

ASCO-CAP Guidelines for Breast Predictive Factor Testing

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Agenda

- Why ASCO-CAP guidelines for breast predictive factors?
- Parallels between the ER Guideline and the current HER2 Guidelines
- Elements of the Guidelines and strategies for implementation
 - Pre-analytic elements
 - Analytic elements
 - Post-analytic (Interpretation) elements
- Summary

Why Guidelines for Breast Predictive Tests?



Defining Factors

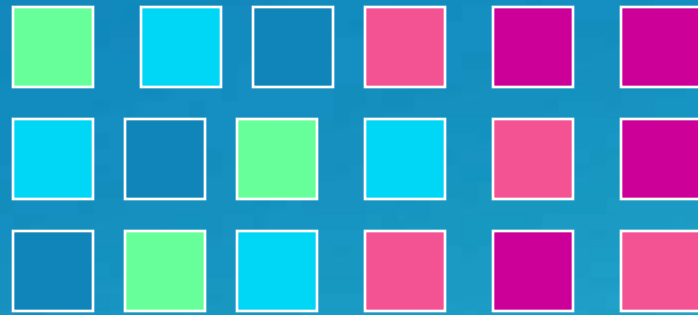
1. Results published from adjuvant breast cancer trials demonstrate great benefit of HER2 targeted therapy for early stage breast cancer patients
2. Results from the same trials showed significant variation in local results of HER2 testing
3. Current quality assurance methods have not impacted testing variation
4. Same issues have long applied to ER and PR testing

Treatment Reality

- HER2 targeted therapy substantially increases the likelihood of an objective response and overall survival for patients with metastatic HER2-positive breast cancer
- The relative risk of recurrence is decreased by about 50% when HER2 targeted therapy is added to adjuvant cytotoxic chemotherapy in patients with HER2-positive breast cancer
- Only patients with ER positive breast cancer respond to hormonal manipulation, but the responses are long lasting and treatments are better tolerated than chemotherapy

Breast Cancer Diversity

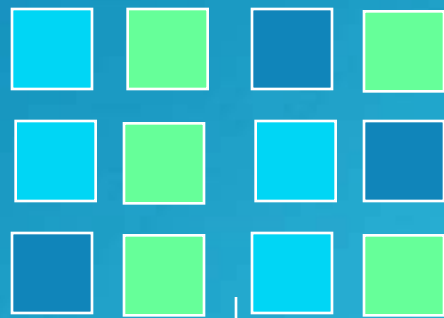
Breast Cancer Patients



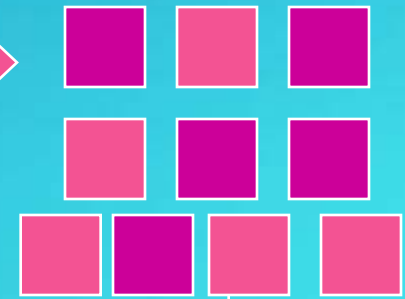
Various Disease Subtypes
Clinical Diversity

Known Breast Cancer Biomarkers

Accurate identification - subsets for therapy



Assessment of tumor biology and molecular drivers of disease progression



Potential targets for therapy



Accurate Predictive Tests Provide Maximum Benefit to Patients

- ER and HER2 testing are more like doing a frozen section than like looking at a special stain: **a single observation leads to a treatment decision**
- **The test is assumed to be accurate and precise every time by both clinician and patient**



Guideline Process

- Teams of experts from ASCO and CAP convened after co chairs defined agenda
- Experts also included from labs, industry, regulators, patient advocates
- One day meeting defined elements of each guideline
- Writing committees developed drafts
- Guidelines approved by both organizations

What are the Guideline Elements?



HER2 Guideline Elements

1. Algorithm for appropriate HER2 testing

- Which tests should be used in various circumstances?
- How should the tests be reported to provide clear guidance about actions required?

HER2 Guideline Elements

2. QA elements will be specified and monitored

- Specimen handling requirements
- Lab validation requirements
- Lab testing requirements
- Test reporting requirements



HER2 Guideline Elements

3. Laboratories and pathologists will be evaluated continuously
 - Lab accreditation
 - Mandatory proficiency testing
 - Ongoing pathologist competency assessment

Algorithm for HER2 Testing

Three categories of test results for each test

- Positive
 - IHC HER2 protein expression of 3+ or
 - FISH HER2 gene/CEP17 ratio of 2.2 or greater or
 - FISH HER2 gene copy number of >6.0
- Equivocal
 - IHC HER2 protein expression of 2+ or
 - FISH HER2 gene/CEP17 ratio of 1.8-2.2 or
 - FISH HER2 gene copy number of 4.0-6.0
- Negative
 - IHC HER2 protein expression of 1+,0 or
 - FISH HER2 gene/CEP17 <1.8 or
 - FISH HER2 gene copy number of <4.0



Caveats

- Algorithm defined by review of literature and expert opinion of panel members
- Depends on assurance that laboratory has established concordance rate between FISH and IHC of at least 95% for both positive and negative categories
- Laboratory must be accredited and participate in mandatory PT

Caveats (cont)

- HER2 IHC category of 3+ refined to assure that 95% would be FISH amplified if tested
 - 30% of cells must show homogeneous dark circumferential staining
 - FDA scoring system required only 10%
 - FDA will accept data from panelists labs to justify change in labeling requirement
 - One published study and one abstract presentation have justified this change

Caveats (cont)

- Definition of equivocal FISH category was controversial
 - Based on manufacturer guidance in training materials
 - Acceptable to FDA who will modify labeling
 - Little data exists about patient outcomes for this group
 - Data from previous trials has been requested to study
 - Ratio of 2.0 is still threshold for Herceptin eligibility based on clinical trials

Compare/Contrast ER and HER2 Guidelines

- Elements that are the same:
 - Pre-analytic, analytic, and post-analytic elements are similar
- Elements that are different:
 - Time to fixation more important for ER, and now must be documented
 - Internal controls for ER are critically important
 - Time of fixation can be up to 72 hours
 - Cytology samples must be fixed in formalin as well

Compare/Contrast with HER2 Guidelines

- Elements that are different (cont):
 - Threshold for positive is different, and there is no equivocal category for ER
 - Major problem with ER testing is false-negatives, not false-positives
 - Validation must be conducted against a clinically validated ER test

Elements of the ER/HER2 Guidelines

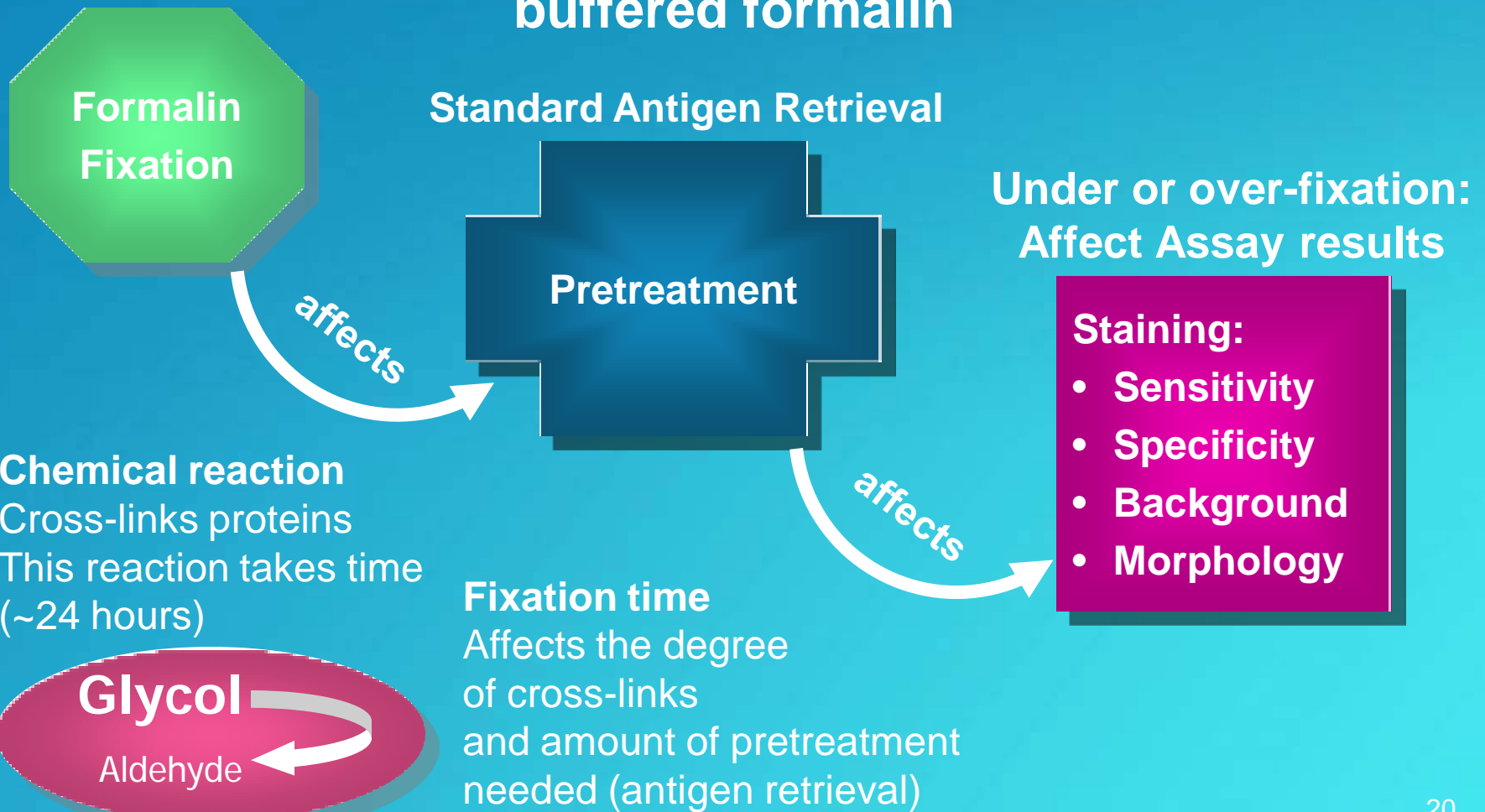
Pre-analytic Overview

- Required to keep time to fixation short (ideally, less than an hour from tissue removal to fixation)
- Required that three time points be recorded and available when you sign out the report
 - Time tissue is removed (OR staff to record)
 - Time tissue is received in grossing room
 - Time tissue is placed in fixative
- Required fixation time is 6-72 hours in neutral buffered formalin fixative without additives

Pre-Analytic Variables

Fixation, Fixation, Fixation

Recommended Fixative Breast Specimens – 10% neutral buffered formalin



Elements of the ER Guideline

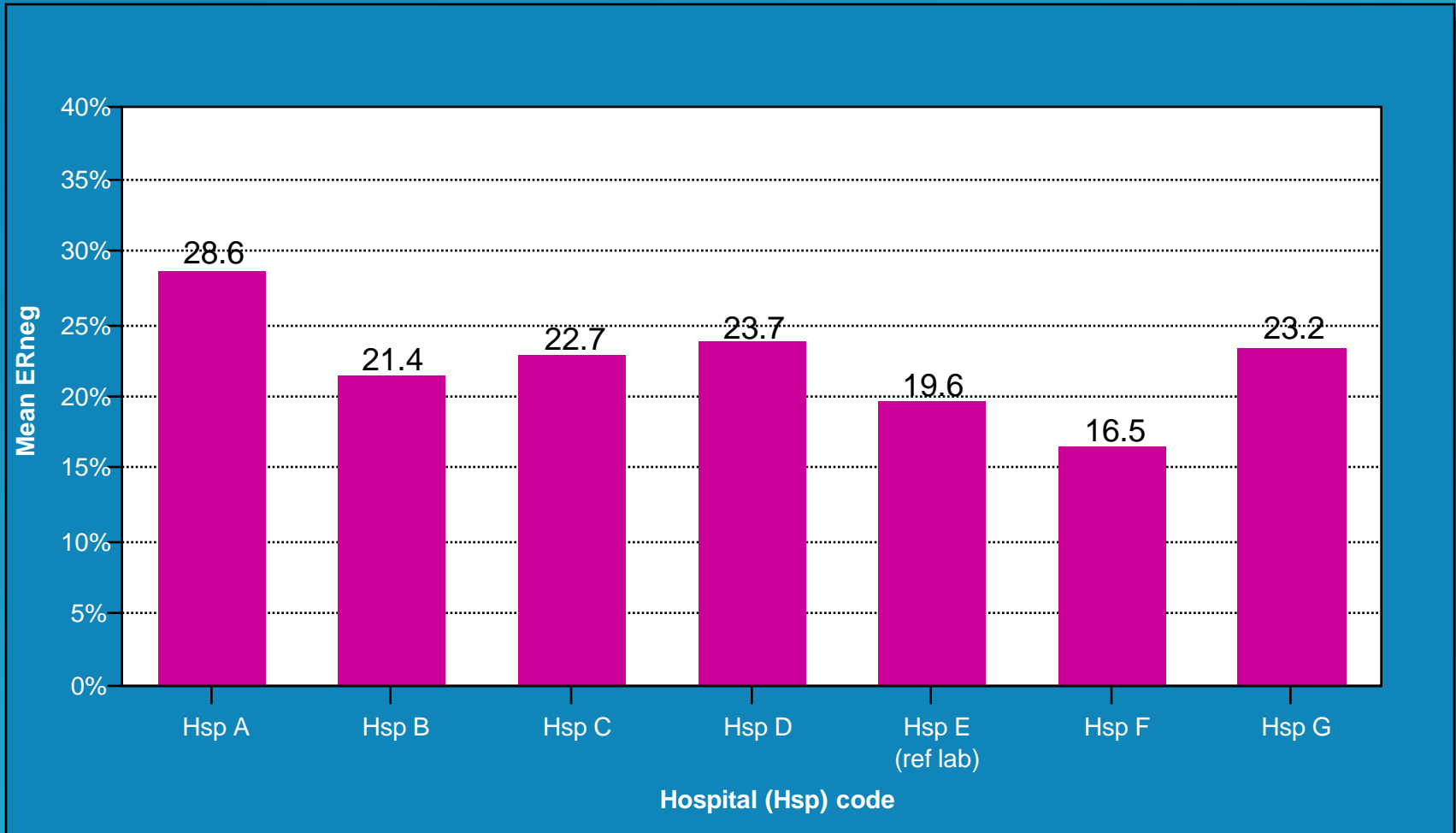
Time to Fixation

- Why is it important to keep the time to fixation to less than one hour?
 - To **prevent loss of ER/HER2 activity**, and to stop the cellular process that destroys the ER/HER2. The test begins when tissue is removed from the patient for processing!
 - Statistically significant data shows **more ER-negative results on weekend cases** when time to fixation is often delayed (see next slide for study results)
 - Testimonial: Time to fixation **can be done** in average of **18 minutes!**

Intermountain Study

- 5044 ER test results (1997-2005)
- All testing performed in central IHC laboratory with standard process, interpretation
- Data analysis controlled for age/stage/specimen type
- Significant variation in ER negative rate by facility and day of week of removal

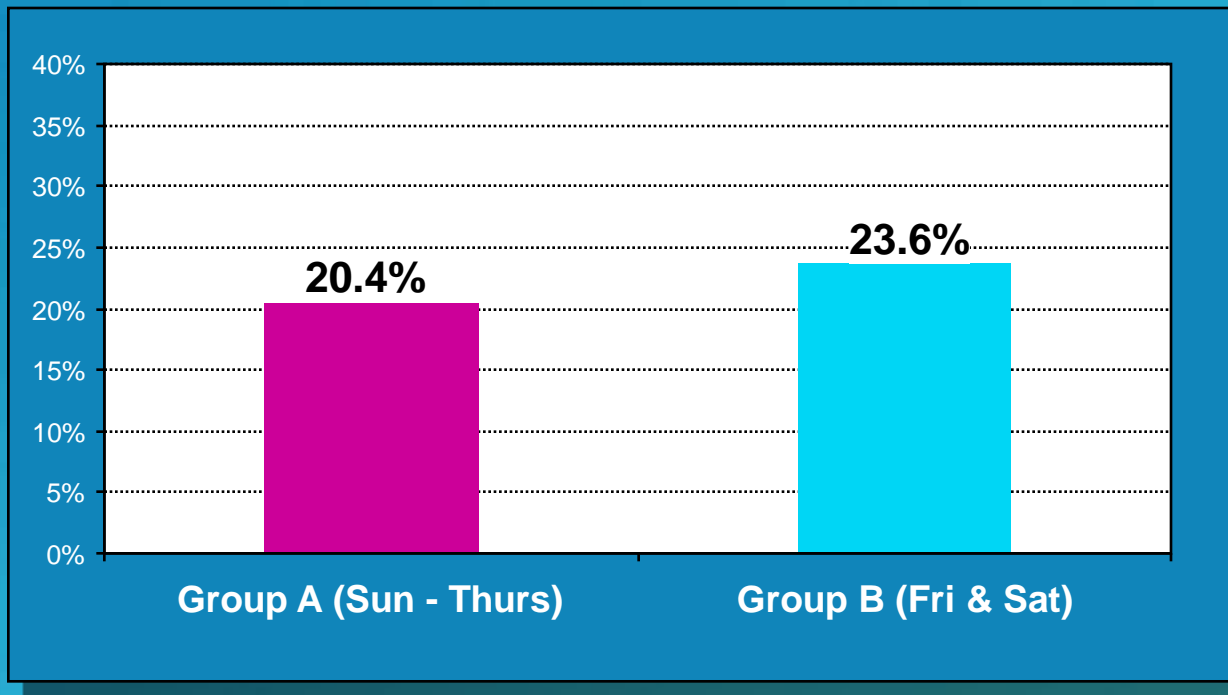
Variation in ER Negative Rate by Hospital of Origin



Elements of the ER Guideline

Time to Fixation

Variation in ER-Negative Rate by Day of Surgery



Specimens removed at the end of the week have higher ER-negative test results!

Elements of the ER Guideline

Recording Time Points

Required that three time points be recorded and available when you sign out the report so time to fixation will be known

1. Time tissue is removed (OR staff to record)
2. Time tissue is received in grossing room
3. Time tissue was placed in fixative



Follow up Study

- Prospectively studied impact of measurement of fixation parameters in 5 Intermountain facilities
 - 2 agreed to prospectively record 3 time points
 - 3 served as controls
- 1054 breast cancer patient samples evaluated
 - Ave time to fixation in test facilities was 18 min
 - ER negative rate higher in test facilities ($p=0.13$)
 - PR negative rate higher in test facilities ($p=0.01$)
- It is feasible and desirable to measure 3 time points and that intervention alone will decrease time to fixation of breast cancer specimens

Goldstein, MINIMUM FORMALIN FIXATION TIME FOR CONSISTENT IMMUNOHISTOCHEMICAL STAINING. Am J Clin Pathol. 2003

Estrogen receptor IHC

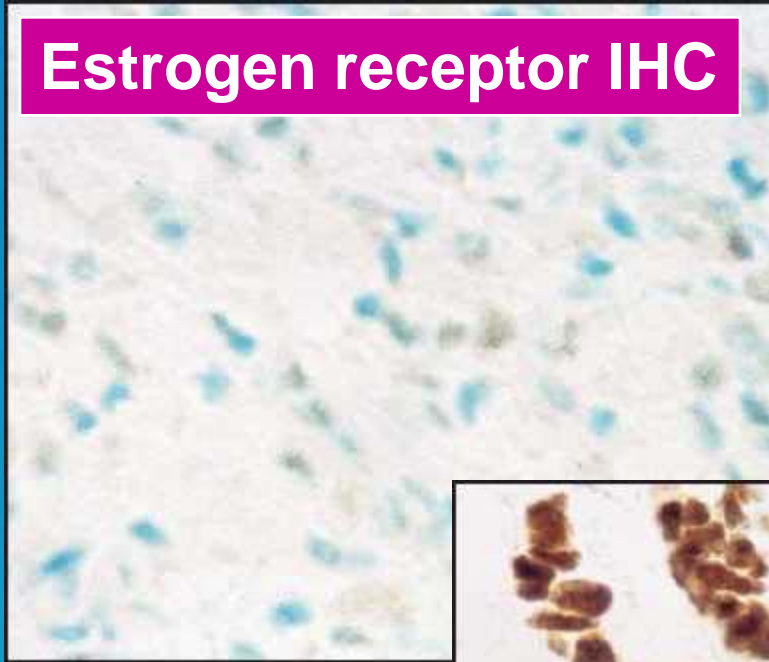


Image 1 Fixation, 3 hrs.
antigen retrieval, 40 min.

One patient sample
formalin fixation for
3 hours
6 hours
8 hours

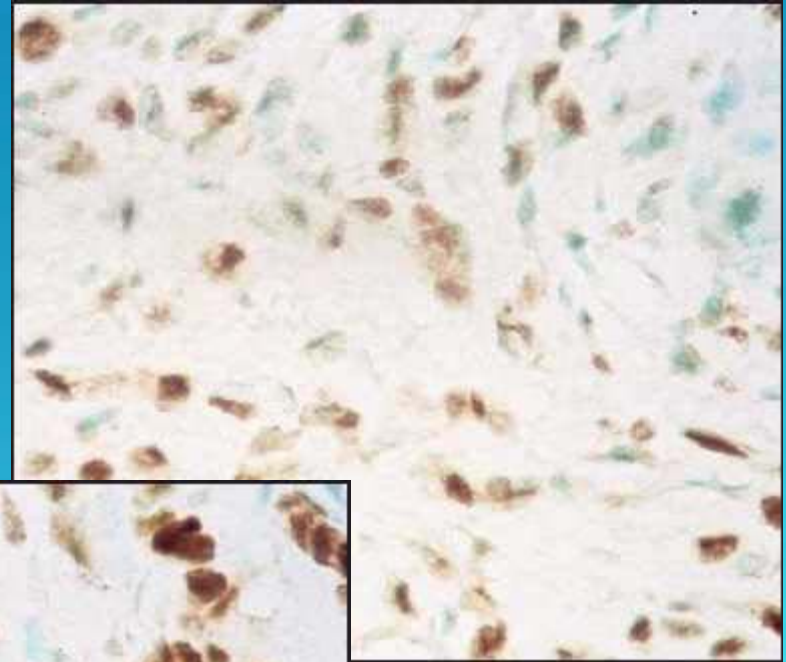


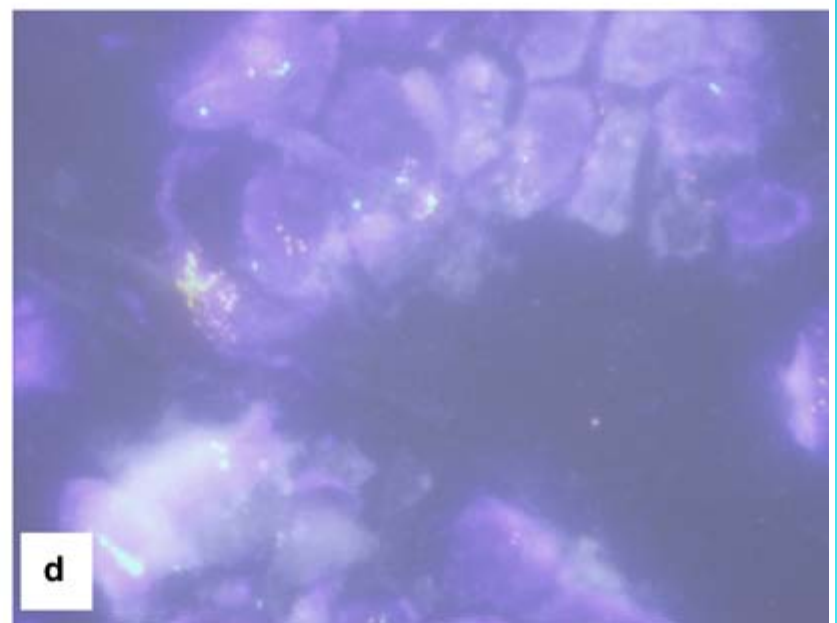
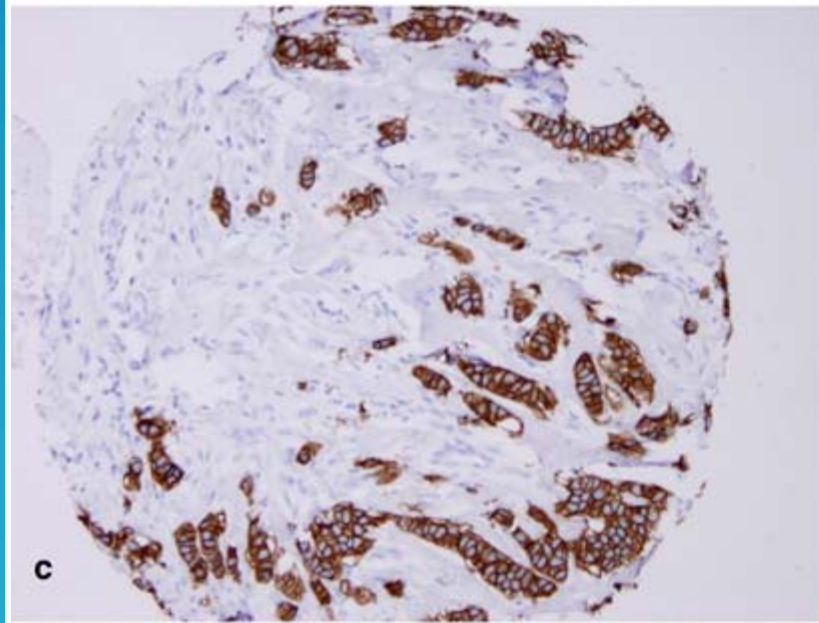
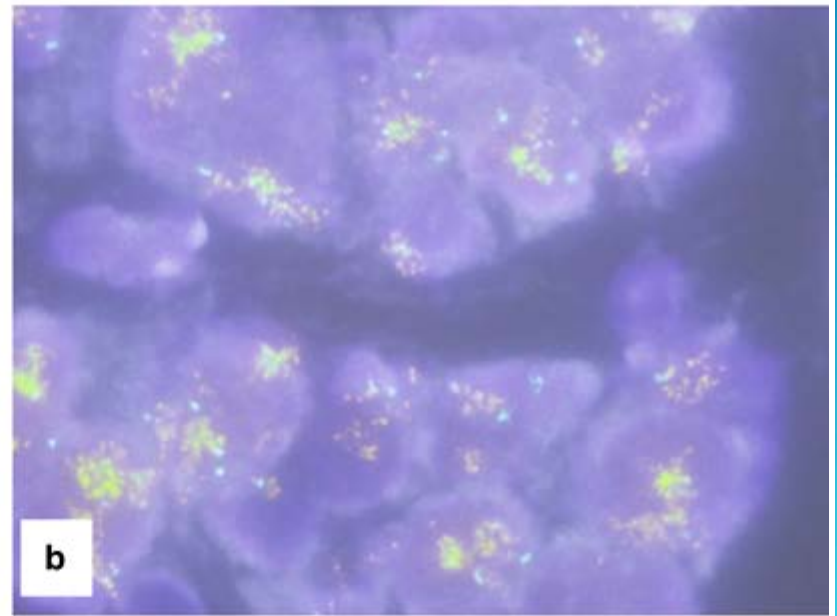
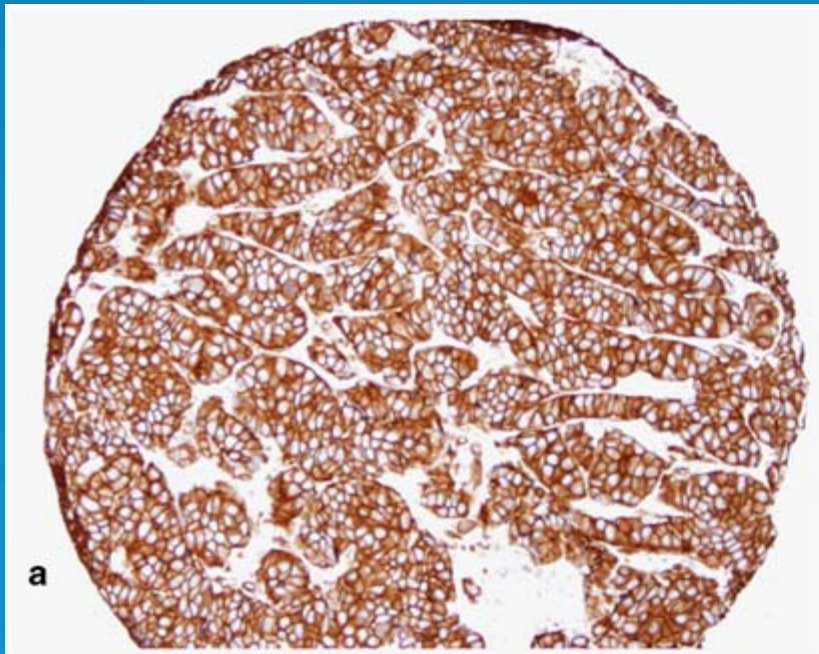
Image 2 Fixation, 6 hrs.
antigen retrieval, 40 min.



Image 3 Fixation, 8 hrs. – antigen retrieval, 40 min.

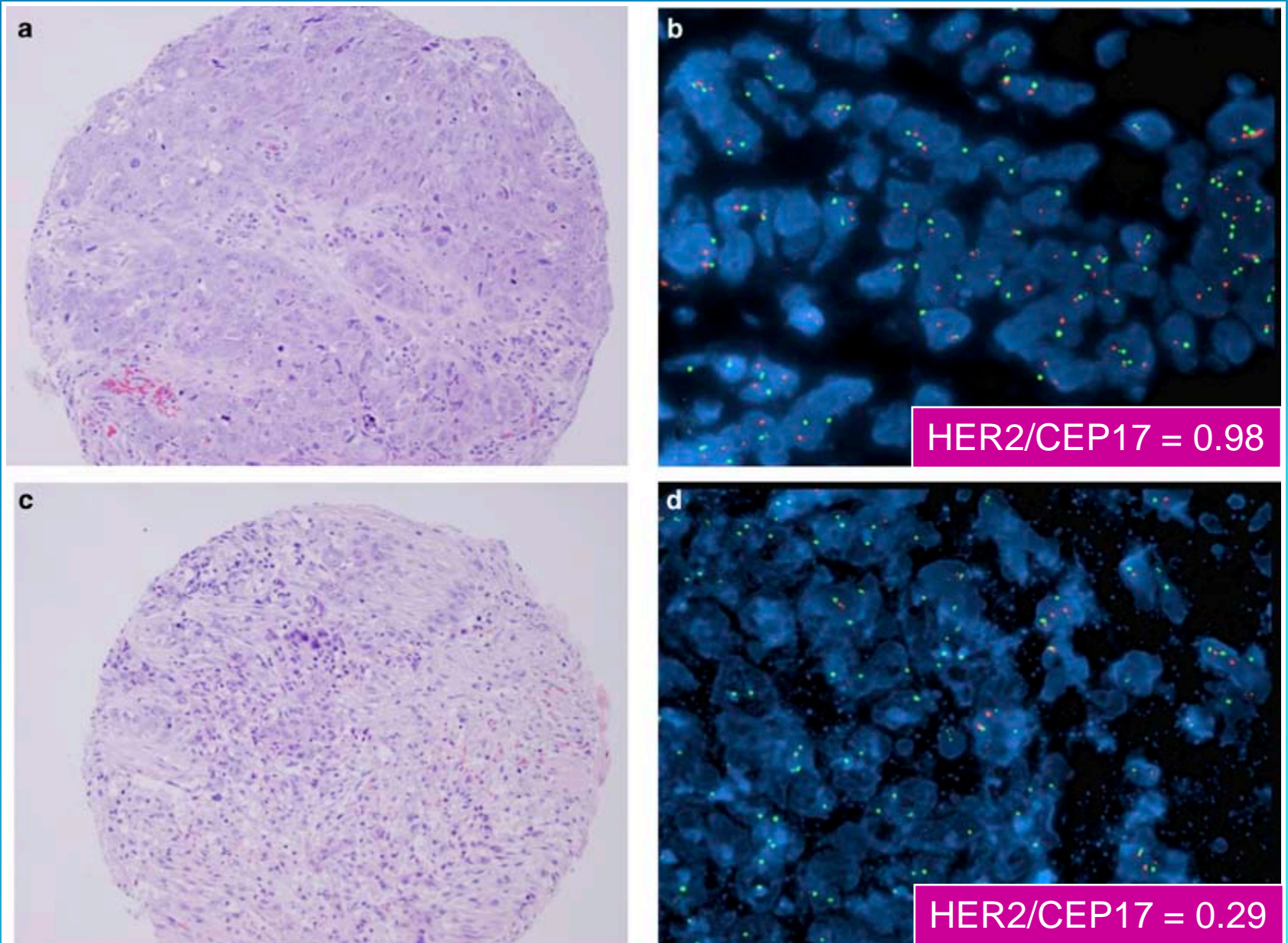
Conclusion:
Minimum of 6-8 hrs
Formalin fixation
Require for reliable
ER/PR assay results

HER2 Testing IHC and FISH



a, 30 min IHC; **b**, 30 min FISH; **c**, 2 h immunohistochemistry; **d**, 2 h FISH

HER2 Testing IHC and FISH



a, 30 min IHC; **b**, 30 min FISH; **c**, 4 h immunohistochemistry; **d**, 4 h FISH

Elements of the ER/HER2 Guideline

Length of Fixation and Type of Fixative

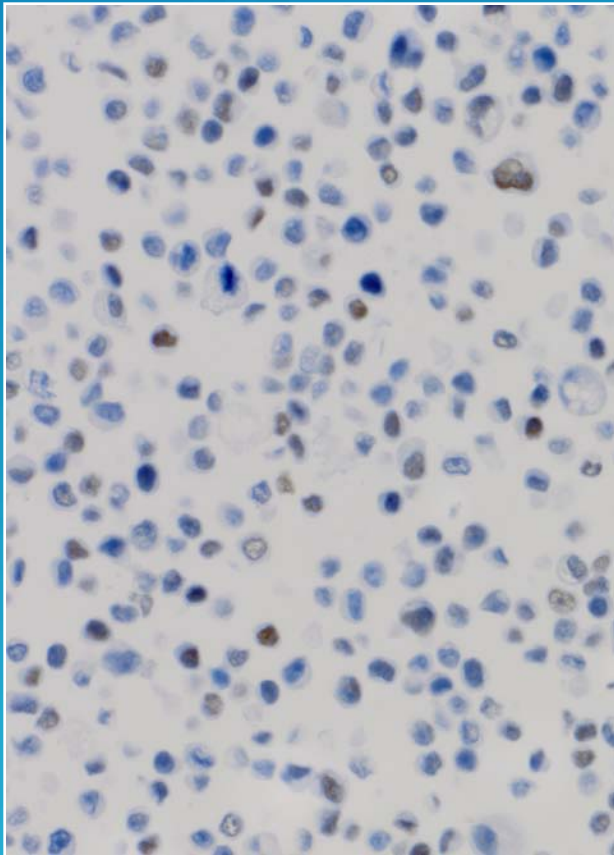
Barriers/challenges to correcting length of fixation:

- Longer fixation will increase turnaround time
- Weekend cases
- Remote cases
- Cases from other laboratories where processes not in your control

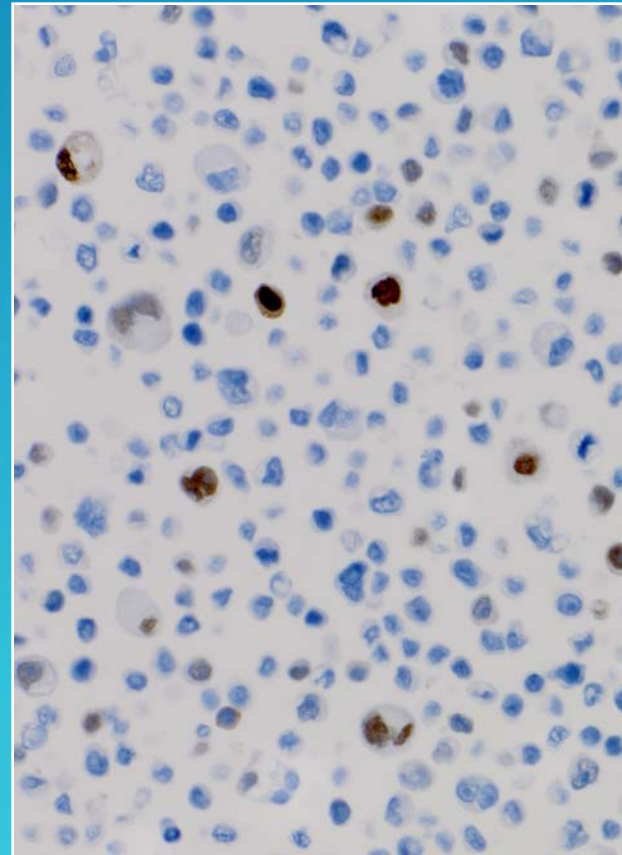
Analytic Elements of the ER/HER2 Guideline

- External controls must be run with each batch including weakly positive controls for each analyte
- Internal lobular elements must be reviewed on each case if they are present
 - Should be positive with ER and negative with HER2
- If possible, normal breast tissue from the patient should also be submitted as ER control

PharmDx FDA Approved ER/PR Kit Cell Line Batch Controls

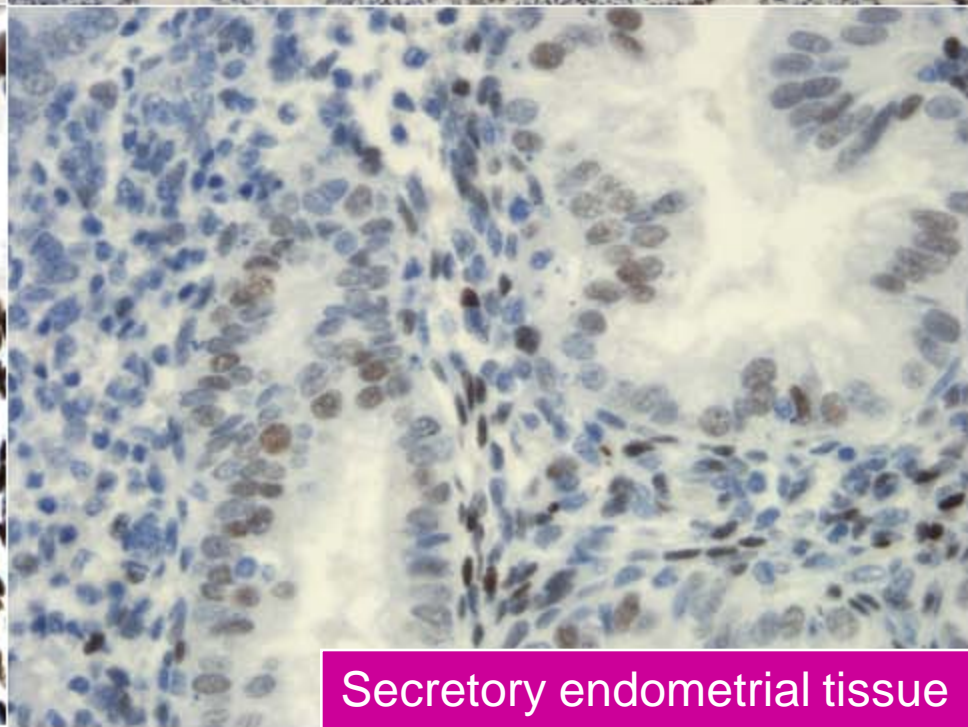
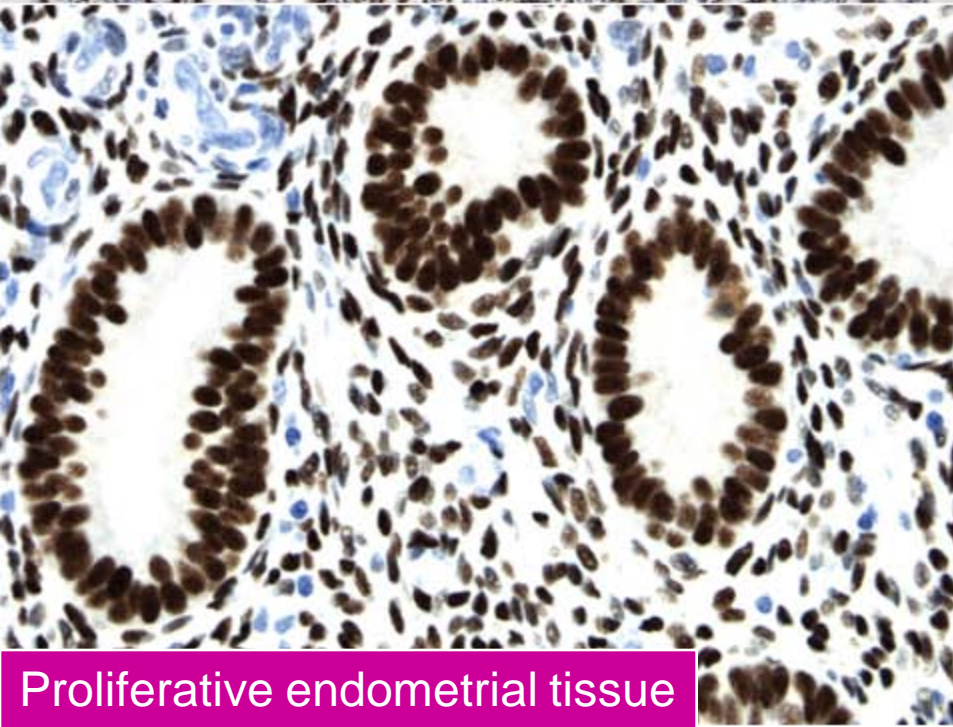
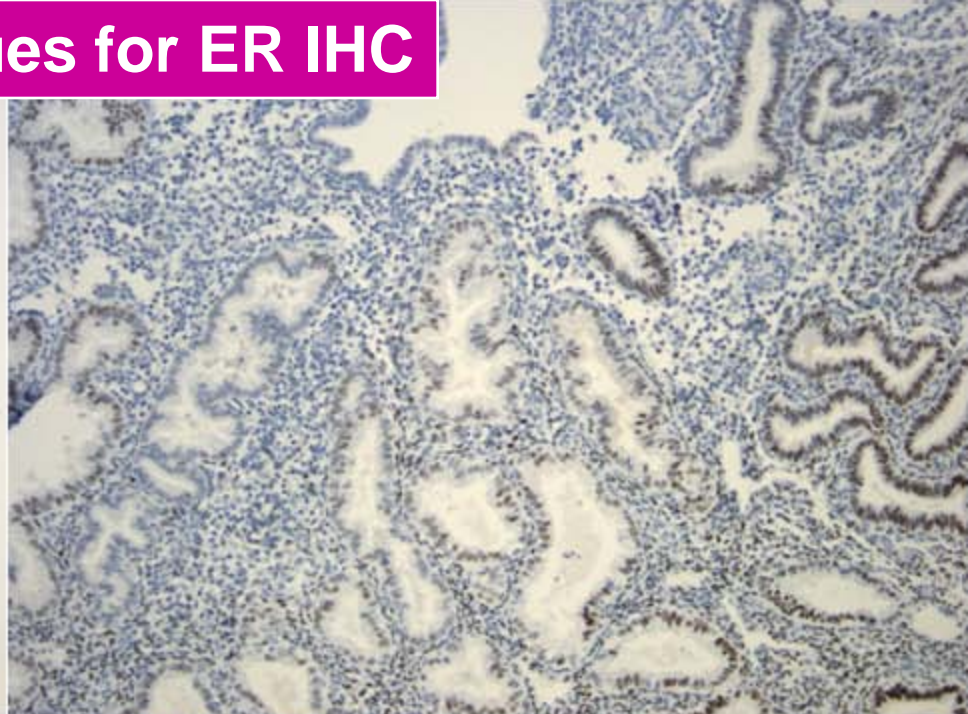
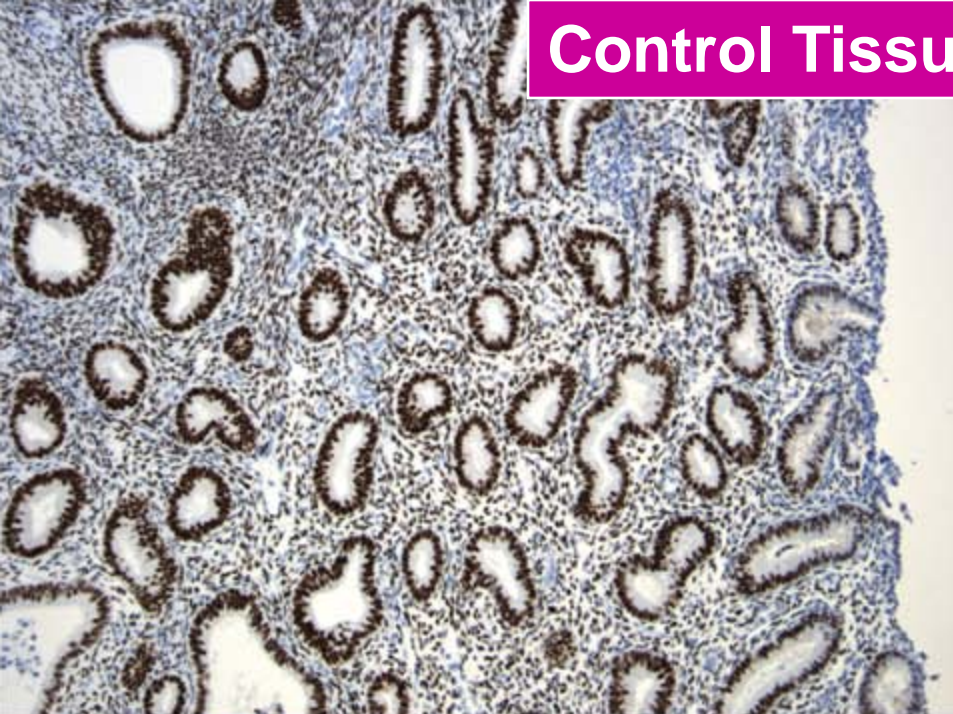


ER+ Cell Line Control



PR+ Cell Line Control

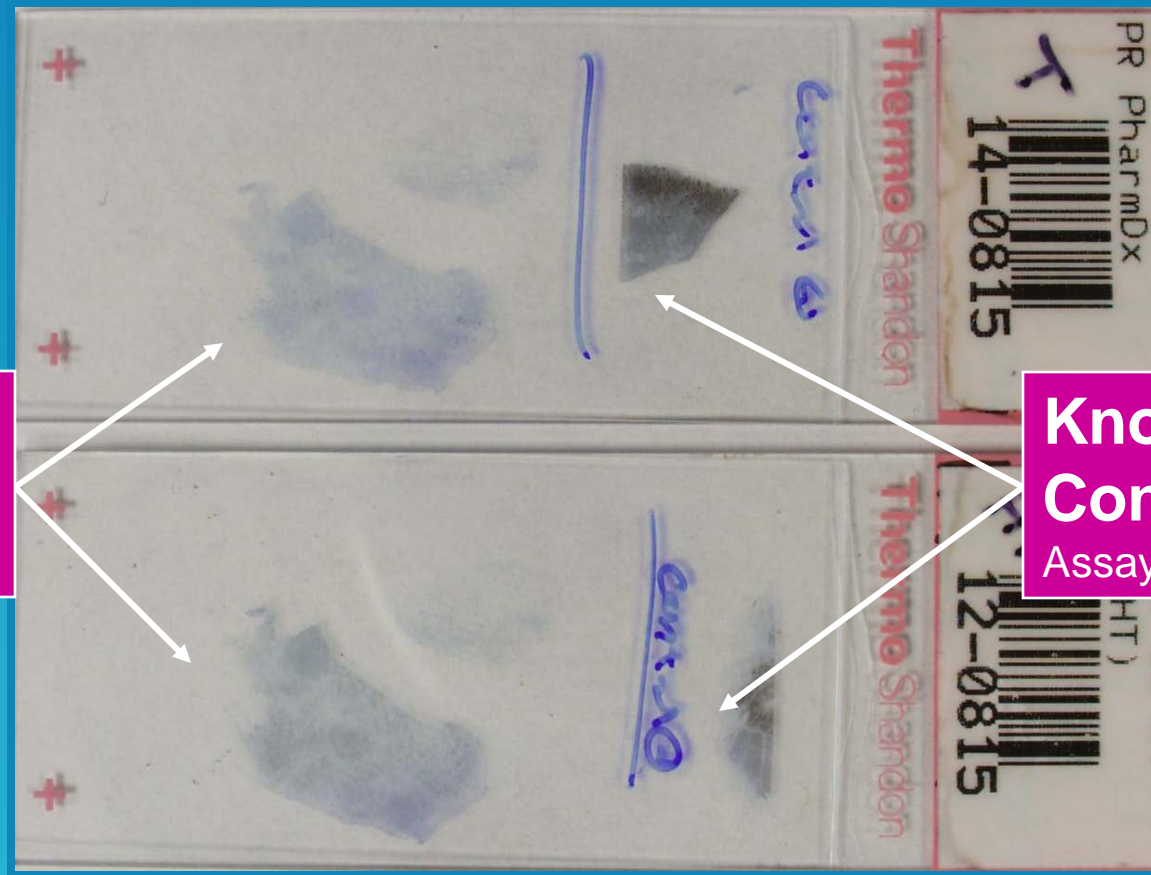
Control Tissues for ER IHC



Proliferative endometrial tissue

Secretory endometrial tissue

“On-Slide” Positive Control HER2, ER and PR IHC



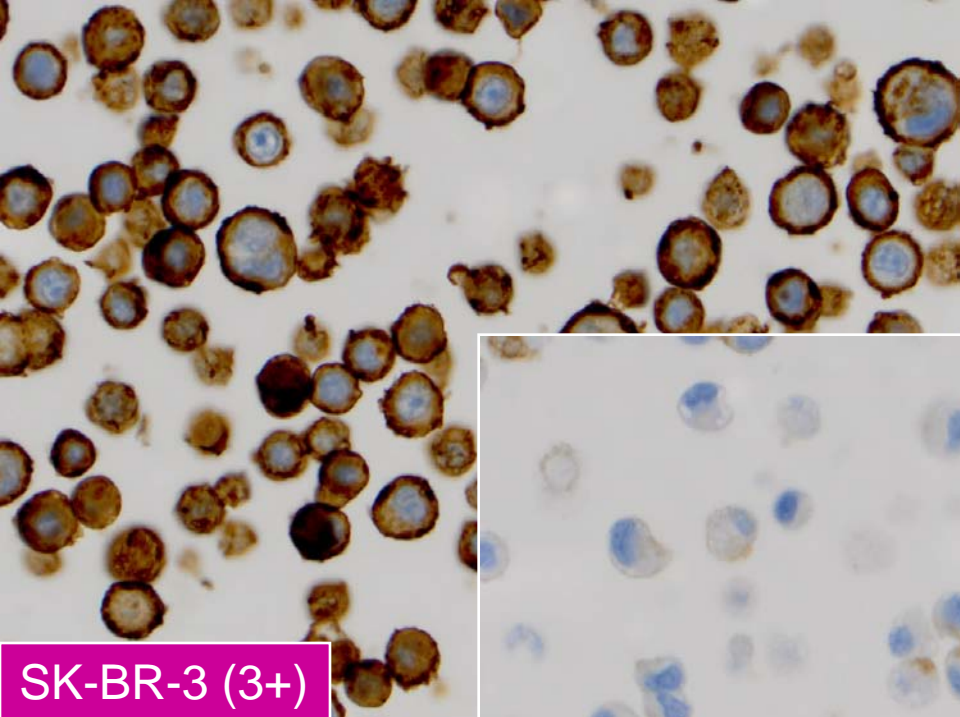
Test Sample
ER-, PR-, HER2-

Known (+) Control
Assay working

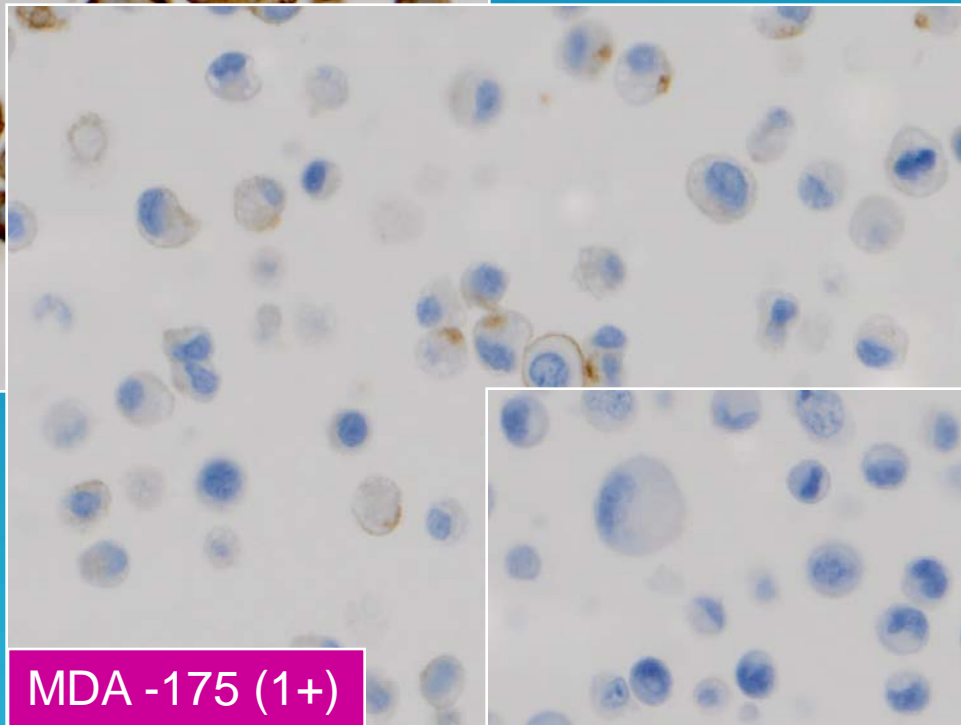
Helps ensure all reagents dispensed properly and assay performing as expected

HER2 Controls

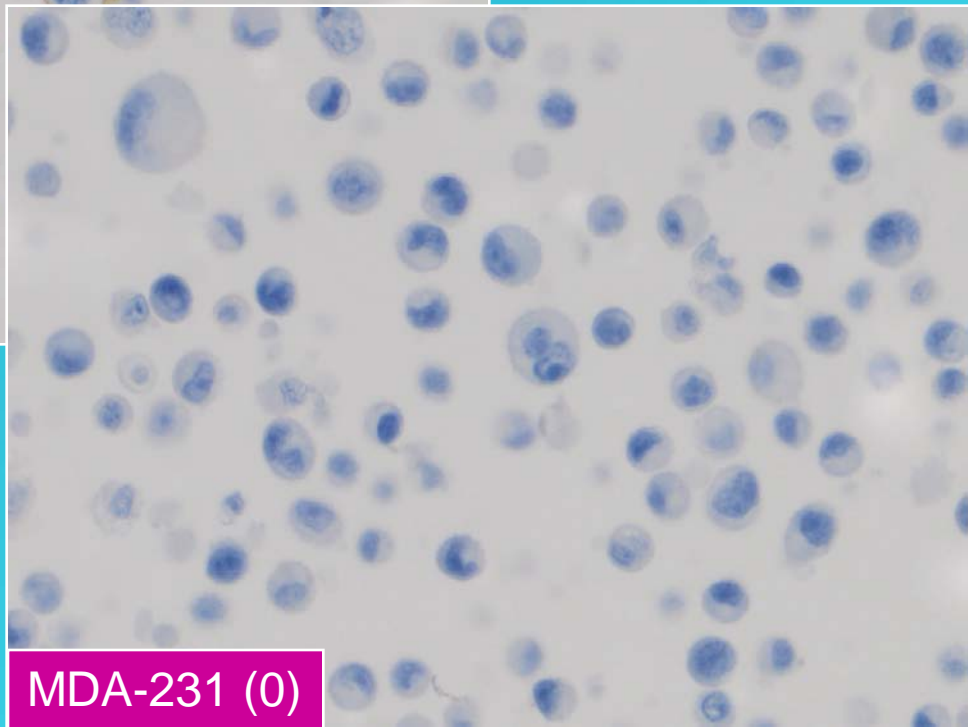
HercepTest kit: Each slide contains section of 3 FFPE breast cancer cell lines representing different levels of HER2 protein expression



SK-BR-3 (3+)



MDA -175 (1+)



MDA-231 (0)

Useful as “Batch Controls”

Helps insure proper assay performance,
Helpful to calibrate the assay sensitivity
and dynamic range for each staining run

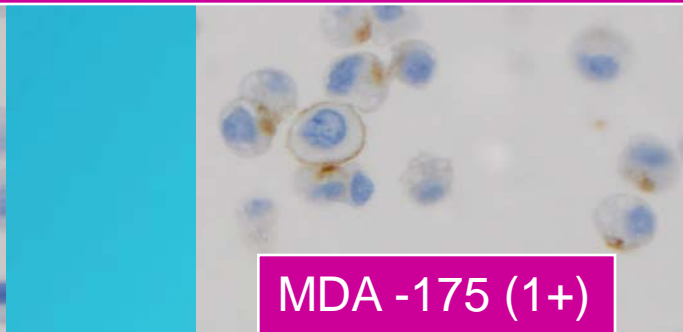
Correlation of # of Receptors, IHC Score and Amplification Status

IHC Score	Cell Line	# of HER2 Receptors	# of Gene Copies per Cell
0	MDA-231	21,600 6,700	2.4 0.2 1.1 0.1 CEP 17 Ratio
1	MDA-175	92,400 12,000	3.0 0.4 1.3 0.1 CEP 17 Ratio
2	MDA-361	500,000 130,000	2.4 CEP 17 Ratio
3	SK-BR-3	2,390,000 1,000,000	15.3 3.9 4.5 1.1 CEP 17 Ratio

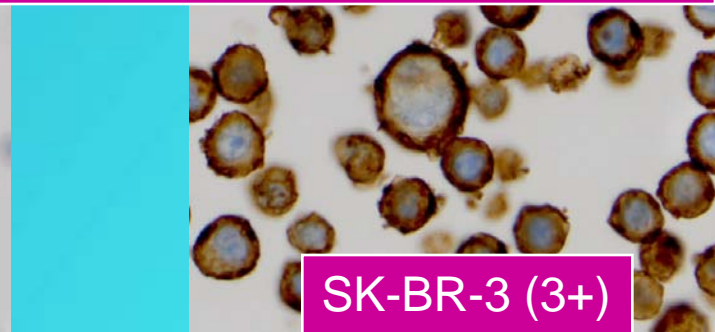
Intensity and distribution of HER2 stain correlates with # of receptors molecules on surface of tumor



MDA-231 (0)



MDA -175 (1+)



SK-BR-3 (3+)

Predictive Factor Interpretation

Standardize criteria for ER, PR and HER2

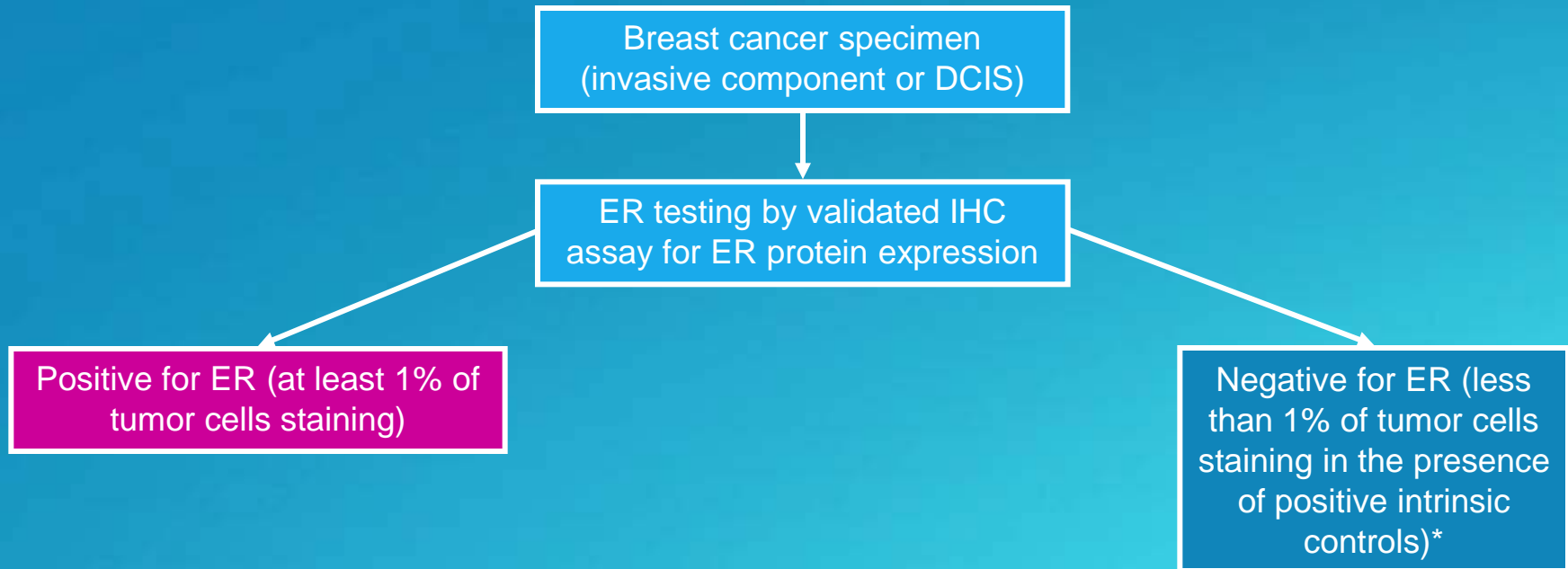
- ER and PR should be positive if 1% of cells are positive
 - Whole slide must be reviewed
 - Record both intensity and percent of cells staining
 - Define on invasive tumor only
- HER2 IHC
 - Use membrane staining as criterion
 - Review entire slide
 - Use chicken wire and uniformity as criteria



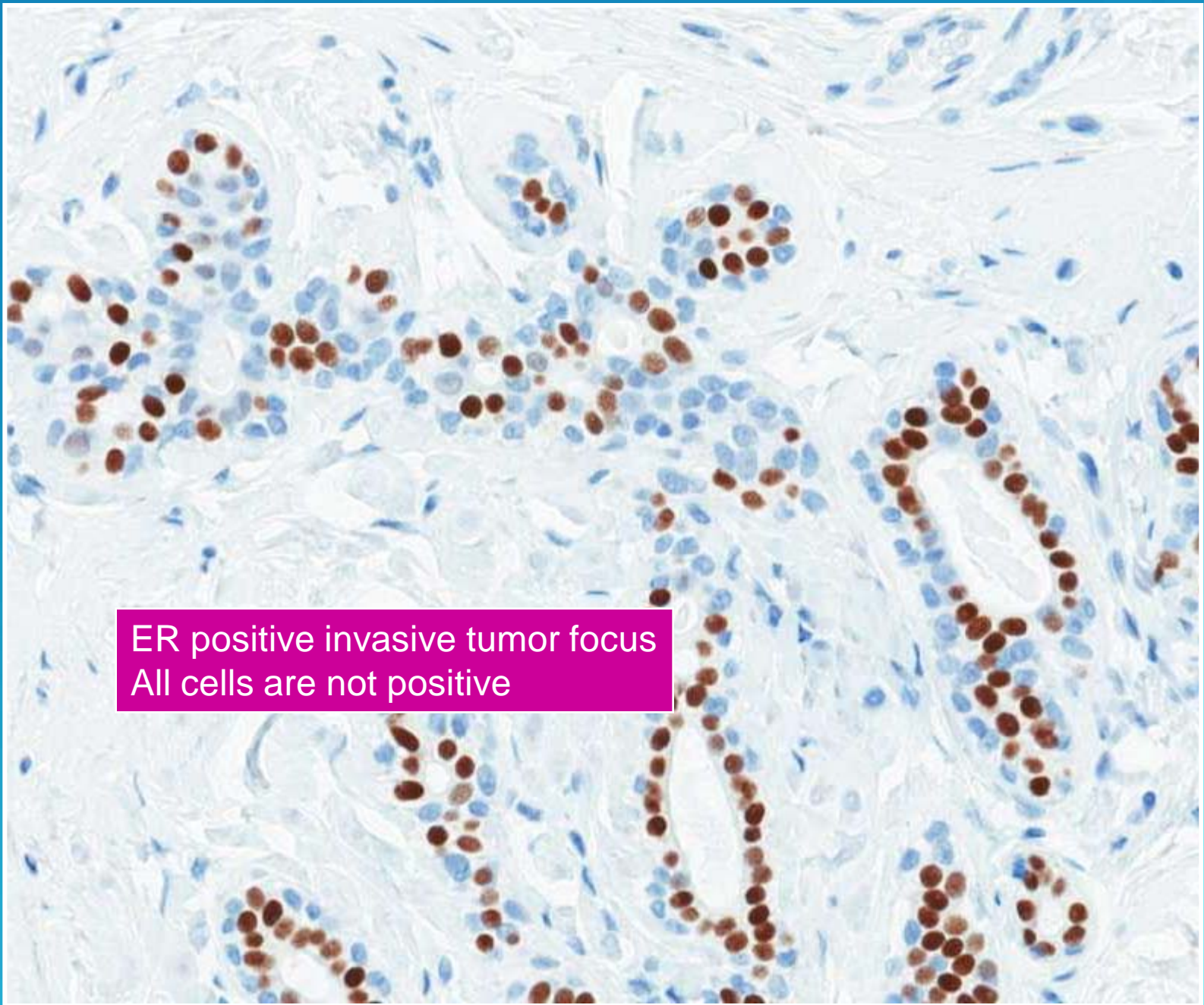
Steps in ER Interpretation

1. Survey the whole H&E slide at low power
 - Where is the tumor?
 - Where are the normal ducts?
 - What kind of tumor is this: high grade, low grade, etc.?
2. Look at the internal controls
 - Are they staining properly?
3. Assess the % of the whole tumor that is positive
4. Assess intensity of staining by comparing with your controls
5. Score the sample

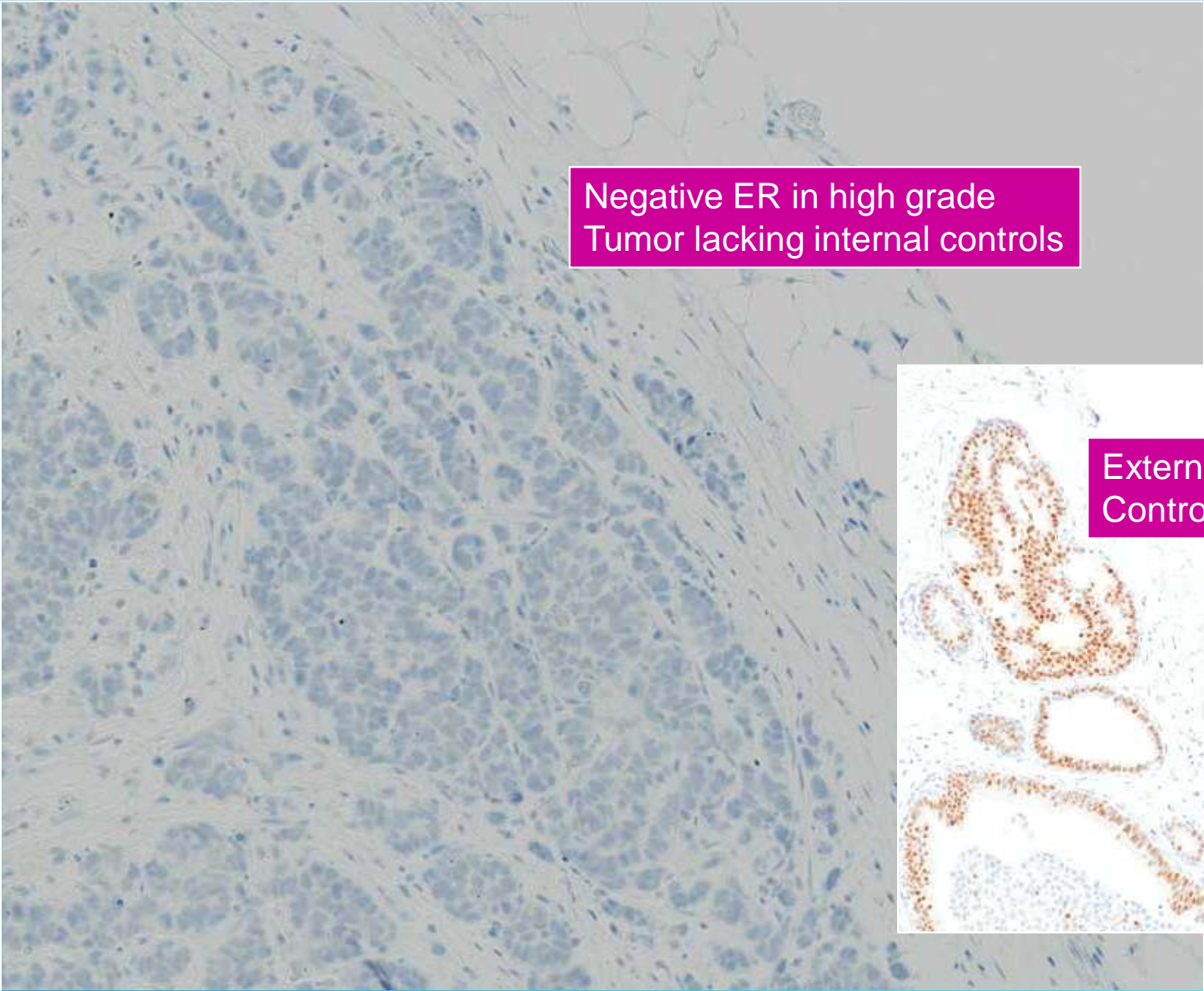
ASCO/CAP Algorithm



* = Negative results in grade 1 tumors should be reported as negative ONLY in the presence of intrinsic positive controls



ER positive invasive tumor focus
All cells are not positive



Negative ER in high grade
Tumor lacking internal controls

This is a large, low-magnification photomicrograph of a tissue section. The tissue is stained with hematoxylin and eosin (H&E). The cells are densely packed and show significant nuclear atypia, including enlarged, hyperchromatic nuclei and prominent nucleoli. The overall architecture is disorganized, consistent with a high-grade malignancy. The cytoplasm is pale, and the nuclei are stained a deep blue-purple. There is no brown staining, indicating a negative result for estrogen receptor (ER) immunohistochemistry.

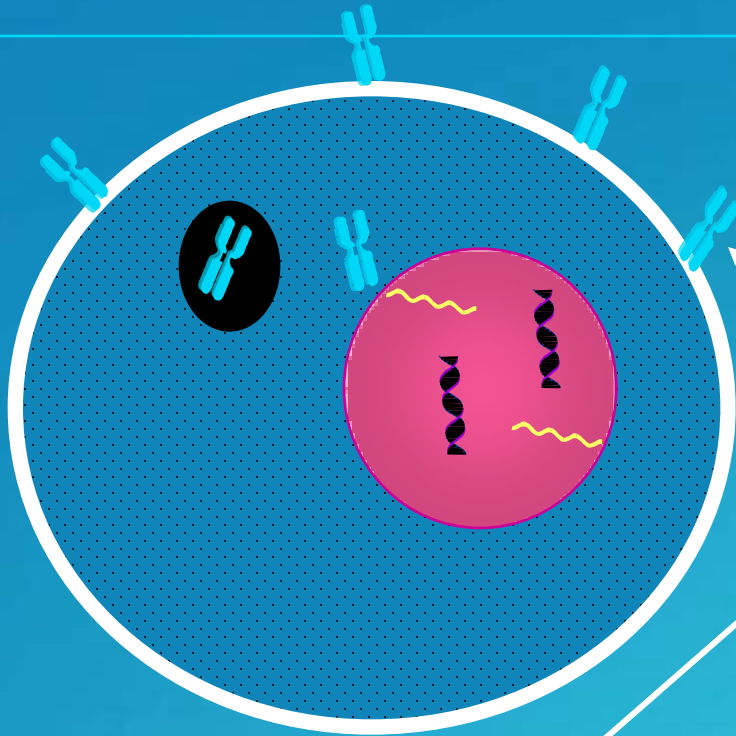


External
Control

This is a high-magnification photomicrograph of an external control tissue section. The tissue shows glandular structures with a clear, organized architecture. The nuclei are stained blue with hematoxylin, and the cytoplasm and glandular lumens are stained pink with eosin. There is prominent brown staining (DAB) within the nuclei of the cells lining the glands, indicating a positive result for estrogen receptor (ER) immunohistochemistry. This serves as a control to demonstrate that the staining procedure is working correctly.

Standardize HER2 IHC Interpretation

Evaluate Staining – HER2 Protein Localization



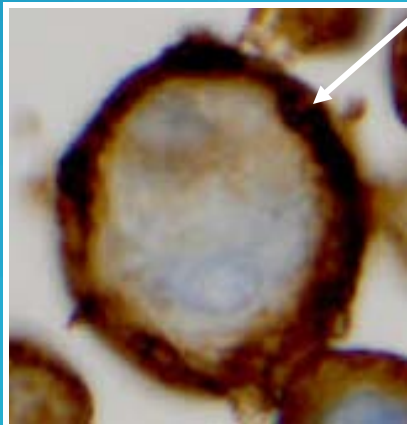
- Membrane

- Predominantly baso-lateral

- Only membrane staining is evaluated (scored) for clinical decision-making

- Cytoplasm

- Transport from cytoplasm to membrane
- Receptor internalization and degradation

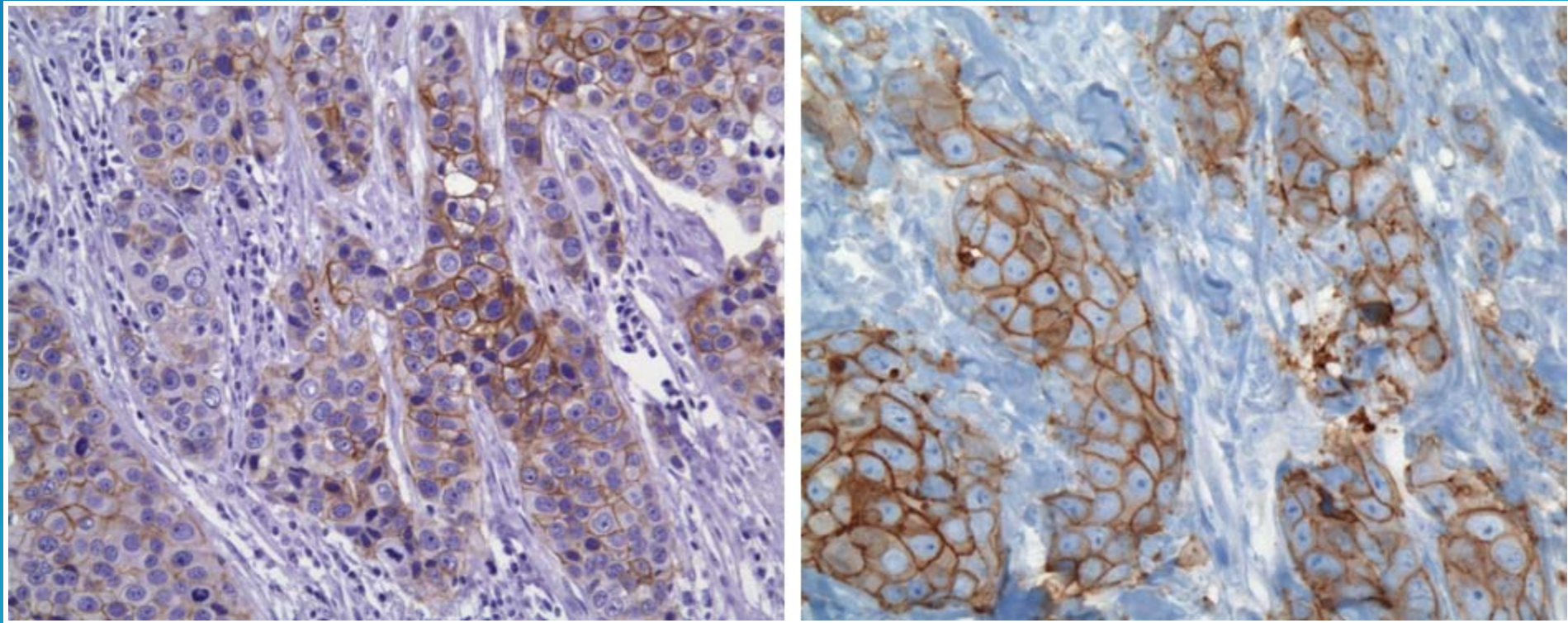


HER2 IHC Interpretation

Evaluate HER2 Protein Expression (Low Power)

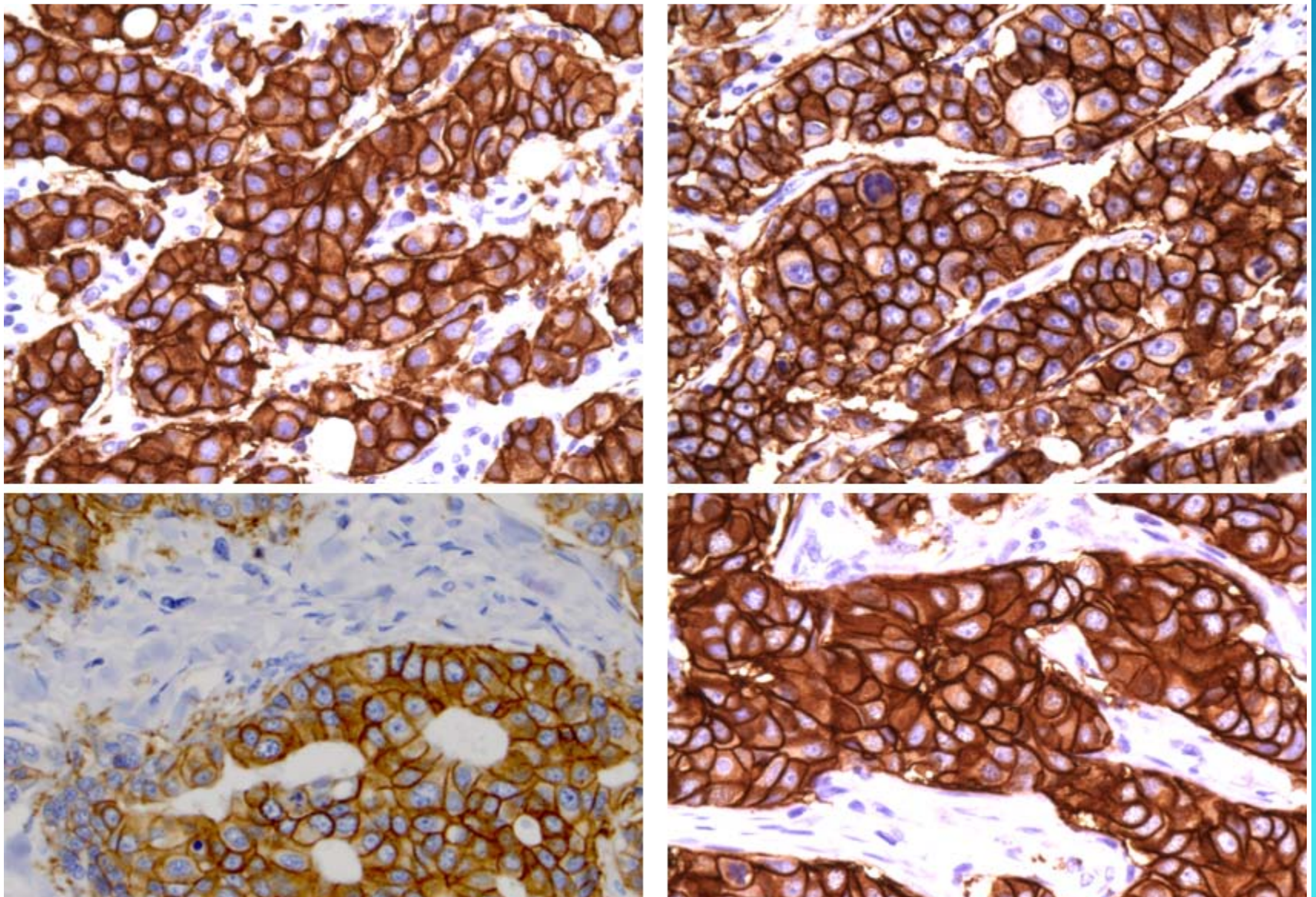
- Be sure no significant staining is present in benign epithelial elements
 - If staining is present assay is too sensitive, repeat the IHC assay, preferably with another tissue block
- Look for “chicken-wire” membrane pattern
 - True 3+, distinct chicken-wire appearance at 10X
 - This staining pattern is typically seen diffusely throughout tumor for HER2+ case by FISH

HER2 IHC: Proper Interpretation of Results



**Complete HER2 membrane staining, non-uniform, thin rims,
score IHC (2+), HER2 equivocal, reflex to FISH**

HER2 IHC: Proper Interpretation of Results



**Chicken-wire pattern, intense staining and uniformity score HER2 (3+)
ASCO/CAP guidelines > 95% gene amplified by FISH**

Cytology Interpretation

- Threshold for ER-positive is 1% of cells with any intensity of staining.
 - Intensity of staining should be recorded.
- For HER2 IHC, same criteria as slides apply.
- Count at least 100 tumor cells. Less than 100 cells might miss someone who is borderline.

Exclusion Criteria for ER/PR IHC

Exercise Caution in Interpretation When:

1. Non-formalin fixatives
2. Excessive delay from collection to fixation
3. Over-fixed tissue – Friday cases (>72 h)
4. Inadequately fixed tissue – (<6 h NBF)
5. Artifacts
6. No staining of weak positive controls
7. No expression in benign elements
8. No invasive tumor seen
9. Tumor is low histologic grade

Critical Evaluation of HER2 IHC: Exclusion Criteria for HER2 Interpretation

Exercise Caution in Interpreting HER2 IHC When:

1. Non-formalin fixatives
2. Excessive delay from collection to fixation
3. Over-fixed tissue – Friday cases (>48 to 72 h in formalin)
4. Inadequately fixed tissue – (<6-8 h NBF)
5. Excessive artifacts
 - Edge, crush, disruption, necrosis
6. Decalcified blocks
7. Inappropriate staining of control cell lines
8. Over-expression in benign elements
9. No invasive tumor seen



Does the Results Fit the Clinical Profile for the Patient?

- ER expression correlates with grade and histology
- HER2 more likely in high grade tumors
- Low grade tumors typically are ER positive and HER2 negative including:
 - Classic infiltrating lobular
 - Mucinous carcinoma
 - Tubular carcinoma
- Negative result for ER or positive result for HER2 in a tumor with these morphologic features
 - Suggests that the assay may be false
 - May be indication for repeat testing

Summary

- ER and HER2 guideline are designed to complement each other and provide uniform standards
- Focus on total test (time of removal from patient to report generation)
- Pay attention to exclusion criteria
- Pay attention to clinical profile of patient's breast cancer
- Helping all stakeholders understand the importance of test accuracy and elements affecting that will enable process changes