Bond™ Oracle™ HER2 IHC System

Interpretation Guide – Breast Tissue

Living up to Life

Leica MICROSYSTEMS

Bond™ Oracle™ HER2 IHC System

Interpretation Guide

For use on Leica Microsystems' 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

For additional information and interactive learning exercises, log-on to:

www.leica-microsystems.com/TA9145-elearning

Contents

Introduction

Bond Oracle HER2 IHC System

The Bond Oracle HER2 IHC System is a semi-quantitative immunohistochemical assay to determine HER2 (Human Epidermal Growth Factor Receptor 2) oncoprotein status in human breast cancer tissue 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). Decisions regarding Herceptin® treatment should be made within the context of the patient's clinical history.

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. Tissue sections are counterstained, dehydrated, cleared and mounted. Results are interpreted using brightfield 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 has been correlated to both HER2 oncoprotein receptor load per cell and HER2 gene status.

Concordance Data

Reliable, consistent, semi-quantitative HER2 clinical data requires a highly reproducible assay that is precision manufactured as a complete system. External Quality Assessment for HER2 immunohistochemistry continues to indicate the benefits of using a standardized system verses an alternative in-house developed assay. Extensive testing within runs, between runs, between laboratories and between observers has shown exceptionally high reproducibility rates for the Bond Oracle HER2 IHC System. Complete component manufacture coupled with full Bond Automation has enabled improved standards in batch-to-batch manufacturing consistency and assay precision (see Bond Oracle HER2 IHC System Instructions for Use for clinical, precision and reproducibility data breakdown).

How does Oracle compare?

Assay	Dako HercepTest	**Leica Bond Oracle HER2 IHC System (CB11)**	Ventana Pathway (CB11)	Ventana Pathway (4B5)
Comparative Device	Herceptin Clinical Trial Assay	**Dako HercepTest**	Dako HercepTest	Ventana Pathway (CB11)
Sample Size	548	**431**	450	321
2x2* Concordance	78.6%	**92.3%**	92.4%	89.4%
3x3# Concordance	69.2%	**86.5%**	88.4%	80.7%

Performance characteristics *2x2: 0 and 1+ = Negative; 2+ and 3+ = Positive #3x3: 0 and 1+ = Negative; 2+ = Equivocal; 3+ = Positive

Bond Oracle HER2 IHC System Interpretation Guide

This document is provided as a guide to help scientists and pathologists achieve accurate, consistent and reproducible results.

The Interpretation Guide will familiarize you with the requirements for scoring breast carcinomas stained with the Bond Oracle HER2 IHC System and interpretation of the Oracle HER2 Control Slides.

The Interpretation Guide includes:

- Technical tips for ensuring high-quality HER2 staining and efficient throughput in your laboratory
- A review of the Bond Oracle HER2 IHC System Instructions For Use
- Guidance for interpretation of the Oracle HER2 Control Slides
- Examples of varying HER2 expression levels in human breast carcinomas

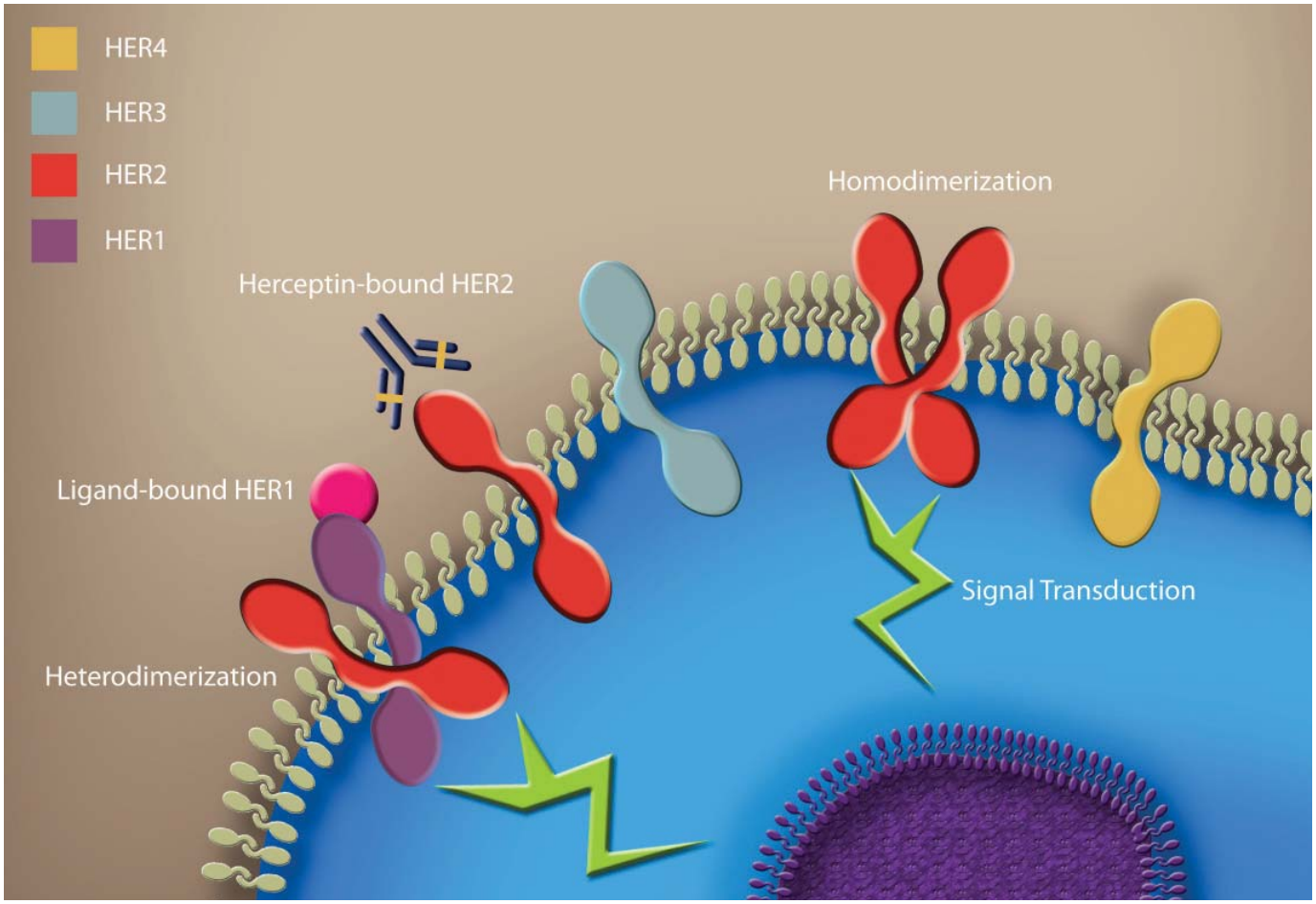
Consultation and continued review of the Bond Oracle HER2 IHC System Interpretation Guide provides a solid foundation for evaluating slides stained with the Bond Oracle HER2 IHC System.

HER2 Overview

HER2, encoded by the c-erb-B2 gene, is one of four oncoproteins belonging to the Human Epidermal Growth Factor Receptor (HER1-4) family of tyrosine kinases and is overexpressed in 10–20% of invasive breast cancer cases[1,2,3]. Members of the HER family of receptors form ligand-mediated homo and heterodimers, where HER2 is the preferred partner for dimerization[4].

Dimerization of the HER family receptors initiates autophosphorylation cascades which in turn activate multiple cellular signalling pathways, including the Ras/Raf/MAPK and P13K/Akt cascades[5]. The resulting modification of gene transcription pathways has been shown to affect processes as diverse as cell division, angiogenesis, motility and adhesion[5].

Overexpression of HER2 leads to excessive activation of these pathways and may contribute to more aggressive growth associated with these tumor cells[6]. HER2 overexpression is associated with poor prognosis, including reduced disease-free survival and resistance to certain chemotherapeutic agents[7].



References

1 Pawlowski V, Revillion F, Hebbar M. et al. Prognostic value of the Type I growth factor receptors in a large series of human primary breast cancers quantified with a real-time reverse transcription-polymerase chain reaction assay. ClinCancer Res. 2000 Nov(6): 4217-4225.

2 Lonardo F, Di Marco E, King CR, et al. The normal erbB-2 product is an atypical receptor-like tyrosinase kinase with constitutive activity in the absence of a ligand. New Biologist. 1990; 2:992-1003.

3 Walker RA, Bartlett JMS Dowsett M, Ellis IO, Hanby AN, Jasani B, Miller K and Pinder SE. HER2 Testing in the UK- Further Update To Recommendations. Journal of Clinical Pathology 2008

4 Piccart-Gebhart MJ, Procter M, Leyland-Jones B et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med. 2005 Oct 20; 353(16): 1652-4.

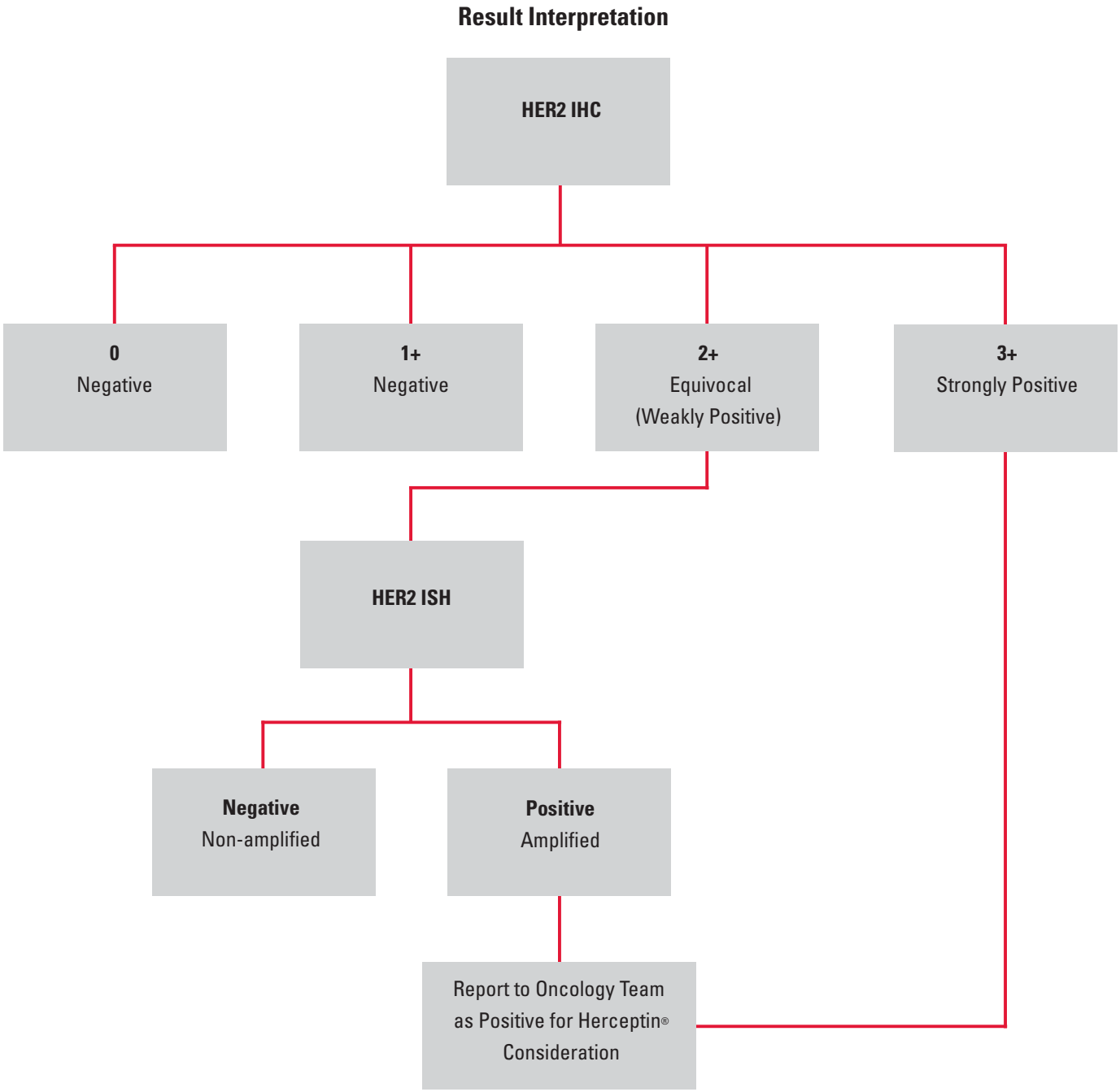
5 Yarden Y, Sliwkowski MX Untangling the ErbB signalling network. Nat Rev Mol Cell Biol. 2001 Feb; 2 (2): 127-37.

6 Graus-Porta D, Beerli RR, Daly JM, Hynes NE. ErbB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signalling. EMBO J. 1997 Apr 1; 16(7): 1647-55.

7 Perez EA, Suman VJ, Davidson NE, et al, HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 intergroup adjuvant trial. J Clin Oncol. 2006 Jul 1;24(19): 3032-8.

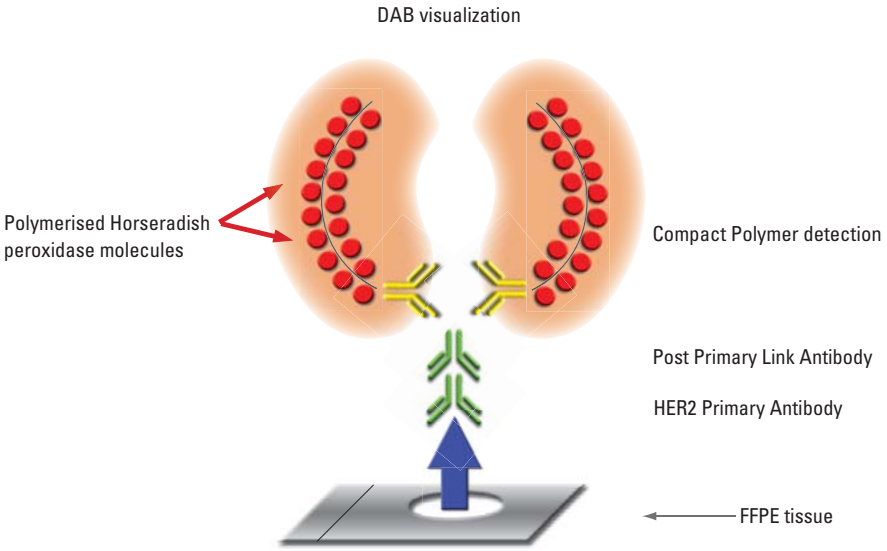
HER2 Testing Algorithm

First line assessment of patient samples using the Bond Oracle HER2 IHC System is used to determine HER2 oncoprotein levels at expression levels of 0, 1+, 2+ and 3+ immunohistochemical (IHC) staining intensities. Cases exhibiting weakly positive (2+) staining may be considered equivocal and reflexed to in situ hybridization (ISH) testing.



HER2 Oncoprotein Expression Determined by Immunohistochemistry

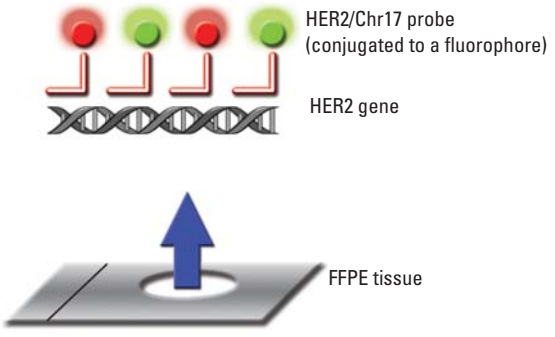
HER2 testing using IHC targets the HER2 oncoprotein located on the cell membrane. The Bond Oracle HER2 IHC System utilizes a target-specific primary antibody to label the HER2 protein. This antibody is then visualized using a multi-step Compact Polymer detection system.



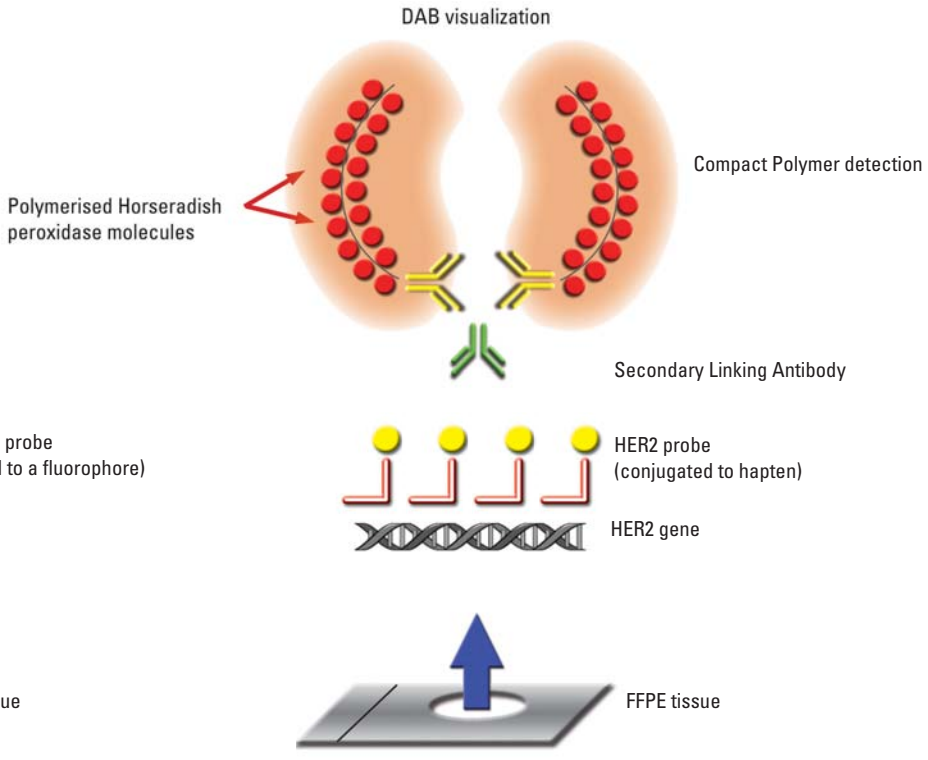
HER2 Gene Status Determined by In Situ Hybridization

Cases found to be equivocal (2+, weakly positive) using the Bond Oracle HER2 IHC System may be further evaluated for HER2 gene status by in situ hybridization (ISH). ISH techniques utilize HER2 labeled probes, detected fluorescently or through chromogenic visualization, to assess gene amplification status. Chromosome 17 signal enumeration to enable HER2 gene ratio is also performed utilizing these labelling techniques.

Fluorescent-based



Chromogenic-based



Bond Oracle HER2 IHC System Components

Peroxide Block

A peroxide block is used in IHC techniques to block endogenous peroxidase within the tissue section. This is important as endogenous peroxidase may cause non specific background by association with the horseradish peroxidase (HRP) of the polymer components of the detection system.

HER2 Negative Control

The Bond Oracle HER2 IHC System contains a ready-to-use mouse IgG at an equivalent concentration to the HER2 Primary Antibody.

It is important to use a negative control antibody on each patient case to confirm the lack of detection system cross-reactivity to specifically targeted cells/cellular components.

HER2 Primary Antibody

The Bond Oracle HER2 IHC System contains the anti-HER2 antibody, clone CB11 affinity purified, mouse-monoclonal, in a fully optimized, ready-to-use format. Clone CB11, originally developed by Corbett et al, manufactured exclusively by Leica Biosystems Newcastle Ltd, is directed against the internal domain of the HER2 oncoprotein.

Performance monitoring of the Bond Oracle HER2 IHC System in an FDA compliant clinical trial has shown a high degree of concordance to the Dako HercepTest, using recommended commercial interpretation guidelines.

Oracle 2x2 Result	Dako 2x2 Result		
	Negative	Positive	Total
Negative	269	23 *	292
Positive	10	129	139
Total	279	152	431

* Concordance of the Bond Oracle HER2 IHC System to HER2 gene amplification status, as assessed by fluorescent in situ hybridization (Vysis PathVysion), showed 100% concordance to FISH for cases in the highlighted critical positive to negative population. NO gene amplified cases were identified in this subgroup.

Compact Polymer technology

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 Oracle product range, the problem of nonspecific endogenous biotin staining, which may be seen with streptavidin/biotin detection systems, does not occur.

DAB visualization

Chromogen and Substrate Buffer combine in a reaction catalyzed by the polymerized enzymes to produce a brown precipitate which is viewed by brightfield microscopy.

Hematoxylin

Hematoxylin nuclear counterstain for IHC assessment of HER2 expression should be light; excessive counterstain can obscure staining results and make interpretation difficult.

HER2 Control Slide

A cornerstone of the Bond Oracle HER2 IHC System, the Oracle HER2 Control Slides, contain four formalin-fixed, paraffin-embedded human breast cancer cell lines expressing the HER2 oncoprotein at staining intensities of 0, 1+, 2+ and 3+. The cells are routinely processed using Leica Peloris™ processing technology to ensure consistent manufacturing from batch to batch.

The HER2 control cell lines are designed as procedural controls, confirming procedural accuracy of the Bond Oracle HER2 IHC System. They validate:

- Reagent optimization and assay performance
- Correct protocol implementation
- Bond Instrument performance

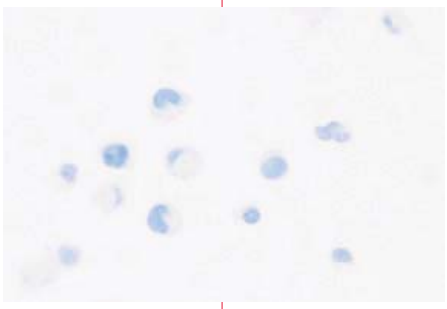
Oracle HER2 Control Slide

Oracle HER2 Control Slide Profiling

Levels of HER2 expression and their associated staining patterns for the HER2 control cell lines are represented below.

The MDA-MB-231 cell line (0) represents the equivalent level of expression found in the normal ductal epithelium.

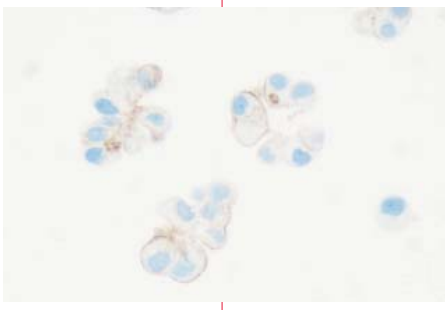
0

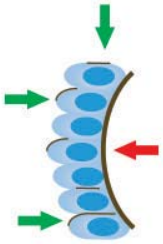


MDA-MB-231

No staining in the MDA-MB-231 cell line (0)

1+

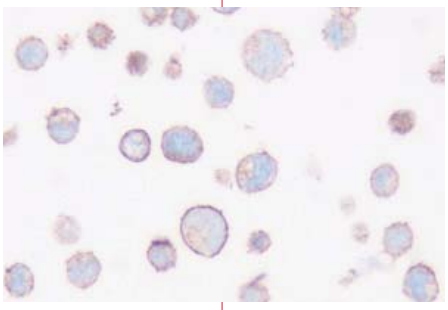




MDA-MB-175

Presence of faint/barely perceptible, brown, incomplete cell membrane staining in the MDA-MB-175 cell line (1+)

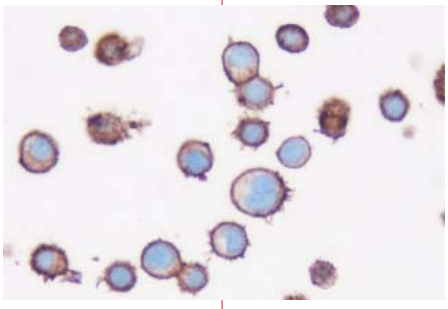
2+



MDA-MB-453

Presence of weak to moderate, brown, complete cell membrane staining in the MDA-MB-453 cell line (2+)

3+



SK-BR-3

Presence of strong brown, complete cell membrane staining in the SK-BR-3 cell line (3+)

Important note: A feature of the MDA-MB-175 cell line (1+) 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 is stronger than that of the rest of the cell membrane.

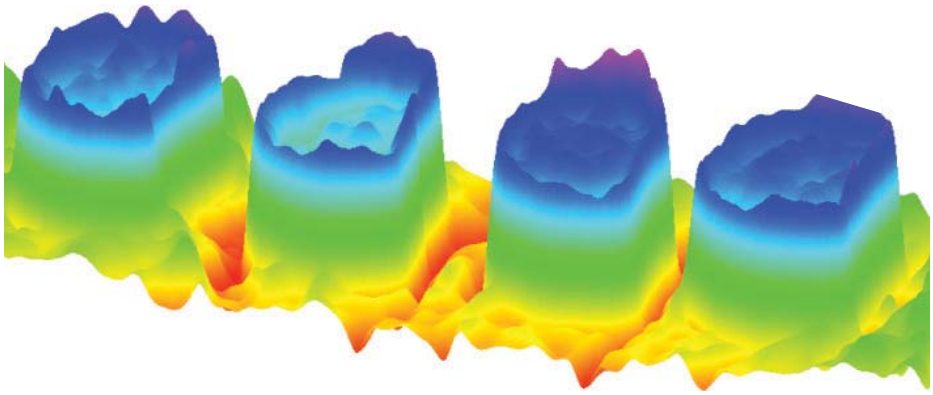
It is the faint/barely perceptible incomplete cell membrane staining that is the correct HER2 oncoprotein (1+) staining pattern.

Uniquely Consistent Control Slides

Oracle HER2 Control Slides provide a comprehensive control method for assessing consistency of assay performance utilizing a unique system of four cell lines.

The addition of a 2+ cell line provides additional confidence, by more closely monitoring the potential for assay variation.

Each Oracle HER2 Control Slide is non-destructively QC tested using a patented white light interferometry system[1]. This unique process means accurate section thickness is maintained and control slides stain consistently. This level of control is critical to achieving accurate HER2 assay validation and continuous batch performance.



3D image of Oracle HER2 control cell line spots on a glass slide as generated by white light interferometry [1]
This technique provides an accurate method for consistent Oracle HER2 Control Slides that stain reproducibly.

Cell Line Characterization Data

The Oracle HER2 control cell lines have been fully characterized for immunohistochemical profile, HER2 gene status and HER2 receptor load.

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 and Chromosome 17) FISH (Vysis PathVysion).

References:
1. Barker C, et al. Non-destructive quality control of HER2 control cell line sections: the use of interferometry for measuring section thickness and implications for HER2 interpretation on breast tissue. Accepted for publication 27 Feb 2009; AIMM

In-house Tissue Controls:

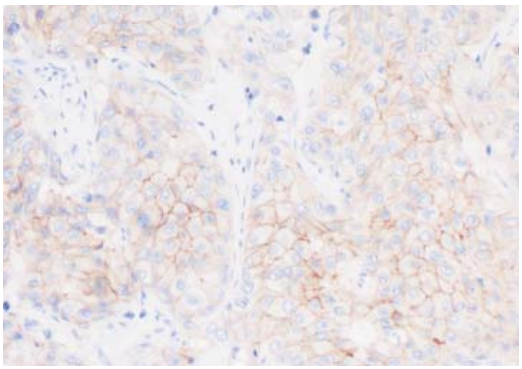
In-house tissue controls should be:

- Included in each staining run
- Biopsy or surgical samples of known HER2 status, fixed, processed and embedded in the same manner as patient samples

In-house Positive Tissue Control

Indicative of correctly prepared tissues and valid staining techniques. An ideal positive control section should demonstrate weak positive staining so as to define subtle changes in primary antibody sensitivity.

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.

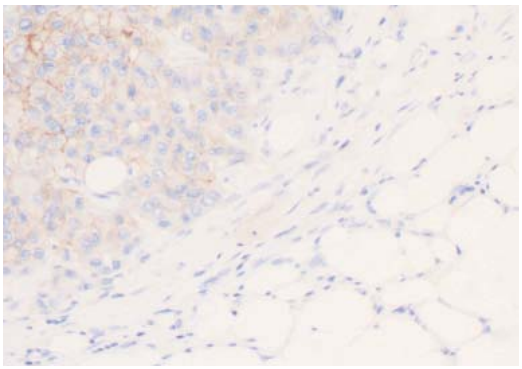


20x magnification

The in-house positive control tissue illustrated is an equivocal (2+) invasive breast tumor.

In-house Negative Tissue Control

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 in the patient specimens should be considered invalid



20x magnification

The in-house negative control component utilized in this case is adipose with stromal cells adjacent to the invasive breast tumor. The adipose and stromal cells are unstained demonstrating no cross reactivity with these normal cell components.

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

Technical Considerations and Recommendations

Specimen Handling

Procedural deviations related to sample handling and processing can compromise HER2 assay performance. Variables that may alter assay performance are:

- Specimens drying prior to fixation
- Type of fixative
- Temperature, age, storage and pH of fixative
- Length of fixation, specimen size, ratio of size to fixative volume
- Length of time in alcohol after primary fixation
- Processing time, temperature, pressure and chemicals used
- Storage of paraffin blocks
- Storage of cut sections

Fixation, Processing and Embedding

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 Section Preparation

Appropriate tissue preparation is integral to the continued performance of the Bond Oracle HER2 IHC System.

Embedded tissue specimens should be sectioned at a thickness between 3–5 µm. Overheating of tissues during embedding or sections during drying can be detrimental to immunostaining and therefore should be avoided.

The slides required for tumor verification (H&E) and HER2 oncoprotein evaluation (Bond Oracle HER2 IHC System) should be prepared at the same time. To preserve antigenicity, tissue sections mounted on slides (Leica Microsystems Plus Slides – product code S21.2113) should be stained within 4–6 weeks of sectioning when held at room temperature (20–25 °C). Following sectioning, slides should be incubated for 12–18 hours (overnight) at 37 °C. Sections that require additional adherence may be incubated at 60 °C for a further hour.

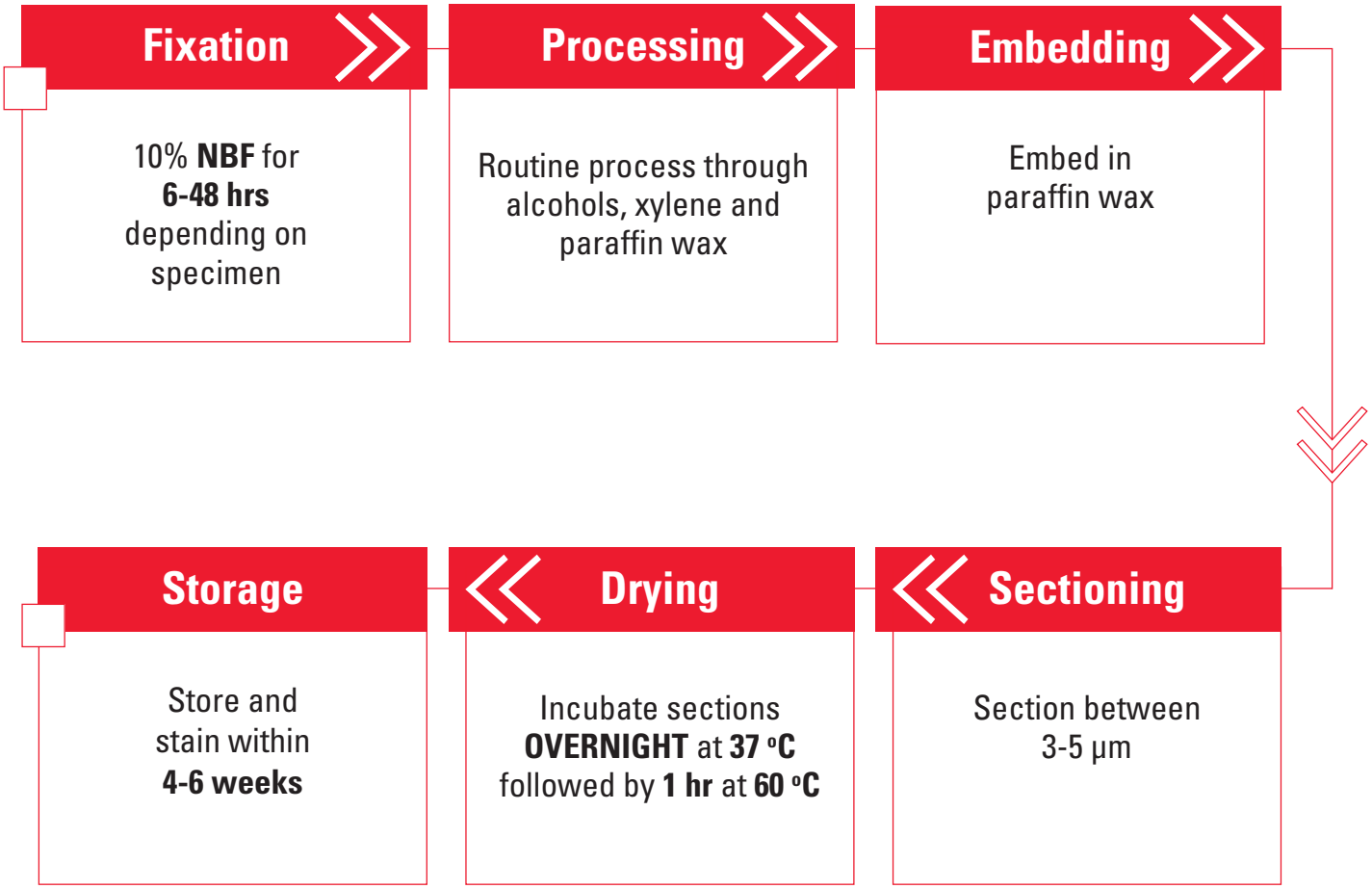
Protocol Defaults

The Bond Oracle HER2 IHC System and the Bond fully automated advanced staining system provide regulated, consistent epitope retrieval and controlled reagent incubations enabling reproducible results.

The following default settings are used with the Bond Oracle HER2 IHC System:

- Onboard dewaxing – *Dewax
- Regulated epitope retrieval – *HIER 25 min with ER1 (97)
- Controlled reagent incubations – *IHC Protocol H

For full details of the Oracle HER2 Bond IHC System protocol please consult the **Instructions for Use** document



A new Bond Universal Covertile™ should be used with each slide+

Using a new Bond Universal Covertile (product code S21.2001.110) every time will help ensure the consistency of this semi-quantitative test. The Covertile system allows gentle application and even flow of reagent across the sections to provide unmatched tissue care.

+The use of Bond Universal Covertiles which have been previously utilized for either immunohistochemical or in situ hybridization staining have not been validated with this test.

Interpretation of Oracle HER2 Staining

For the determination of HER2 oncoprotein overexpression, only membrane-staining pattern and intensity should be evaluated. A pathologist using a brightlight microscope should perform the slide evaluation. For evaluation of immunohistochemical staining and scoring, an objective of 10x magnification is appropriate. The use of 20x–40x objective magnification should be used to confirm the score. Cytoplasmic staining should be considered as non-specific staining and should not be included in the assessment of membrane staining intensity. To aid in the differentiation of 0, 1+, 2+, and 3+ staining, refer to the Bond Oracle HER2 IHC Staining Atlas for representative images of the staining intensities. Only specimens from patients with invasive breast carcinoma should be scored. In cases where carcinoma in situ and invasive carcinoma are found in the same specimen, only the invasive component should be scored.

Oracle HER2 IHC Scoring Guidelines

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

The Bond Oracle HER2 IHC System is not intended to provide prognostic information to the patient 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 to enable semi-quantitative assessment of the staining intensity of the sample tissue.

1. HER2 Control Slide
2. In-house positive control
3. In-house negative control
4. Patient tissue – HER2 Negative Control
5. Patient tissue – HER2 Primary Antibody

HER2 Expression in Normal Tissue

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
Heart	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

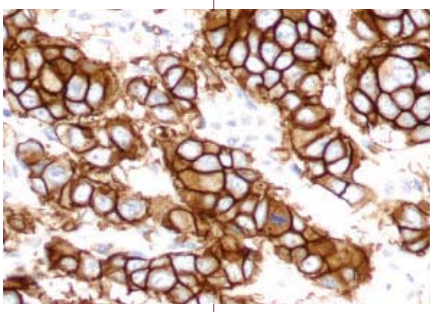
Bond Oracle HER2 IHC Staining Atlas

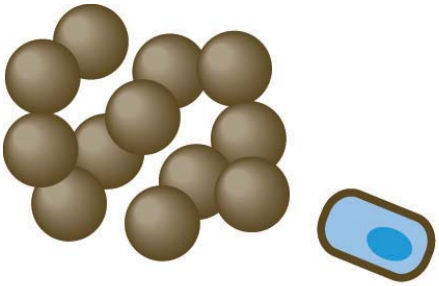
Oracle HER2 Profiling in Tumors

Levels of HER2 expression and associated staining patterns in tumor cells are represented below.

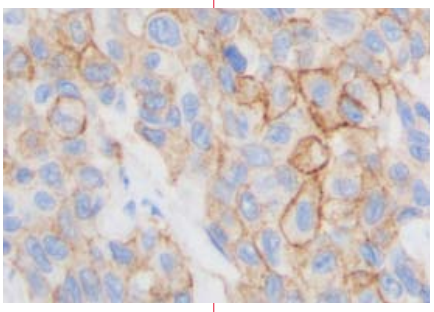
The normal benign ductal epithelium provides a baseline (0) internal control level of HER2 protein in the tumor tissue. Tumors that do not overexpress the HER2 protein will have a similar level of HER2 expression.

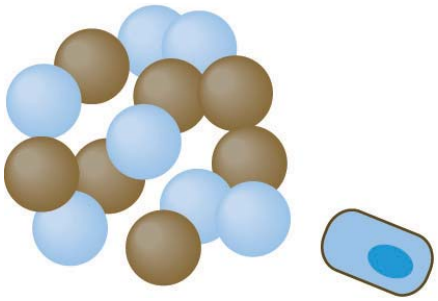
3+



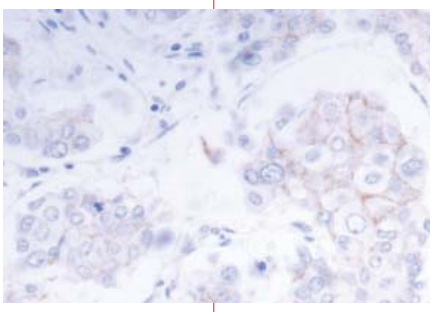


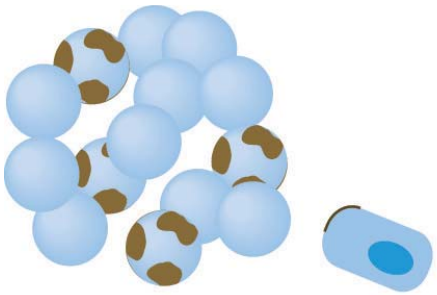
2+



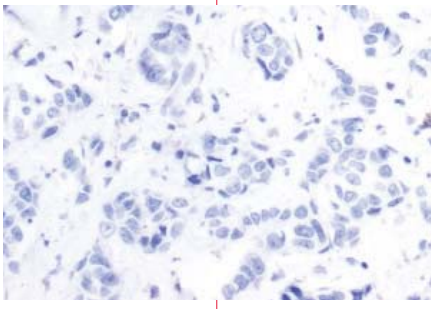


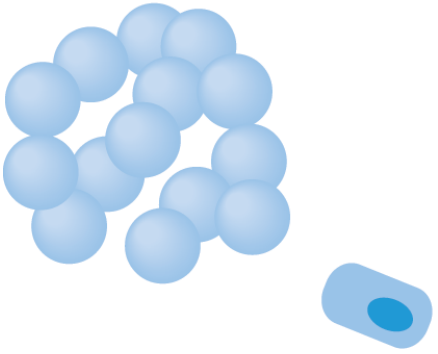
1+





0

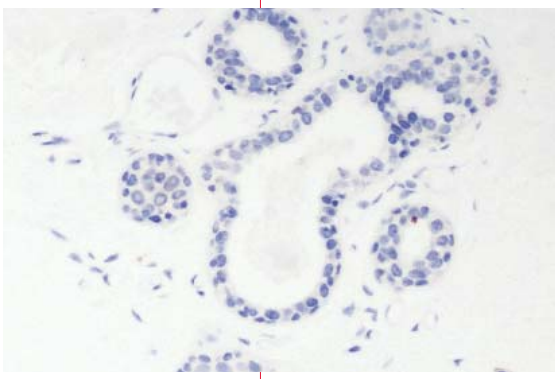




Tumor Profiling – Non-reportable Staining

Non-neoplastic Epithelium

Case 1



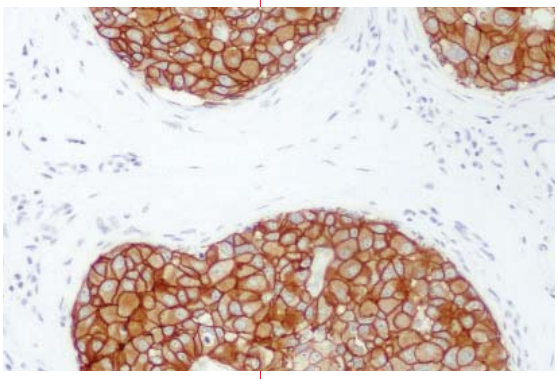
20x magnification.
Normal breast epithelium

Normal unassociated benign epithelium rarely expresses HER2. However, occasional staining of the normal ductal epithelium may be observed.

- The sensitivity of the Bond Oracle HER2 IHC System has been optimized to stain normal ductal epithelia at an intensity of 0/1+
- If the normal ductal epithelia is staining >1+, the assay should be repeated
- Cells undergoing columnar change may express HER2 at a raised level

Ductal Carcinoma In Situ (DCIS)

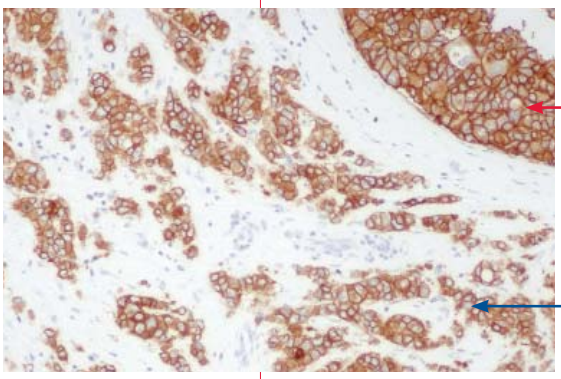
Case 2



10x magnification
DCIS breast tumor

The initial evaluation of invasive vs DCIS using H&E is an integral step in efficient HER2 testing. Despite a high percentage of DCIS cases staining positive for HER2, they should not be reported for Herceptin® immunotherapy.

Case 3



10x magnification
Breast tumor with DCIS and invasive components

For cases that contain both DCIS and invasive components, only the invasive component should be interpreted and reported for Herceptin® immunotherapy.

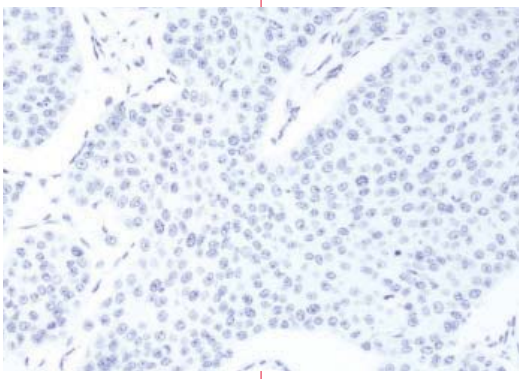
DCIS with invasive components.

Tumor Profiling - Reportable Staining

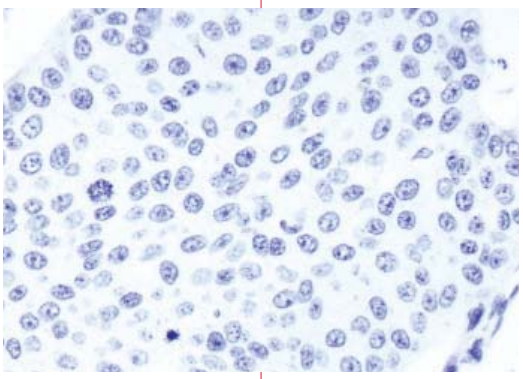
Invasive Breast Carcinoma (0)

No staining is observed or membrane staining is observed in less than 10% of the tumor cells.

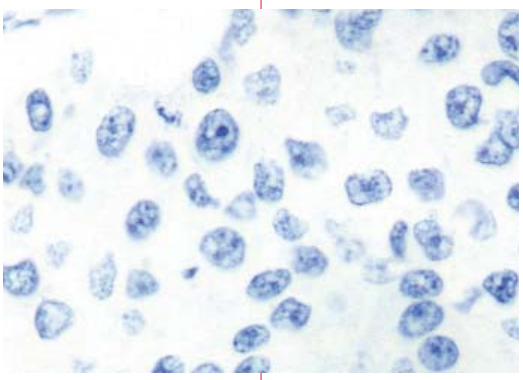
Case 1



10x magnification

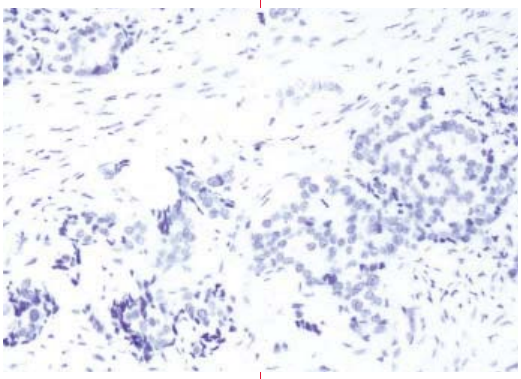


20x magnification

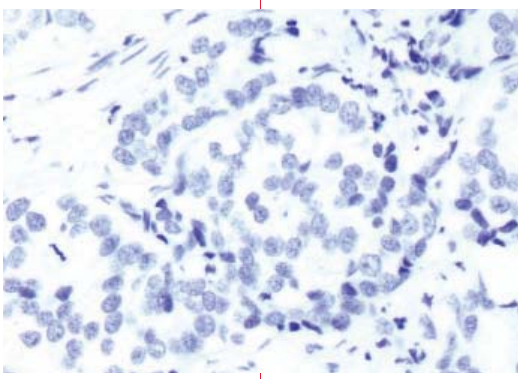


40x magnification

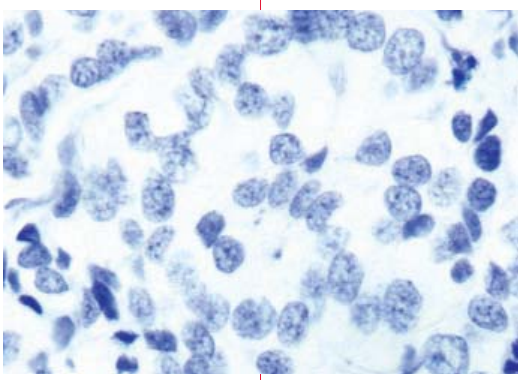
Case 2



10x magnification



20x magnification

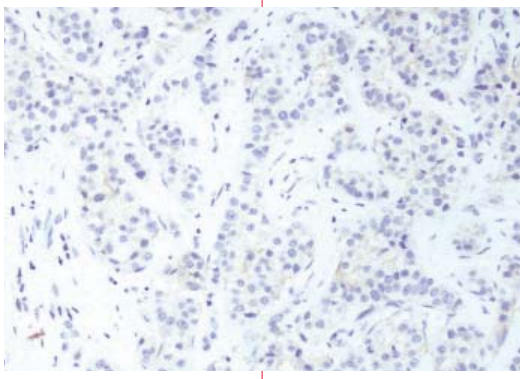


40x magnification

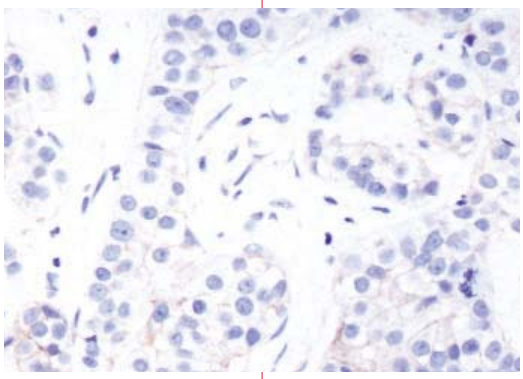
Invasive Breast Carcinoma (1+)

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.

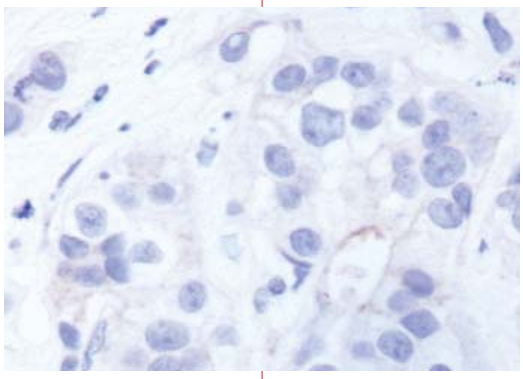
Case 3



10x magnification

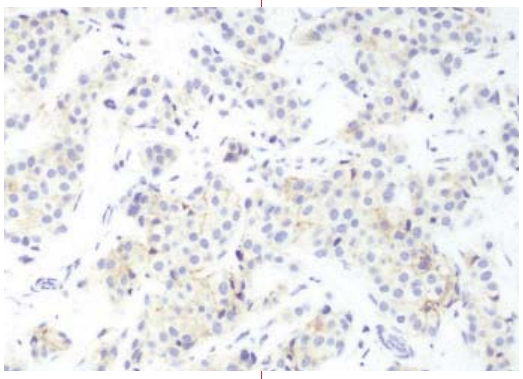


20x magnification

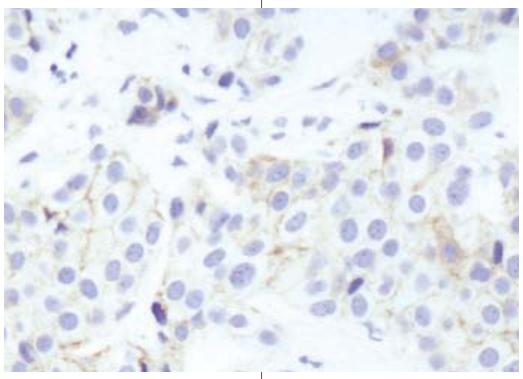


40x magnification

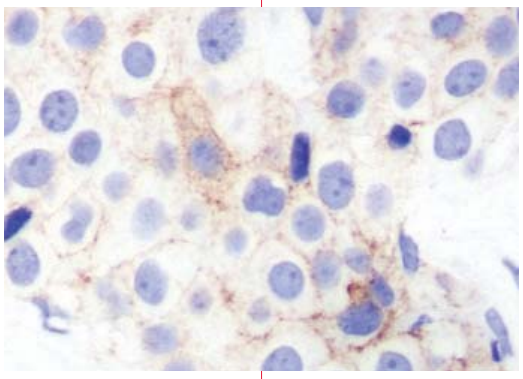
Case 4



10x magnification



20x magnification

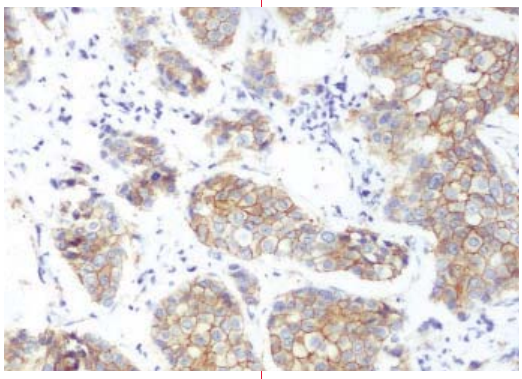


40x magnification

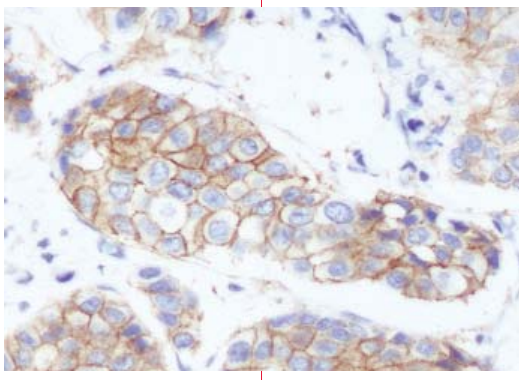
Invasive Breast Carcinoma (2+)

Weak to moderate complete membrane staining is observed in more than 10% of the tumor cells

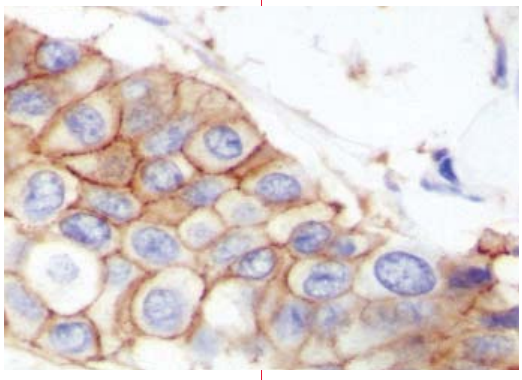
Case 5



10x magnification

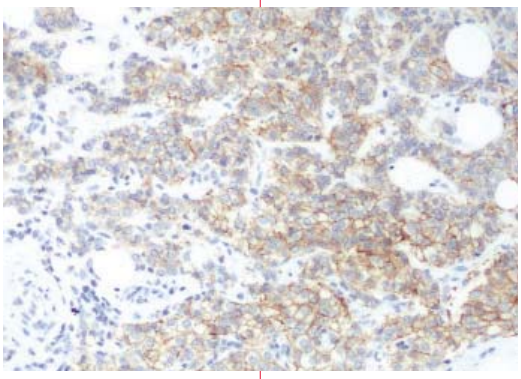


20x magnification

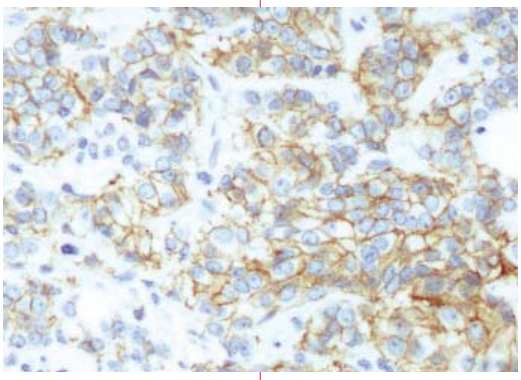


40x magnification

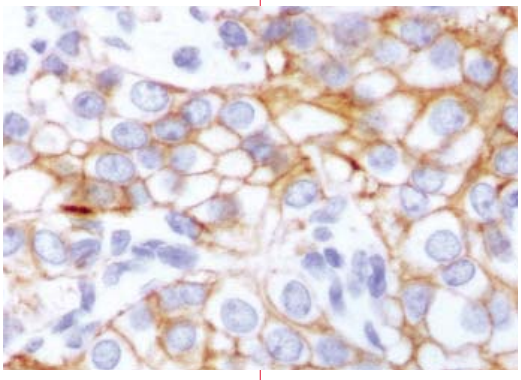
Case 6



10x magnification



20x magnification

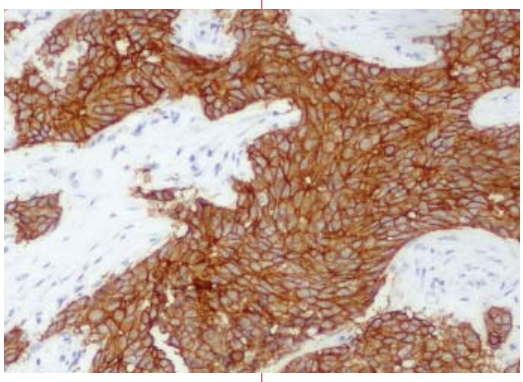


40x magnification

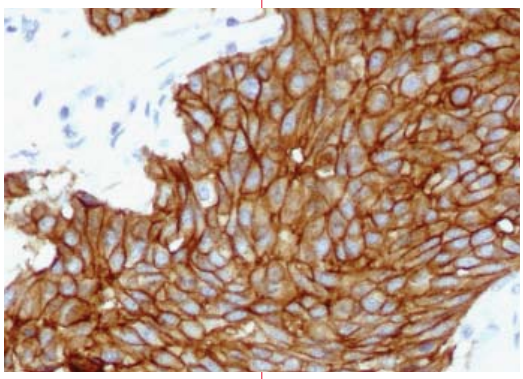
Invasive Breast Carcinoma (3+)

Strong complete membrane staining is observed in more than 10% of the tumor cells.

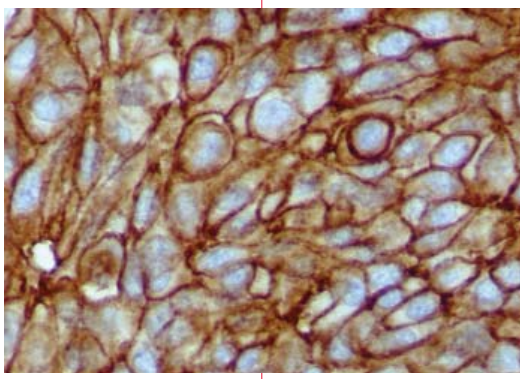
Case 7



10x magnification

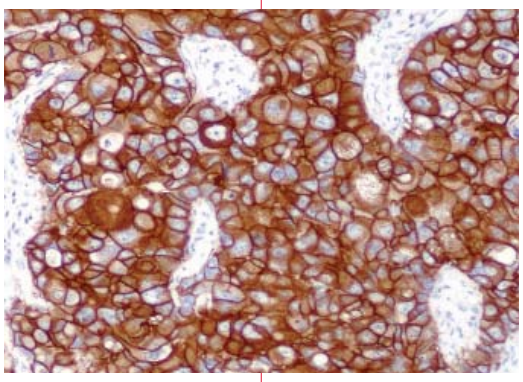


20x magnification

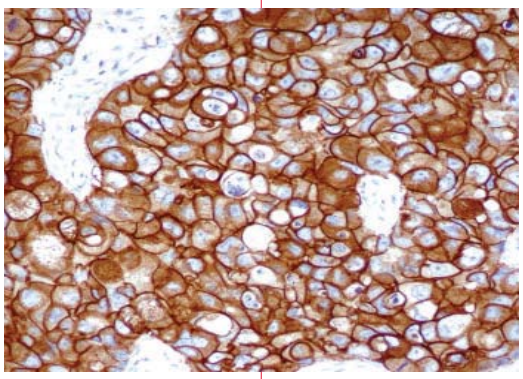


40x magnification

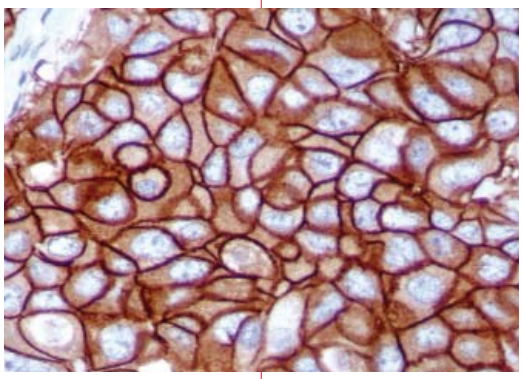
Case 8



10x magnification



20x magnification



40x magnification

Staining Artifacts

Edge Artifacts

Fixation Related Edge Artifact

Increased staining at the edge of the tissue or decreased staining observed in more centrally located tumor regions.

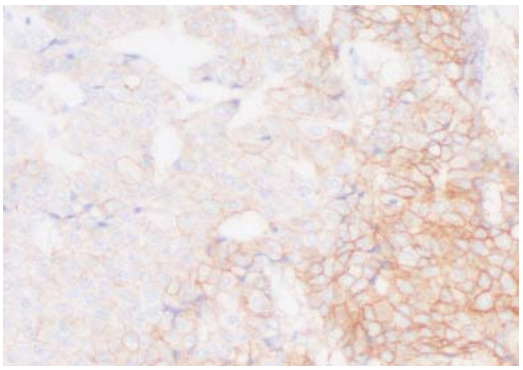
Causes

Commonly caused by inappropriate fixation of specimens during the pre-analytical phase, this artifact can be seen both in H&E and IHC preparations. Inadequate fixation and/or processing of poorly prepared samples can manifest as suboptimal staining in the central portions of the tissue relative to the periphery. This suboptimal, inadequately processed zonal staining may be misinterpreted as a false negative.

Interpretation Tips

Increased staining around the edge of a sample can be due to drying prior to fixation. Staining confined to, or signal amplified only in, the periphery of the sample should be interpreted with caution.

Decreased staining in central locations may be due to inadequate fixation. Appropriate areas for interpretation should be assessed in conjunction with a corresponding H&E stained section.



20x magnification. Breast tumor

The specimen shows increased HER2 IHC profile in tumor regions close to the periphery of the specimen, it is likely that this is due to inadequate diffusion of fixative to central regions of the tumor resulting in suboptimal protein fixation. This artifact should be interpreted with caution as loss of HER2 expression at the central core of the tumor may also be associated with cellular down regulation of HER2 associated with tumor growth.

Handling Related Edge Artifact

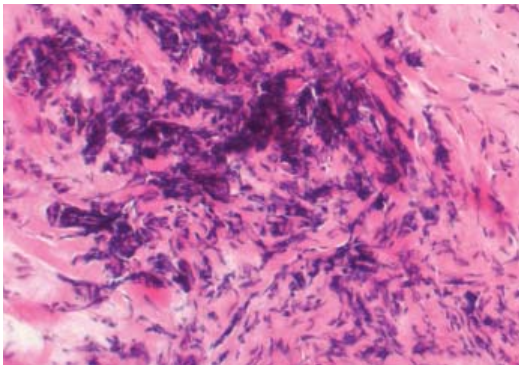
Poor cellular morphology at the edge of the tissue results in staining patterns that are difficult to interpret.

Causes

Commonly caused by inappropriate handling of specimens during the pre-analytical phase, this is most commonly seen in stereotactic needle core biopsies, this artifact can be seen both in H&E and IHC preparations.

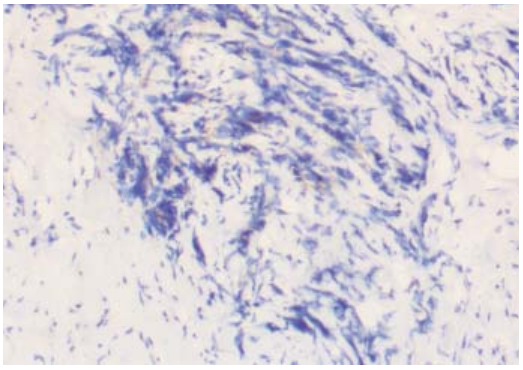
Interpretation Tips

Ensure interpretation and assessment is performed on tumor regions showing optimal morphology. Avoid areas that show crushed nuclei and cellular damage.



20x magnification. Breast tumor

Handling related edge artifact – H&E stained section illustrating crushed nuclei and damaged connective tissue morphology. Note intense deposition of hematoxylin in overlapping nuclei as a result of tissue crush damage.



20x magnification. Breast tumor

Handling related edge artifact – HER2 IHC stained section illustrating crushed nuclei and damaged connective tissue morphology. Note some DAB trapping in overlapping nuclei as a result of tissue crush damage.

Cytoplasmic Staining or Dot Artifact

Staining specific to the cytoplasm.

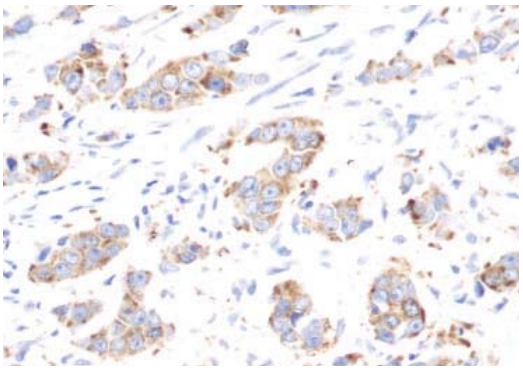
Causes

Cytoplasmic elements of HER2 staining may be observed when:

- The specimen shows high levels of expression of the HER2 protein. Low level cytoplasmic staining is commonly observed when membrane intensity is 3+.
- Case specific cytoplasmic staining or dot-like staining may be observed. This staining pattern may be present in the absence of membrane staining.

Interpretation Tip

The correlation between cytoplasmic staining response to Herceptin therapy has not been determined. Only the membrane component of the HER2 immunohistochemical stain should be interpreted. Appropriate areas for interpretation should be assessed in conjunction with a corresponding H&E stained section.



20x magnification. Breast tumor

Oracle HER2 IHC stained slide illustrating specific cytoplasmic staining in the absence of membrane staining: HER2 score 0.

Thermal Artifact

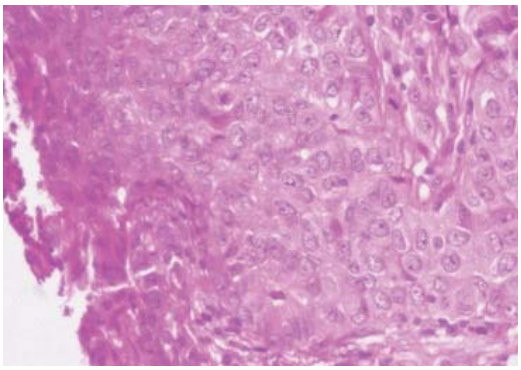
On tissue dissection this artifact presents as hard tissue, often at the peripheral margins of the resected specimen.

Causes

Thermal artifact may be seen as a result of the use of high temperature/laser based techniques during a range of surgical and histological procedures.

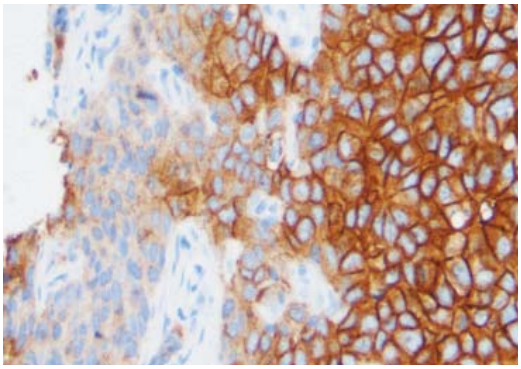
Interpretation Tips

Heat induced cellular damage may be observed under H&E evaluation and presents as condensed and acidophilic staining with a loss of cellular detail. Interpretation of immunohistochemical staining within these areas should be avoided.



20x magnification. Breast tumor

The specimen shows enhanced acidophilic staining at the periphery resulting in increased staining with the acid dye eosin. The specimen also shows a poor affinity for hematoxylin with little or no nuclear detail visible.



20x magnification. Breast tumor

The specimen shows thermal damage to the HER2 protein at the periphery of the specimen (this area of tumor should not be interpreted). The remainder of the tumor shows good HER2 protein preservation and is interpreted as 3+.

Retraction Artifact

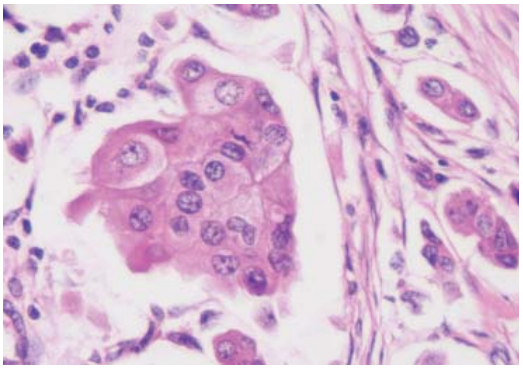
Retraction artifact is the separation of tumor islands from surrounding stromal tissue, resulting in aggregation or non-specific binding of chromogen at the detached cellular surfaces.

Causes

Retraction artifact is generally caused by inadequate fixation and processing of the tissue sample. However, this artifact should be interpreted with caution. In specific cases loss of cellular adhesion due to an underlying pathology, for example, loss or down regulation of cellular adhesion molecules, may result in separation of tumor from surrounding structures mimicking this artifact.

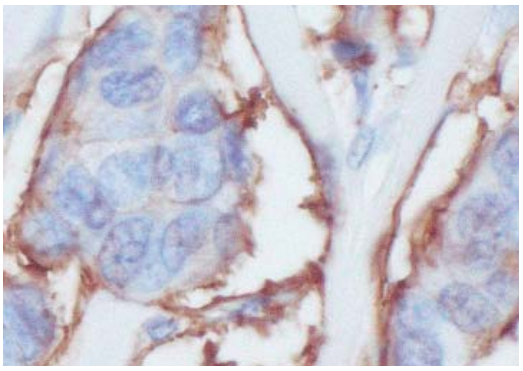
Interpretation Tip

In IHC stained sections, retraction artifact often presents as diffuse large deposits adhering to both stromal and adjacent tumor cell surfaces.



40x magnification. Breast tumor

H&E stained section showing retraction artifact, note the detachment of tumor cells from surrounding stromal surfaces

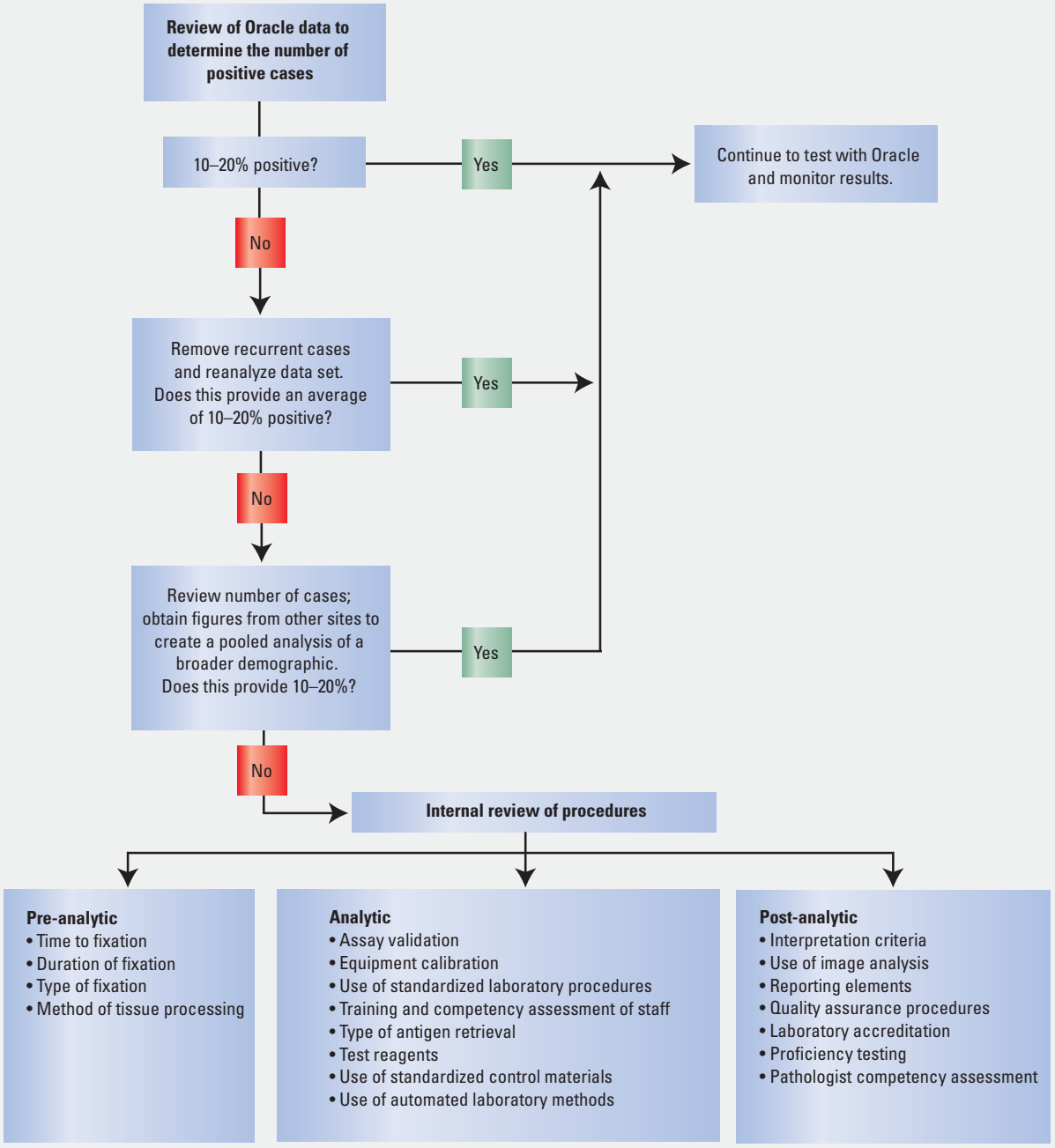


40x magnification. Breast tumor

Oracle HER2 IHC staining showing non-specific aggregation of DAB between retracted surfaces.

Data Monitoring System

Each laboratory performing Bond Oracle™ HER2 IHC System testing should monitor its rate of positivity. If the positive rate is outside the 10–20% range, a review of interpretation and technical procedures should be undertaken.



Leica Bond Oracle™ HER2 IHC System – Training Checklist

Trainee Name:_____ **Institution**_____

Equipment for Training

- ❑ Bond Oracle HER2 IHC System
- ❑ BOND Ancillary reagents and consumables
  - ❑ BOND ER1
  - ❑ BOND Wash
  - ❑ BOND Dewax
  - ❑ BOND Universal Covertiles

- ❑ Bond Oracle HER2 IHC System Instructions for Use
- ❑ Bond Oracle HER2 IHC System Interpretation Guide
- ❑ Bond Oracle HER2 IHC System Scoring Guide
  These can be downloaded from website: www.leica-microsystems.com/TA9145-IFU:
- ❑ Sufficient tissue of known HER2 status for procedural and interpretation training

Before Training

- ❑ Trainee has completed Bond Oracle HER2 IHC System e-Learning module

www.leica-microsystems.com/TA9145-elearning Certificate number: _____

- ❑ The Leica BOND instrument software version: _____
- ❑ Leica BOND instrument date of last PM: _____
- ❑ The Leica BOND instrument is equipped with a new/clean FTP probe
- ❑ There is a cleaning schedule for the Leica BOND instrument

Storage of Reagents

- ❑ Storage of Bond Oracle HER2 IHC System at 2-8 °C
- ❑ Check storage of bulk reagents
  - ❑ ER1 at 2-8 °C
  - ❑ Bond Wash at 2-8 °C
  - ❑ Dewax at 18-25 °C
- ❑ The lab uses de-ionised H_2O

Tissue Handling, Fixation and Processing

- ❑ Appropriate formalin fixation and processing schedule (see IFU)
- ❑ Sectioning at 3-5 µm
- ❑ Sufficient drying and adherence

Procedure

- ❑ Check Bulk reagents, hazardous and non-hazardous waste
- ❑ Register Bond Oracle HER2 IHC System components on Leica BOND
- ❑ Set up label profiles
- ❑ Set up a case
- ❑ Slide set up and optimal layout for the most efficient usage of the system
  Reference: Table 3 Bond Oracle IHC System IFU

Quality Control

- ❑ The importance of positive and negative control components
- ❑ The importance of in-house tissue controls
- ❑ The importance of using control cell lines (highlighting 1+ and 2+ cell lines)
- ❑ The importance of always using new BOND Universal Covertiles

Interpretation - Use slides stained in training run (on a pre-stained set)

- ❑ Slide Screening Order Rationale – the order in which to screen slides (see IFU)
- ❑ Review of Table 4: (IFU) "Interpretation of HER2 staining"
- ❑ What to expect of the Bond Oracle HER2 IHC System Control Cell Slides
  - ❑ Special attention to brush border staining of 1+ control cell line
- ❑ Interpretation of test slides:
  1. Invasive v DCIS?
  2. What percentage of the tumor stains?
  3. What is the intensity?
  4. Artifacts?

See IFU, Interpretation Guide and Scoring Guide

Signed on behalf of Leica Microsystems (Trainer):

……(Signature)

……(Name and Title)

……(Date)

Signed by End User (Trainee):

……(Signature)

……(Name and Title)

……(Date)

Please keep a copy of this checklist and the e-Learning certificate for each trainee for your training records

Leica Microsystems operates globally in four divisions, where we rank with the market leaders.

Life Science Division

The Leica Microsystems Life Science Division supports the imaging needs of the scientific community with advanced innovation and technical expertise for the visualization, measurement, and analysis of microstructures. Our strong focus on understanding scientific applications puts Leica Microsystems' customers at the leading edge of science.

Industry Division

The Leica Microsystems Industry Division's focus is to support customers' pursuit of the highest quality end result. Leica Microsystems provide the best and most innovative imaging systems to see, measure, and analyze the microstructures in routine and research industrial applications, materials science, quality control, forensic science investigation, and educational applications.

Biosystems Division

The Leica Microsystems Biosystems Division brings histopathology labs and researchers the highest-quality, most comprehensive product range. From patient to pathologist, the range includes the ideal product for each histology step and high-productivity workflow solutions for the entire lab. With complete histology systems featuring innovative automation and Novocastra™ reagents, Leica Microsystems creates better patient care through rapid turnaround, diagnostic confidence, and close customer collaboration.

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The Leica Microsystems Medical Division's focus is to partner with and support surgeons and their care of patients with the highest-quality, most innovative surgical microscope technology today and into the future.

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For detailed contact information about European sales offices or distributors please visit our website.

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Total Reagent Solutions

Leica HER2 FISH System for BOND™

The Leica HER2 FISH System fully automates PathVysion®* Dual HER2 FISH probes, creating an easy, efficient and accurate assessment of HER2 status in breast cancer.

- Easy – reduce errors and lower repeat rates
- Efficient – Lean workflows for maximum productivity and minimal costs
- Accurate – deliver diagnostic confidence

Novocastra™ Reference Range™

The Reference Range is a group of superior products selected and organized to assist pathologists and scientists in choosing the ideal antibodies for optimal tissue staining. Reference Range breast markers include:

- Cytokeratin 20 (clone RW31)
- Estrogen Receptor (clone 6F11)
- Ki67 Antigen (clone MM1)
- P53 Protein (DO-7) (clone DO-7)
- P63 Protein (clone 7JUL)
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TOTAL HISTOLOGY

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