumab) therapy, based upon its high concordance with the HercepTest.

Clinical Concordance of Bond Oracle HER2 IHC System to PathVysion HER-2 DNA Probe Kit - Breast

Part 2 of the study was designed to examine the concordance between the Bond Oracle HER2 IHC System and the Abbott Molecular PathVysion HER-2 DNA Probe Kit, considered as the 'gold standard' for gene assessment reflex assay used in conjunction with HER-2 immunohistochemistry.

This study was performed at the same investigational sites and used the same study cohort as in Part 1. All cases were stained with the Abbott Molecular PathVysion HER-2 DNA Probe Kit according to the manufacturers' instructions as specified in the package insert. Sequential sections from each case were stained with the Bond Oracle HER2 IHC System on board a BOND fully automated, advanced staining system (from Part 1 of the clinical study). Of the 431 cases stained no result was obtained on three occasions due to insufficient probe hybridization resulting in a total cohort of 428 cases.

All stained slides were scored by trained observers at two investigational sites. For concordance analysis the scores were interpreted as negative if the HER2/CEP17 gene amplification ratio was less than (<) 2.0 and positive if greater than or equal to (>) 2.0 following a 20 tumor cell count.

3x2 Concordance Results

The observed agreement for 428 samples between the two tests in a 3x2 analysis show a concordance of 87.6% (375/428) with a 95% CI of 84.1% to 90.6%.

The percentage Positive Agreement (sensitivity) or the ability of Bond Oracle HER2 IHC System to correctly identify PathVysion positive cases (the percentage of specimens scored positive by both Bond Oracle HER2 IHC System and PathVysion out of all the PathVysion positive cases) was 93.8% (61+30/97) with a 95% CI of 87.0% to 97.7%.

The percentage Negative Agreement (specificity) or the ability of the test to correctly identify PathVysion negative cases (the percentage of specimens scored negative by both Bond Oracle HER2 IHC System and PathVysion out of all the PathVysion negative cases) was 85.8% (284/331) with a 95% CI of 81.6% to 89.4%. See Table 10.

		PathVysion HER-2 DNA Probe Kit		
		Negative	Positive	Totals
Bond Oracle HER2 IHC System	0/1+	284	6	290
	2+	41	30	71
	3+	6	61	67
	Totals	331	97	428

Overall Concordance (95% CI) = 87.6% (84.1% to 90.6%)

Table 10. 3x2 concordance of Bond Oracle HER2 IHC System staining v PathVysion HER-2 DNA Probe kit.

Clinical Concordance of Bond Oracle HER2 IHC System v Ventana Medical Systems Inc. PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody - Gastric

The aim of Part 3 of the study was to generate data from formalin fixed, paraffin-embedded advanced stage gastric cancer specimens, to examine the concordance between the fully automated Bond Oracle HER2 IHC System on the BOND fully automated, advanced staining system, using the Ventana Medical Systems Inc. PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody. The acceptance criterion for part three was defined as greater than 75% overall concordance between the two tests.

For 2x2 concordance analysis, scores were interpreted as negative if the staining intensity was 0 or 1+, and positive for scores of 2+ or 3+. For 3x3 concordance analysis, scores were interpreted as negative if the staining was 0 or 1+, equivocal for scores of 2+ and positive for scores of 3+. Data was also analyzed for positive staining agreement and negative staining agreement.

Results - Bond Oracle HER2 IHC System vs. Ventana Medical Systems Inc. PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody - Gastric

2x2 Concordance Results

Concordance between the fully automated Bond Oracle HER2 IHC System, using the BOND fully automated, advanced staining system and the Ventana PATHWAY anti-HER-2/neu (4B5) was conducted on 287 specimen samples.

The observed concordance between the two tests in a 2x2 analysis was 95.12% (273/287) with a 95% CI of 91.95% - 97.31%. This data supports rejection of the null hypothesis (H0) that agreement was no greater than 75% with a p-value<0.0001. The percentage positive agreement (sensitivity) or the ability of Bond Oracle HER2 IHC System to correctly identify Ventana PATHWAY anti-HER-2/neu (4B5) positive specimens (the percentage of specimens scored positive by both Bond Oracle HER2 IHC System and Ventana PATHWAY anti-HER-2/neu (4B5) out of all the Ventana PATHWAY anti-HER-2/neu (4B5) positive specimens) was 90.79% (138/152) with a 95% CI of 85.03%-94.87%. The percentage negative agreement (specificity) or the ability of the test to correctly identify Ventana PATHWAY anti-HER-2/neu (4B5) negative specimens (the percentage of specimens scored negative by both Bond Oracle HER2 IHC System and Ventana PATHWAY anti-HER-2/neu (4B5) out of all the Ventana PATHWAY anti-HER-2/neu (4B5) negative specimens) was 100% (135/135) with a 95% CI of 97.30%-100% (see Table 11).

		Ventana PATHWAY anti-HER-2/neu (4B5)		
		Negative	Positive	Totals
Bond Oracle HER2 IHC System	Negative	135	14	149
	Positive	0	138	138
	Totals	135	152	287

Overall Concordance (95% CI) = 95.12% (91.95%-97.31%)

Table 11. 2x2 Concordance of Bond Oracle HER2 IHC System staining v Ventana Medical Systems Inc. PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody on gastric tissue.

3x3 Concordance Results

The observed concordance between the two tests in a 3x3 analysis was 89.90% (258/287) with a 95% CI of 85.81% to 93.13%. Therefore, the null hypothesis (H_0) that agreement was no greater than 75% was rejected with a p-value <0.0001. The percentage positive agreement for 3+ or the ability of Bond Oracle HER2 IHC System to correctly identify Ventana PATHWAY anti-HER-2/neu (4B5) positive specimens (the percentage of specimens scored 3+ positive by both Bond Oracle HER2 IHC System and Ventana PATHWAY anti-HER-2/neu (4B5) out of all the 3+ Ventana PATHWAY anti-HER-2/neu (4B5) positive specimens) in this study was 85.94% (110/128) with a 95% CI of 78.69% to 91.45%. The percentage negative agreement (specificity) or the ability of the test to correctly identify Ventana PATHWAY anti-HER-2/neu (4B5) negative specimens (the percentage of specimens scored negative by both Bond Oracle HER2 IHC System and Ventana PATHWAY anti-HER-2/neu (4B5) out of all the Ventana PATHWAY anti-HER-2/neu (4B5) negative specimens) was 100% (135/135) with a 95% CI of 97.30% to 100%. See Table 12.

		Ventana PATHWAY anti-HER-2/neu (4B5)			
		Negative (0 or 1+)	2+	3+	Totals
Bond Oracle HER2 IHC System	Negative (0 or 1+)	135	11	3	149
	2+	0	13	15	28
	3+	0	0	110	110
	Totals	135	24	128	287

Overall Concordance (95% CI) = 89.90% (85.81%-93.13%)

Table 12. 3x3 Concordance of Bond Oracle HER2 IHC System staining v Ventana Medical Systems Inc. PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody on gastric tissue.

Immunoreactivity – Normal Panel

Normal Tissue Type	Staining Pattern	
	HER2 Primary Antibody	HER2 Negative Control
Adrenal	Negative	Negative
Brain, Cerebellum	Negative	Negative
Brain, Cerebrum	Negative	Negative
Breast	Negative	Negative
Bone Marrow	Negative	Negative
Colon	Negative	Negative
Esophagus	Negative	Negative
Eye	Negative	Negative
Hypophysis	Moderate cytoplasmic staining observed in hypophyseal cells (1/3)	Negative
Kidney	Negative	Negative
Larynx	Negative	Negative
Liver	Negative	Negative
Lung	Negative	Negative
Mesothelium	Negative	Negative
Ovary	Negative	Negative
Pancreas	Negative	Negative
Parathyroid	Negative	Negative
Peripheral Nerve	Negative	Negative
Prostate	Negative	Negative
Salivary Gland	Negative	Negative
Skin	Negative	Negative
Small Intestine	Negative	Negative
Spleen	Negative	Negative
Stomach	Weak cytoplasmic staining observed in gastric glands (2/3)	Negative
Striated Muscle	Negative	Negative
Testis	Negative	Negative
Thymus	Negative	Negative
Thyroid	Negative	Negative
Tonsil	Negative	Negative
Uterine Cervix	Negative	Negative
Uterus	Negative	Negative

Table 13. Normal Panel Staining

Reproducibility Study

Within and Between Precision Testing

Precision testing was performed at Leica Biosystems, Newcastle Ltd. The tissue used was a formalin-fixed, paraffin-embedded composite tissue micro array (TMA) supplied by Isu Abxis (Yonsei University Medical Center 134 Shinchon-dong, Seoul, 120-752 Korea), comprising of 20, 4mm diameter invasive breast carcinoma tissue cores. The 20 cases were selected based on previously assigned HER2 scores. On this basis, x5 cases of HER2 3+, x5 cases of HER2 2+, x5 cases of HER2 1+ and x5 cases of HER2 0, were included.

A. Within Run Precision Testing

Within run precision testing of the Bond Oracle HER2 IHC System was evaluated on a total of 40 consecutive sections from a TMA comprising of 20 invasive breast tumors and 40 HER2 Control Slides. All slides were stained with the Bond Oracle HER2 IHC System on the BOND fully automated advanced staining system. Sections were stained during one continuous period using a Bond Oracle HER2 IHC System from the same manufacturing batch. Stained sections were blinded and assessed in a randomized fashion by a single experienced observer to determine within run precision.

An evaluation of the slides from the within run investigation indicated that 733/800 (91.63%) test data points could be interpreted. 40 data points were excluded due to presence of DCIS only, and a further 27 data points could not be interpreted due to a loss of invasive tumor (specific to 3 cores). Variation in staining occurred 61 (8.32%) out of a possible 733 staining events. On 37 occasions, variation from 3+ to 2+ (n = 20) and from 1+ to 0 (n = 17) was observed and would therefore not represent a change from clinically positive to clinically negative or vice versa in a 2x2 data assessment. The remaining 24 (3.27%) occasions represented a change from clinically negative (0 or 1+) to clinically positive (2+ or 3+). Pass value = 96.7% (95% CI = 95.15% to 97.81%).

B. Between Run Precision Testing

Between run precision testing of the Bond Oracle HER2 IHC System was evaluated on a total of 24 consecutive sections taken from a TMA comprising of 20 invasive breast tumors and 24 HER2 Control Slides. All slides were stained with the Bond Oracle HER2 IHC System on the BOND fully automated advanced staining system. The slides were evaluated in 8 independent runs, performed within the same laboratory, on three separate occasions using a Bond Oracle HER2 IHC System from the same manufacturing batch. Stained slides were blinded and assessed in a randomized fashion by a single experienced observer to determine between run precision.

An evaluation of the slides from the between run investigation indicated that 456/480 (95.00%) test data points could be interpreted. 24 data points could not be interpreted due to a loss of invasive tumor (specific to 5 cores). Variation in staining occurred 42 (9.21%) out of a possible 456 data points. On 30 occasions, variation from 3+ to 2+ (n = 10) and from 1+ to 0 (n = 20) were observed and would therefore not represent a change from clinically positive to clinically negative or vice versa in a 2x2 data assessment. The remaining 12 (2.63%) represented a change from clinically negative (0 or 1+) to clinically positive (2+ or 3+). Pass value = 97.37% (95% CI = 95.90% to 98.77%).

C. Lot-to-Lot Reproducibility

To determine Lot-to-Lot reproducibility, 3 lots of Bond Oracle HER2 IHC System were manufactured under GMP on 3 separate occasions and evaluated on 24 breast tumor sections (24 test data points) taken from four different formalin-fixed, paraffin-embedded tissue blocks (representing 0, 1+, 2+ and 3+ HER2 staining intensities) and three HER2

Control Slides (12 control data points). Three independent runs were performed within the same laboratory on three separate occasions, each using a separate manufacturing lot of Bond Oracle HER2 IHC System. All slides were stained with the Bond Oracle HER2 IHC System on board a BOND fully automated advanced staining system. Stained slides were masked and assessed in a randomized fashion by a single trained observer to determine Lot-to-Lot reproducibility.

An evaluation of the slides (tests and controls) from the lot-to-lot investigation indicated that 36/36 data points could be interpreted. No variation in staining occurred in the 36 data points between the three different manufacturing lots of the Bond Oracle HER2 IHC System. Staining with the Bond Oracle HER2 IHC System is consistent across manufacturing batches.

D. Between Laboratory Reproducibility

Between laboratory reproducibility testing of the Bond Oracle HER2 IHC System was evaluated at 3 sites, Leica Biosystems Newcastle Ltd (Site A), and two independent laboratories (Sites B and C) on a total of 192 sections from a TMA comprising of 20 invasive breast tumors and 24 HER2 Control Slides. Of the 192 TMA sections stained, 96 were stained with the HER2 Primary Antibody and 96 with the HER2 Negative Control reagent. All slides were stained with the Bond Oracle HER2 IHC System on the BOND fully automated advanced staining system. The slides were evaluated in 8 independent runs performed within each of the 3 different investigational sites using a Bond Oracle HER2 IHC System from the same manufacturing batch. Stained slides were blinded and assessed in a randomized fashion by a single experienced observer at Leica Biosystems, Newcastle Ltd to determine between laboratory reproducibility.

An evaluation of the slides from the between laboratory reproducibility investigation indicated that 1477/1920 (76.93%) test data points could be interpreted. 443 test data points could not be interpreted due to:

- a) Inadequate performance of the HER2 Control slide on 2/24 occasions resulting in 2 runs/160 test data points being removed. This event occurred once at Site A and once at Site B (80 data test points per investigational site removed).
- b) Deviation from the test plan at Site C, in which 24 slides in total were manually counterstained with hematoxylin following Bond Oracle HER2 IHC System staining. This resulted in excessive counterstaining of both HER2 control slides and TMA test data points resulting in 240 data points being removed.
- c) Loss of invasive tumor resulting in 23 test data points being removed. This event occurred on 23 occasions at Site A and was a direct result of loss of tissue in the TMA block on production of the 192 consecutive TMA sections required to complete this investigation.
- d) Uninterpretable staining due to inadequate washing by the BOND fully automated advanced staining system resulting in 20 data points being removed.

An evaluation of the interpretable slides in the between laboratory precision investigation indicated that variation in staining occurred 79 (5.28%) out of a possible 1477 staining events. Of these, 14/1477 (0.95%) occasions represented variations from 0 to 1+ or 2+ to 3+ and as such did not represent a change from clinically positive to clinically negative or vice versa in a 2x2 data assessment. Pass value = 99.05% (95% CI = 98.42% to 99.46%). Of the 14 staining events, 5/1477 (0.34%) staining events occurred at Leica Biosystems, Newcastle, Ltd (Site A), 8/1477 (0.54%) occurred at Site B and 1/1477 (0.07%) occurred at Site C.

The remaining 65/1477 (4.40%) staining events showed variation from 2+ to 1+ or 2+ to 0 and therefore would represent a change from clinically positive to clinically negative or vice versa in a 2x2 data assessment. Pass value = 95.6% (95% CI = 94.42% to 96.54%). Of the 65 clinically significant changes, 11/65 (16.9%) occurred at Leica Biosystems, Newcastle, Ltd (Site A), 24/65 (36.9%) occurred at Site B and 30/65 (46.1%) occurred at Site C. Of the clinically significant changes on no occasions did a 3+ change to a negative (0 or 1+) result or vice versa.

E. Inter-Observer Reproducibility

40 randomly selected invasive breast cancer cases, providing an equal distribution of each of the HER2 IHC grades (resection specimens) were consecutively sectioned and provided to Leica Biosystems, Newcastle Ltd (Site A), Site B and Site C for staining and interpretation. The sections were blinded and randomized at each site prior to scoring. Inter observer agreement between the two independent clinical sites, Site B and Site C, was 87.5% (95% CI = 73.3% to 95.8%). The agreement between Site B and Site C and Leica Biosystems Newcastle, Ltd was 92.5% (95% CI = 79.6% to 98.4%) and 85% (95% CI = 70.1% to 94.29%) respectively. The analysis of total concurrence between the three observers (A, B, C) is 82.50%.

F. Between Instrument Precision (BOND-MAX v BOND-III)

Between instrument precision testing using the Bond Oracle HER2 IHC System was performed at a single European investigational site. The samples tested were obtained from formalin-fixed, paraffin-embedded whole sections from one hundred and thirty eight invasive breast cancer cases (resection specimens).

Identical lot numbers of Bond Oracle HER2 IHC System and BOND Instrument ancillary reagents were used across each instrument. Sections were stained retrospectively. Slides were interpreted at the investigational site by a single experienced observer to determine between instrument precision.

An evaluation of the slides from between instrument precision showed a 2x2 concordance between positive (2+, 3+) and negative (0, 1+) of 94.2% (130/138) with a 95% CI of 88.9 to 97.5% and a 3x3 concordance between positive (3+), equivocal (2+) and negative (0, 1+) concordance of 87.0% (120/138) with a 95% CI of 80.2 to 92.1%.

		BOND-MAX		
		Negative (0/1+)	Positive (2/3+)	Totals
BOND-III	Negative (0/1+)	80	1	81
	Positive (2/3+)	7	50	57
	Totals	87	51	138

Overall Concordance (95% CI) = 94.2% (88.9 to 97.5%)

Table 14. 2x2 concordance of Bond Oracle HER2 IHC System staining on BOND-MAX v BOND-III platforms.

		BOND-MAX			
		Negative (0/1+)	Equivocal (2+)	Positive (3+)	Totals
BOND-III	Negative (0/1+)	80	1	0	81
	Equivocal (2+)	6	5	1	12
	Positive (3+)	1	9	35	45
	Totals	87	15	36	138

Overall Concordance (95% CI) = 87.0% (80.2 to 92.1%)

Table 15. 3x3 concordance of Bond Oracle HER2 IHC System staining on BOND-MAX v BOND-III platforms.

In conclusion, the data generated in this study demonstrates a high level of concordance between the Leica Biosystems' BOND-MAX and BOND-III Systems when evaluated using the Bond Oracle HER2 IHC System.

Troubleshooting

Problem	Probable Cause	Remedial Action
No immunohistochemical staining	Run aborted prior to completion	Using BOND software, confirm the presence of any reportable errors during the staining run and address as instructed by the BOND software.
	Incorrect protocol selection	Ensure appropriate default to *IHC Protocol H in the staining protocol field of the Add slide dialog.
	Inadequate deparaffinization of slides	Ensure *Dewax mode is selected in the Preparation field of the Add slide dialog.
	Inappropriate bulk reagents dispensed	Ensure all BOND reagents have been allocated to appropriate bulk containers and placed into appropriate positions on the instrument.
	Contamination of BOND Wash Solution with sodium azide	Use fresh BOND Wash Solution prepared to appropriate working strength.
Weak specific immunohistochemical staining	Inappropriate epitope retrieval	Ensure appropriate BOND Epitope Retrieval reagents have been allocated into correct bulk containers, and BOND software has defaulted to the appropriate epitope retrieval protocol, *HIER 25 min with *ER1 (97) .
	Inappropriate fixation or processing of test specimen	Ensure a formalin-based fixative is used and that processing schedules are suitable for the specimen undergoing testing.
	Bond Oracle HER2 IHC System is being used outside its expiry date	Ensure the Bond Oracle HER2 IHC System used is within its specified expiry date.
Excessive specific immunohistochemical staining	Inappropriate epitope retrieval	Ensure appropriate BOND Epitope Retrieval reagents have been allocated into appropriate bulk containers, and the BOND software has defaulted to *HIER 25 min with ER1 (97) .
	Variation in fixation	Ensure a formalin-based fixative is used and that processing schedules are suitable for the specimen undergoing testing. If possible, retest case using another block. If this is not possible, assess the areas which show best fixation patterns in conjunction with a corresponding H&E stained section.

Problem	Probable Cause	Remedial Action
Nonspecific background staining	Inappropriate bulk reagents dispensed	Ensure all BOND reagents have been allocated into appropriate bulk containers and placed into appropriate positions on the instrument.
	Inadequate deparaffinization of slides	Ensure *Dewax is selected in the Preparation field of the Add slide dialog.
	Nonspecific immunohistochemical cross-reaction in tissue	Refer to Bond Oracle HER2 IHC System description of normal tissue cross reactivity (refer to Table 13).
	Nonspecific immunohistochemical cross-reaction with areas of tissue necrosis	Ensure a formalin-based fixative is used and that processing schedules are suitable for the specimen undergoing testing. If possible, retest case using another block. If this is not possible, assess in conjunction with a corresponding H&E stained section, areas which show best fixation patterns.
	Drying artifact following completion of a staining run	If slides are to be placed on an overnight run it is recommended that the BOND delayed start functionality is used. Ensure that there is an adequate volume of distilled or de-ionized water available to dispense on the slides for this period to ensure the slides do not dry out.
	Sections adhered to slides with the aid of starch additives	Use unstarched slides (Leica BOND Plus Slides – product code S21.2113 or Apex BOND Slides - product code 3800040).
Tissue detached from patient/control slide(s)	Use of incorrect type of slides or inadequate draining of section	Ensure appropriate slides are used for patient/control sections (Leica BOND Plus Slides – product code S21.2113 or Apex BOND Slides - product code 3800040). Ensure slides receive adequate draining and are incubated at 12–18 hours at 37 °C (overnight). Sections which need further adherence may be incubated at 60 °C for a further hour.

Table 16. Bond Oracle HER2 IHC System Trouble Shooting Guide.

If any problems associated with the Bond Oracle HER2 IHC System fall outside the scope of the troubleshooting guide (refer to Table 16) please contact your local Leica Biosystems' Technical Services Department or Distributor for assistance.

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









Amendments to previous issue

Components Provided, Symbol Identification.

Date of issue

16 July 2020

Symbol Identification

	Batch Code		Storage		Catalog number
	In vitro diagnostic medical device		Manufacturer		Fragile
	eIFU - consult instructions for use		Contains sufficient for <n> tests		Use by YYYY-MM-DD
SN	Serial Number		Prescription only		

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