Breast Predictive Factor Update

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Disclosure

I have no relevant financial or commercial relationships to disclose

Agenda

Background
Comparison of ASCO/CAP Breast Cancer Guideline Elements for ER,PgR and HER2 (2013 Update)
Updated HER2 Testing Algorithms
Updated HER2 Interpretation Criteria
Handling of Difficult Cases
Summary

Why Are We Here?

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

Accurate ER, PgR,HER2 Testing Provides Maximum Benefit to Patients

The Role of Guidelines

- Evidence suggests that guidelines can improve testing accuracy by:
  - Clarifying areas of confusion
  - Standardizing elements where evidence is clear
  - Providing specific recommendations that can be measured and monitored
- NCCN and ASCO/CAP accepted the challenge to make guidelines to improve breast cancer testing.
- The 2013 HER2 update is the latest effort in that process.

The Guidelines Are Living Documents

- Evidence continually emerges about issues with testing and the effect of the guidelines on performance.
- Evidence must be re-collated, analyzed and addressed in guideline updates at least every 3 years.
- Iterative guidelines lead to more effective guidance in testing.
- Measuring changes in testing accuracy will help guide these efforts.

2013 HER2 Testing Guideline Update

What Remains the Same and Parallels ER/PgR Guideline?

	Recommendations
No Change from 2007	• Optimal tissue specimen handling procedures – Tissue acquisition (i.e., minimize cold ischemic time) < 1 hour – Fixative: 10% neutral buffered formalin (NBF) – Minimum duration of fixation: 6 hours – Must document fixation time points in accession or report
	• Laboratory quality assurance processes, including proficiency testing and lab accreditation

Wolff AC et al. *Arch Pathol Lab Med*. 2014;138:241-256.

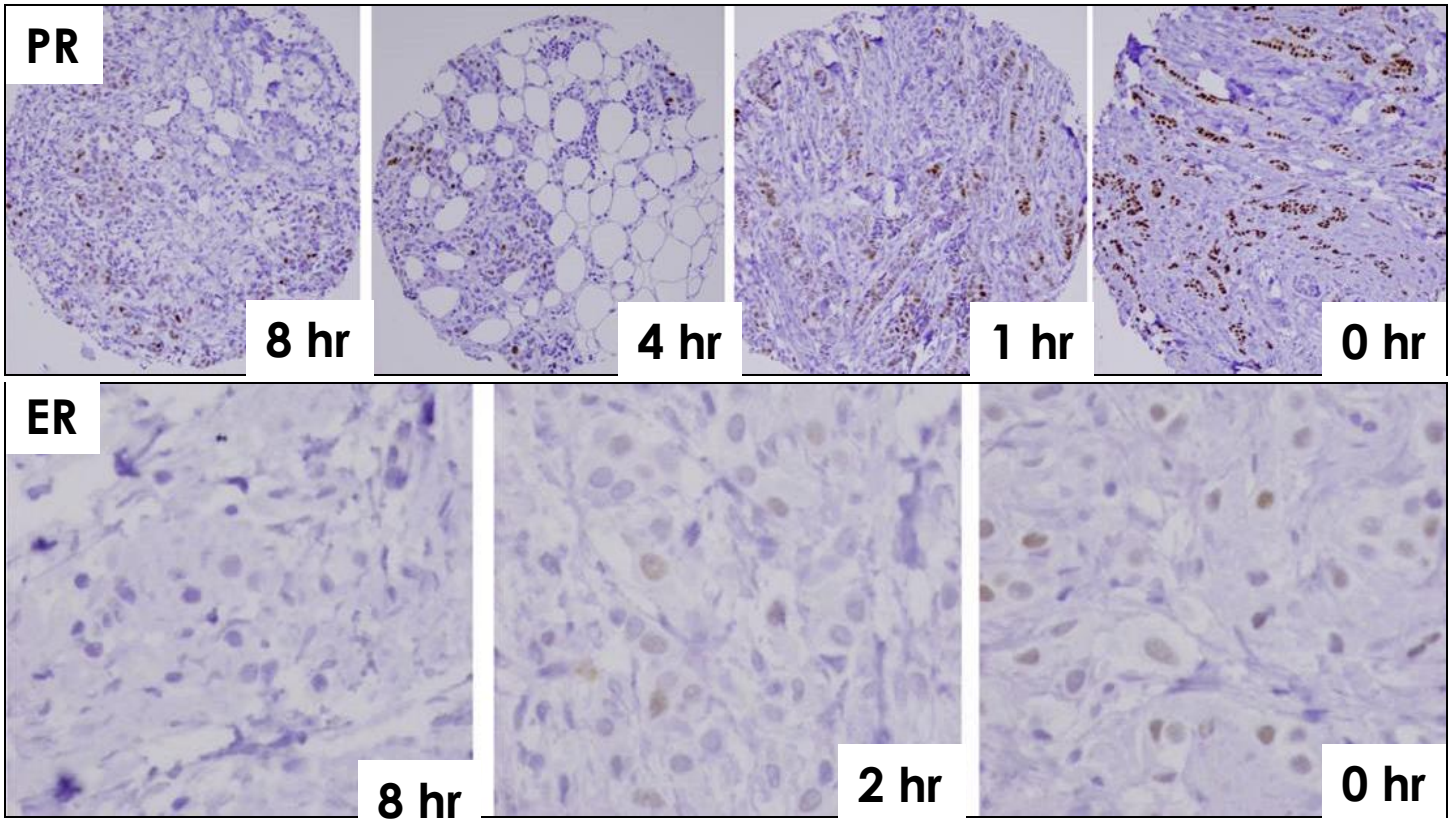
Accurate Results Depend on Standardized Pre-analytic Variables

- **Tissue handling**
  - Avoid delay in start of fixation
  - Responsibility of surgeon and pathologist
- **Type of fixation used in laboratory**
  - 10% NBF should be used for breast specimens
  - Decades of clinical experience & studies using NBF fixed tissue
  - Commercial assays have been developed and optimized for NBF
- **Fixation time**
  - Chemical fixation takes 12-24 hrs to complete
  - Commercial assay developed & optimized - fixation time interval
  - Standard antigen retrieval assumes set time in formalin

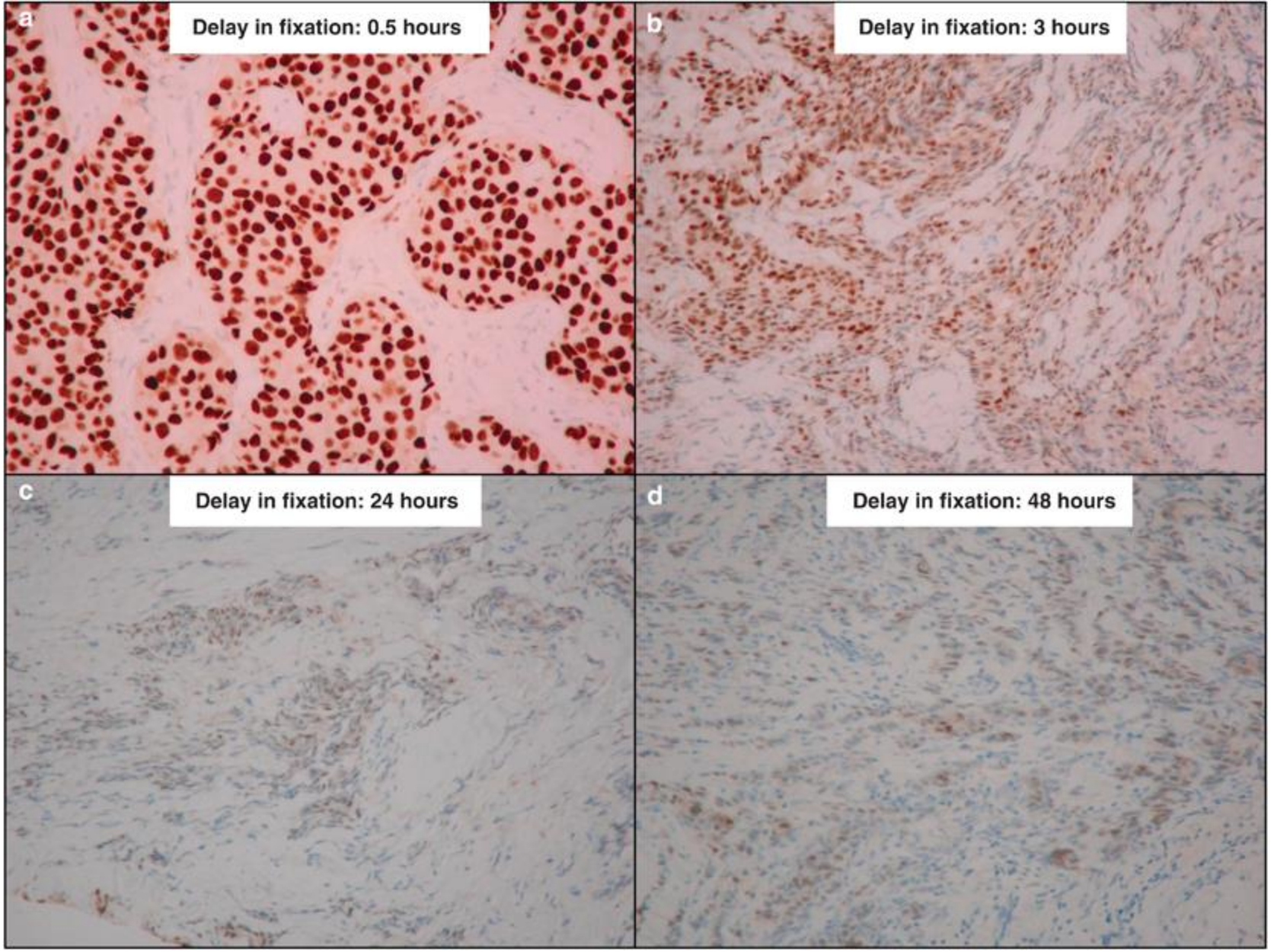
Time to Fixation: Important for Preservation of ER Reactivity

ER, PR Testing by IHC

Mean score started to decline at 1 hr mark for progesterone receptor and 2 hr mark for estrogen receptor



Khoury T, et al., *Mod Pathol.* 2009 Nov;22(11):1457-67.

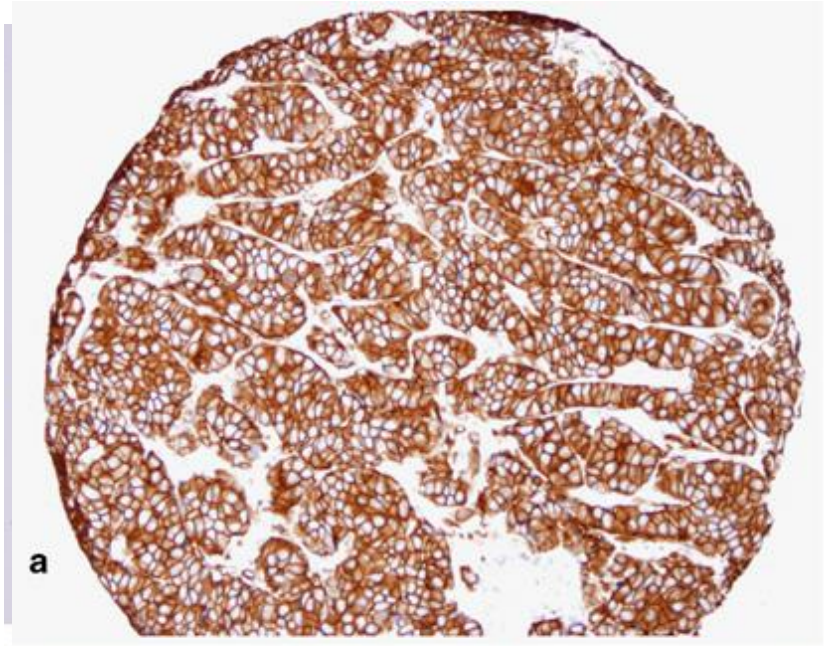


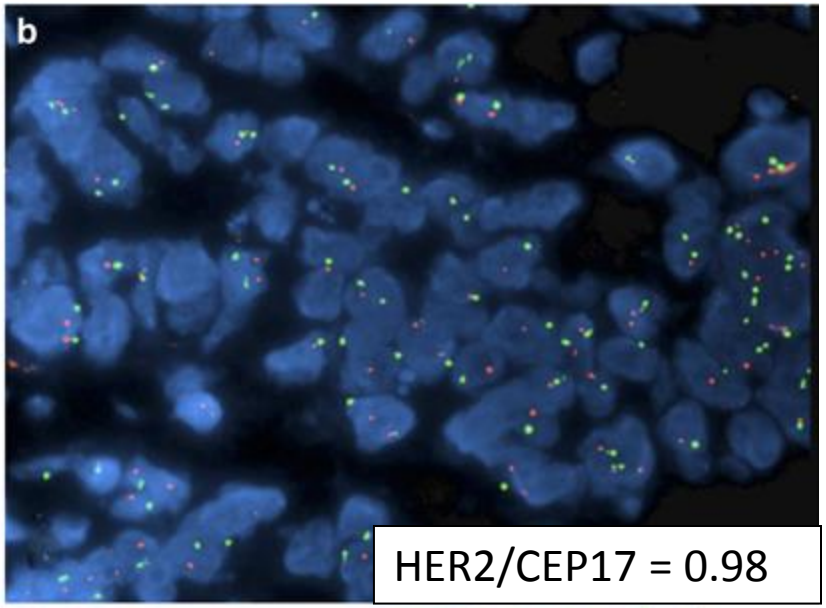
ER IHC at 0.5 h of delayed fixation (a), significant reduction at 3 h (b), 24 h (c), and 48 h (d).

Yildiz-Aktas IZ, et al. *Mod Pathol*. 2012 Aug;25(8):1098-105.

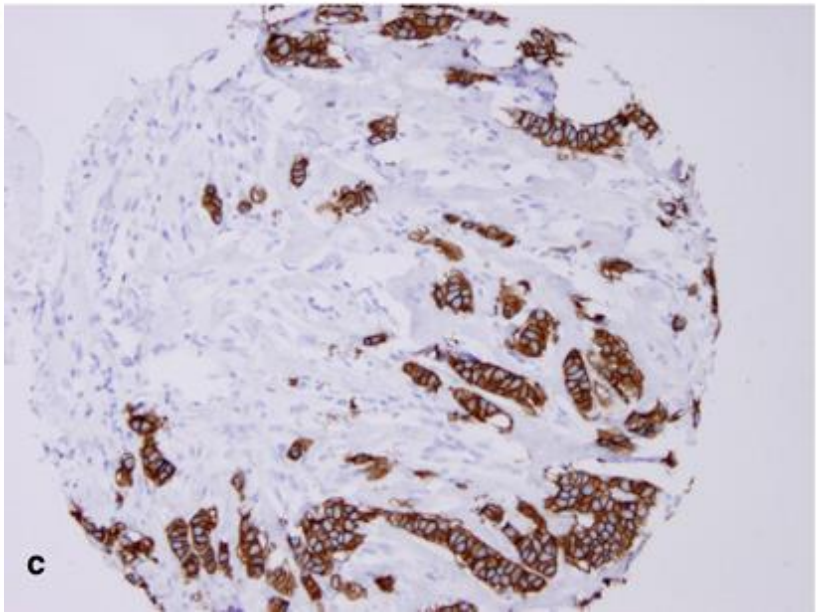
Time to Fixation: HER2 Testing IHC and FISH

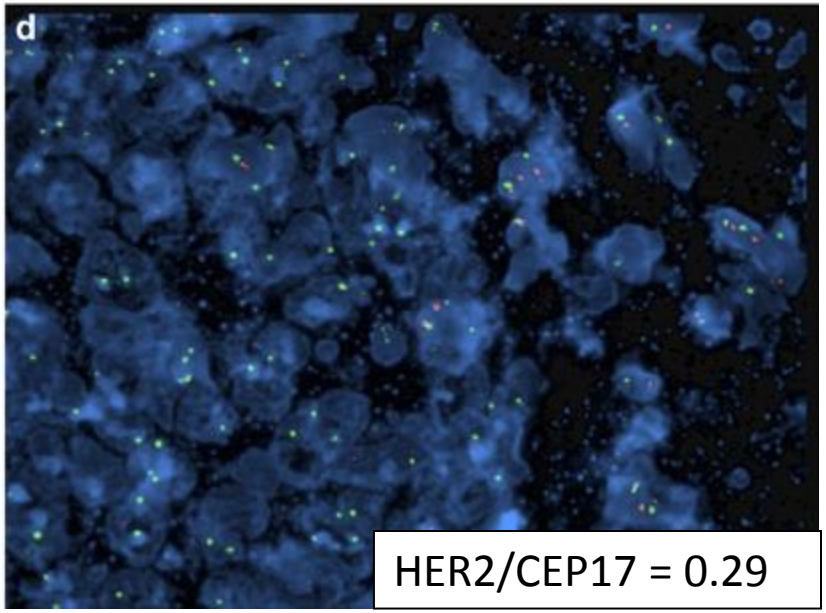
30 minutes





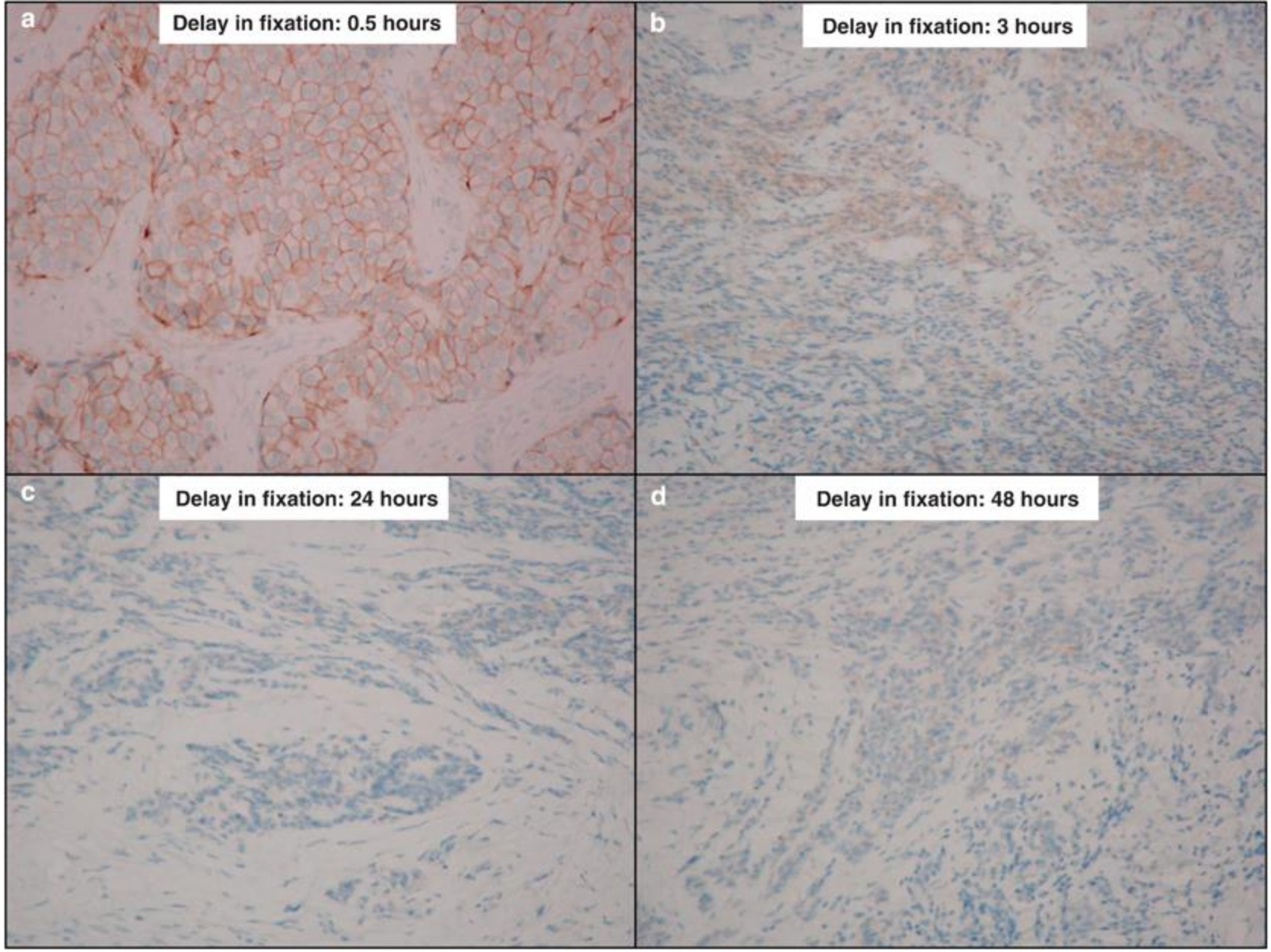
4 hours





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

Khoury T, et al., *Mod Pathol*. 2009 Nov;22(11):1457-67



Tumor stained as '2+' for HER2 at 0.5 h of delayed fixation (a), but demonstrated reduction in staining at 3 h (b) and was completely negative at 24 h (c) and 48 h (d).

Yildiz-Aktas IZ, et al. *Mod Pathol*. 2012 Aug;25(8):1098-105.

2013 HER2 Testing in BC Guideline Update

What Changed?

	Recommendations
2013 Updates	• Perform HER2 testing on every primary invasive tumor and any subsequent reoccurrence including metastatic sites.
	• Optimal tissue specimen handling procedures – Maximum time in fixative: 72 hours like ER
	• New algorithms for test interpretation and reporting
	• Language on repeat testing (reflex and new tests)
	• Need for enhanced communication between pathologists and oncologists • Guidance for communicating with patients
	• Revised test validation requirements to align with ER/PgR recommendations

Wolff AC et al. *Arch Pathol Lab Med*. 2014;138:241-256.

2013 HER2 Testing in BC Guideline Update

Duration of Fixation

2007 Guidelines
Time in fixative • 6 – 48 hours

2013 Guideline Update
Time in fixative • **6 – 72 hours** *Applies to both excision and core specimens*

Both the ASCO/CAP HER2 and ER/PgR Testing Guidelines now share the same recommendation for the duration of fixation.

2013 HER2 Testing in BC Guideline Update

Tumor Specimens to be Tested

2007 Guidelines[1]	2013 Guideline Update[2]
• Resection specimens preferred sample for HER2 testing • More representative sample of the patient's tumor, more tumor tissue for evaluation	• Increasing use of core for testing • Core biopsies can be used for initial test (likely better pre-analytics) • Repeat testing on the excision may be necessary **if a HER2 result is negative on the core in certain circumstances**

ASCO/CAP HER2 and ER/PgR Testing Guidelines now share the same recommendation for preferred specimen type and action to be taken if negative

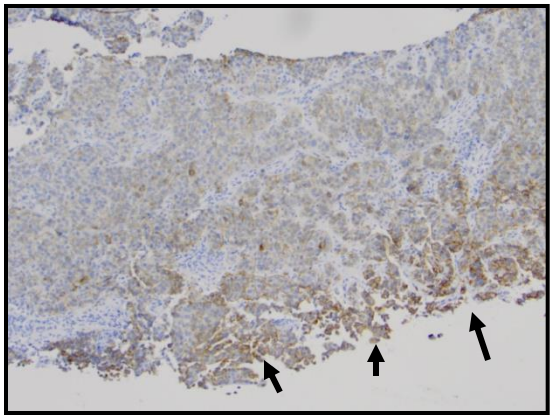
1. Wolff AC, et al., *Arch Pathol Lab Med*. 2007;131:18-43.

2. Wolff AC, et al. *Arch Pathol Lab Med*. 2014;138:241-256.

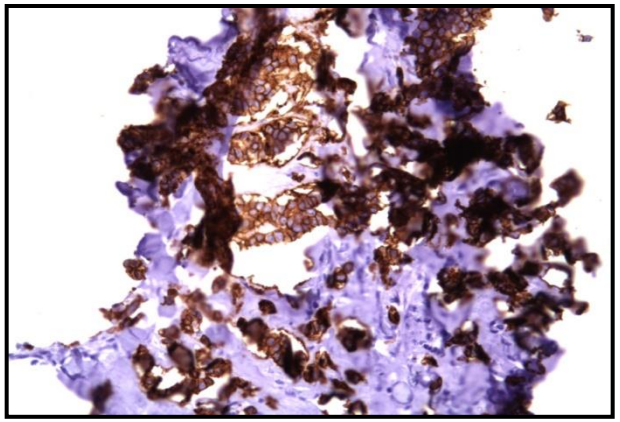
2013 HER2 Testing Update

Tumor Specimen Selection

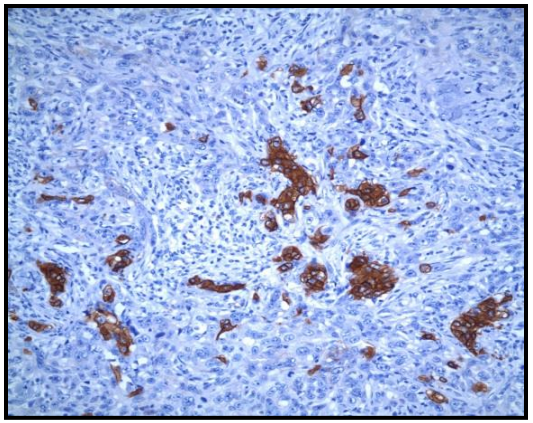
- Core samples may not be optimal in some situations
  - Crushing and surface artifacts in cores may hamper interpretation
  - Tumor on resection may show morphologic heterogeneity
  - Tumor on resection may show intratumoral heterogeneity
  - Tissue is not fixed for adequate length of time



Edge Artifact (HER2 IHC)



Crush(HER2 IHC)



Intratumoral Heterogeneity (HER2 IHC)

If core results are questionable, excision should be tested just as for ER/PgR

2013 HER2 Testing Repeat Testing for Histopathologic Discordance

If the initial test result is HER2 NEGATIVE on Core:	
Order a New Test on the Excision if:	**DO NOT order a New Test if:**
•Tumor is Histologic Grade-3	•Tumor is Histologic Grade-1
•Small amount of invasive tumor on core	•Infiltrating ductal or lobular carcinoma that is strongly ER/PR positive
•Resection contains high-grade component not present on core	•Tubular carcinoma (>90% pure)
•Core biopsy equivocal diagnosis by both IHC and ISH	•Mucinous carcinoma (>90% pure)
•Questionable specimen handling of core or result is suspect to be negative due to testing error	•Cribriform carcinoma (>90% pure)
	•Adenoid cystic carcinoma (typically these tumors are triple negative)

Wolff AC, et al. *Arch Pathol Lab Med*. 2014;138:241-256.

2013 HER2 Test Algorithms

HER2 Positive

IHC: > 10% of invasive tumor cells display staining

ISH: Amplified ratio of HER2/CEP17 of ≥ 2.0 or average HER2 signals ≥6 signals/cell (regardless of ratio) in population of >10% of tumor cells

HER2 Equivocal
(Must Reflex Test)

IHC: 2+ based on circumferential membrane staining, incomplete, weak, or moderate within >10% of the invasive tumor cells; *or* complete & circumferential membrane intense staining within ≤10% of the invasive tumor cells

ISH: Dual Probe *HER2*/CEP17 ratio <2.0 with an average *HER2* copy number ≥4.0 and <6.0 signals/cell

HER2 Negative

IHC (0): No staining observed or membrane staining that is incomplete, faint/barely perceptible and within ≤10% of the invasive tumor cells

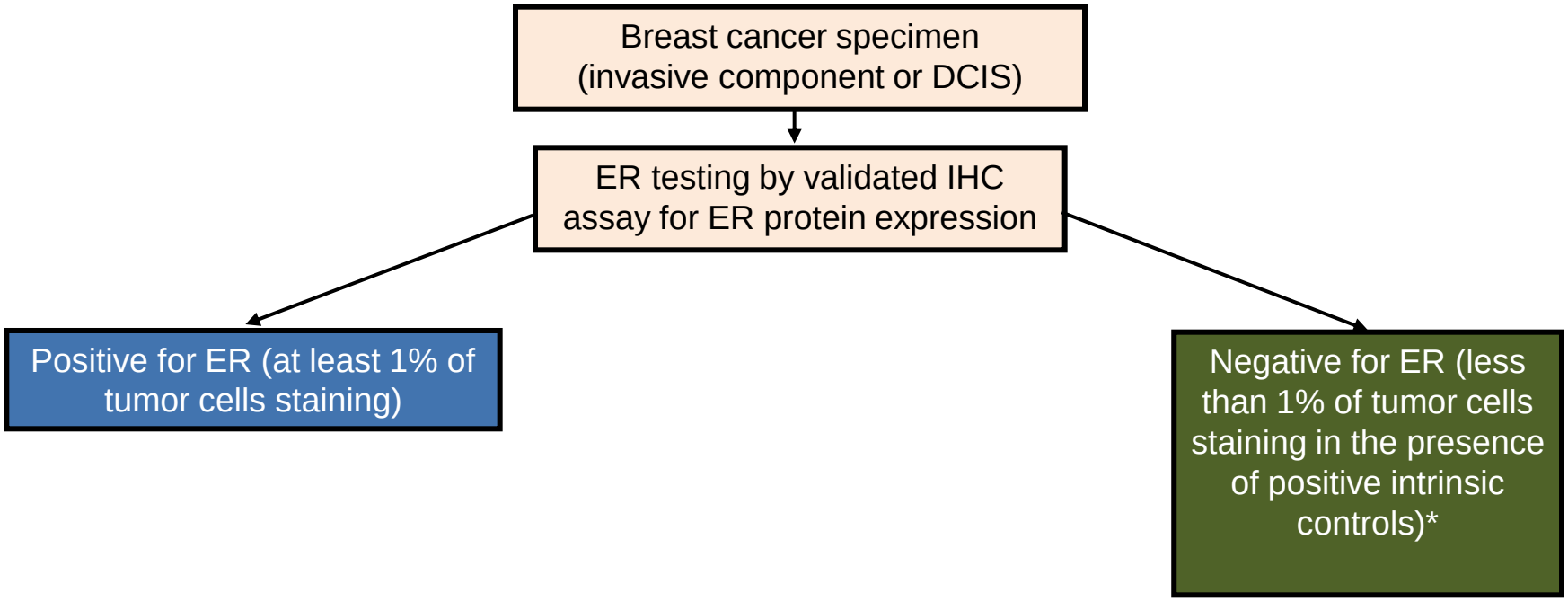
IHC (1)+: Incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumor cells ±

ISH: HER2/CEP17 ratio < 2 or HER2 signals/cell < 4, regardless of ratio

Indeterminate

Technical issues prevent assay from being conclusive (e.g., issues with controls, specimen handling, artifacts, or analytical failure). Assay must be repeated before final diagnosis can be rendered.

2011 ER/PgR Guideline Algorithm



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

Contrasting Elements of ER/PgR and HER2 Update Guidelines

- Threshold for positive is different, and there is no equivocal category for ER
- Major problem with ER testing is false-negatives mostly related to specimen handling; HER2 can be either falsely negative or positive
- Internal control is critical for interpretation of ER and PgR testing

2011 ER/PgR Testing Interpretation Criteria for IHC

- **Threshold for ER positive: >1% of cells with any intensity of staining in presence of positive internal control**
- **Record:**
  - **% of ER+ invasive tumor cells**
  - **Average intensity of staining**
- **Assay should be optimized to capture broad dynamic range of ER expression (not just positive and negative)**

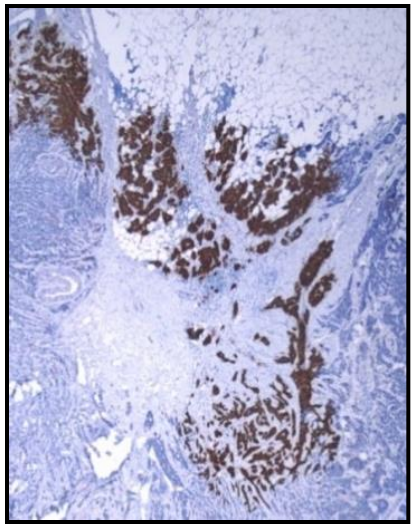
2013 HER2 Testing Positive Interpretation Criteria for IHC

2007 Guidelines

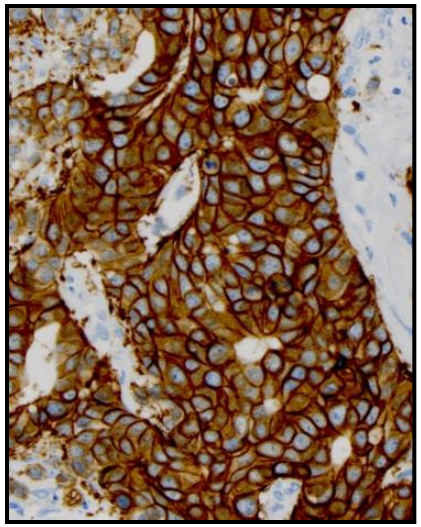
- **Positive for HER2** is 3+ (defined as uniform **intense membrane staining of > 30%** of invasive tumor cells).

2013 Guideline Update

- **Positive for HER2** is **3+** (defined as uniform **intense membrane staining of >10%** of invasive tumor cells*.



HER2 (3+) in 10% of tumor (HER2 Positive)



HER2 (3+) in >90% of tumor (HER2 Positive)

*Readily appreciated at low power.

2013 HER2 Testing Negative Interpretation Criteria for IHC

2007 Guidelines

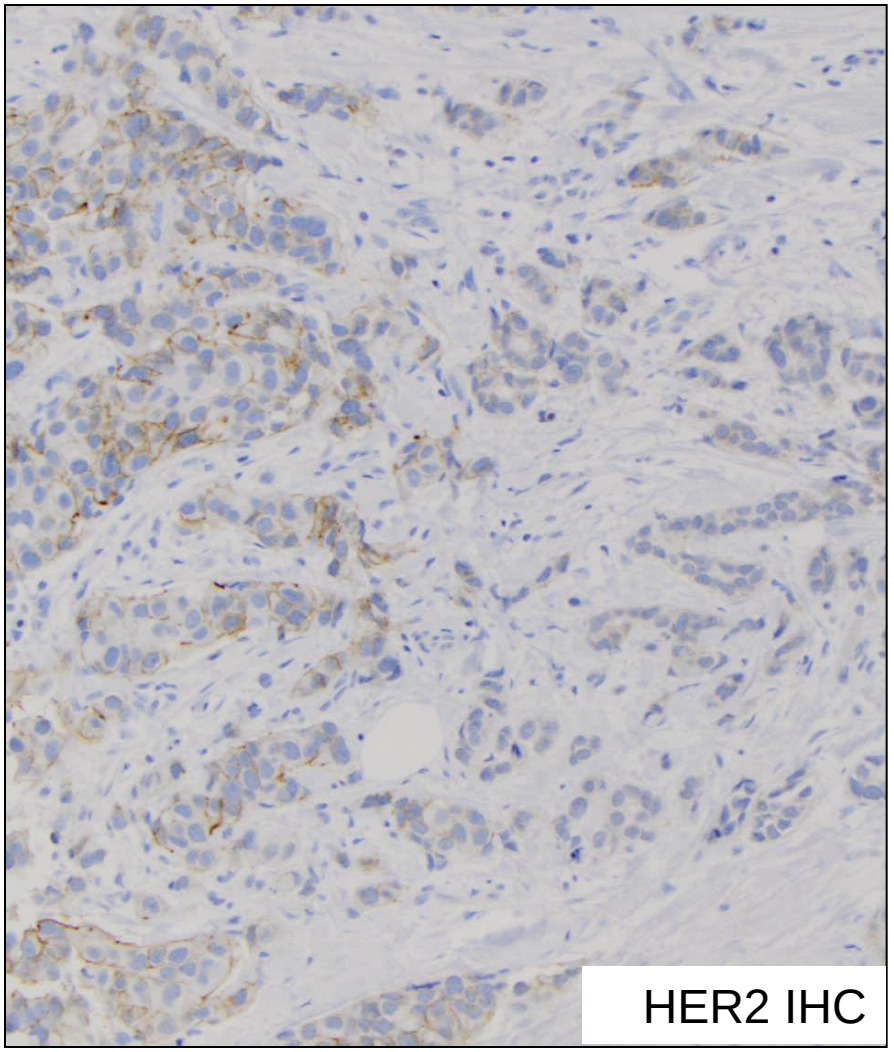
Negative result for HER2 IHC is 0 or 1+

- IHC 0: no staining
- IHC 1+: **weak, incomplete membrane staining in any proportion of tumor cells** or weak, complete staining in <10% of cells

2013 Guideline Update

Negative result for HER2 IHC is 0 or 1+

- **IHC 0**: No staining* or **incomplete membrane staining** (faint/barely perceptible) and **within ≤ 10%** of tumor cells
- **IHC 1+**: **Incomplete membrane** staining (faint/barely perceptible) and **within > 10%** of tumor cells



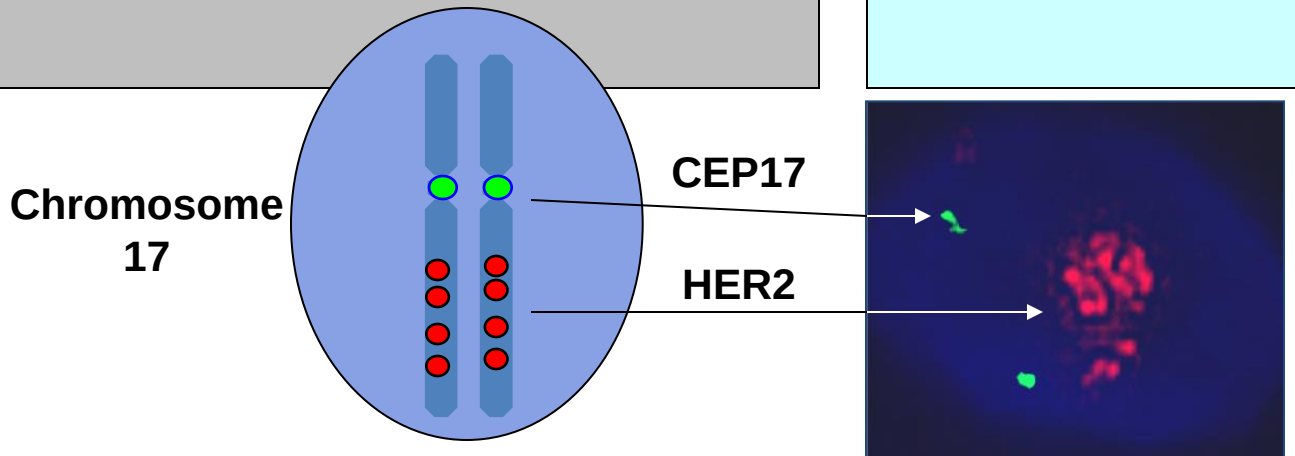
2013 HER2 Testing Positive Interpretation Criteria for ISH

2007 Guidelines

Positive for HER2 is FISH amplified (ratio of *HER2* to CEP17 of > 2.2 or average *HER2* gene copy number > six signals/nucleus for those test systems without an internal control probe).

2013 Guideline update

Positive for HER2 is ISH amplified ratio of HER2/CEP17 of **≥ 2.0** (with average HER2 signals >4) or if **average HER2** signals are **≥6 signals/cell** (regardless of ratio) in population of >10% of tumor cells.



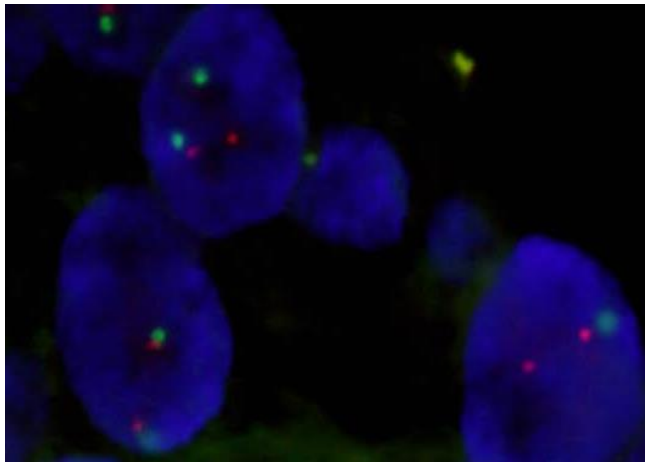
2013 HER2 Testing Negative Interpretation Criteria for ISH

2007 Guidelines

Negative for HER2 is FISH HER2/CEP17 ratio of < 1.8 or average *HER2* gene copy number of < 4 signals/nucleus for test systems without an internal control probe.

2013 Guideline update

Negative for HER2 ISH is HER2/CEP17 **ratio < 2 or HER2 signals/nucleus < 4**, regardless of ratio.



2013 HER2 Testing Equivocal Interpretation Criteria for IHC and ISH

2007 Guidelines

Equivocal for HER2 IHC is 2+

ISH: FISH HER2/CEP17 ratio of 1.8-2.2 or average *HER2* gene copy number 4-6 HER2 signals/nucleus for test systems without an internal control probe

2013 Guideline update

Must report **HER2 test result** as **Equivocal (HER2 tumor status Unknown)** and order reflex test using the alternative test if:

IHC: (2+) circumferential membrane staining, incomplete and/or weak/ moderate in >10% of the invasive tumor cells; *or* complete and circumferential membrane intense staining within ≤10% of the invasive tumor cells

ISH: Dual Probe *HER2*/CEP17 ratio <2.0 with an average *HER2* copy number ≥4.0 and <6.0 signals/cell

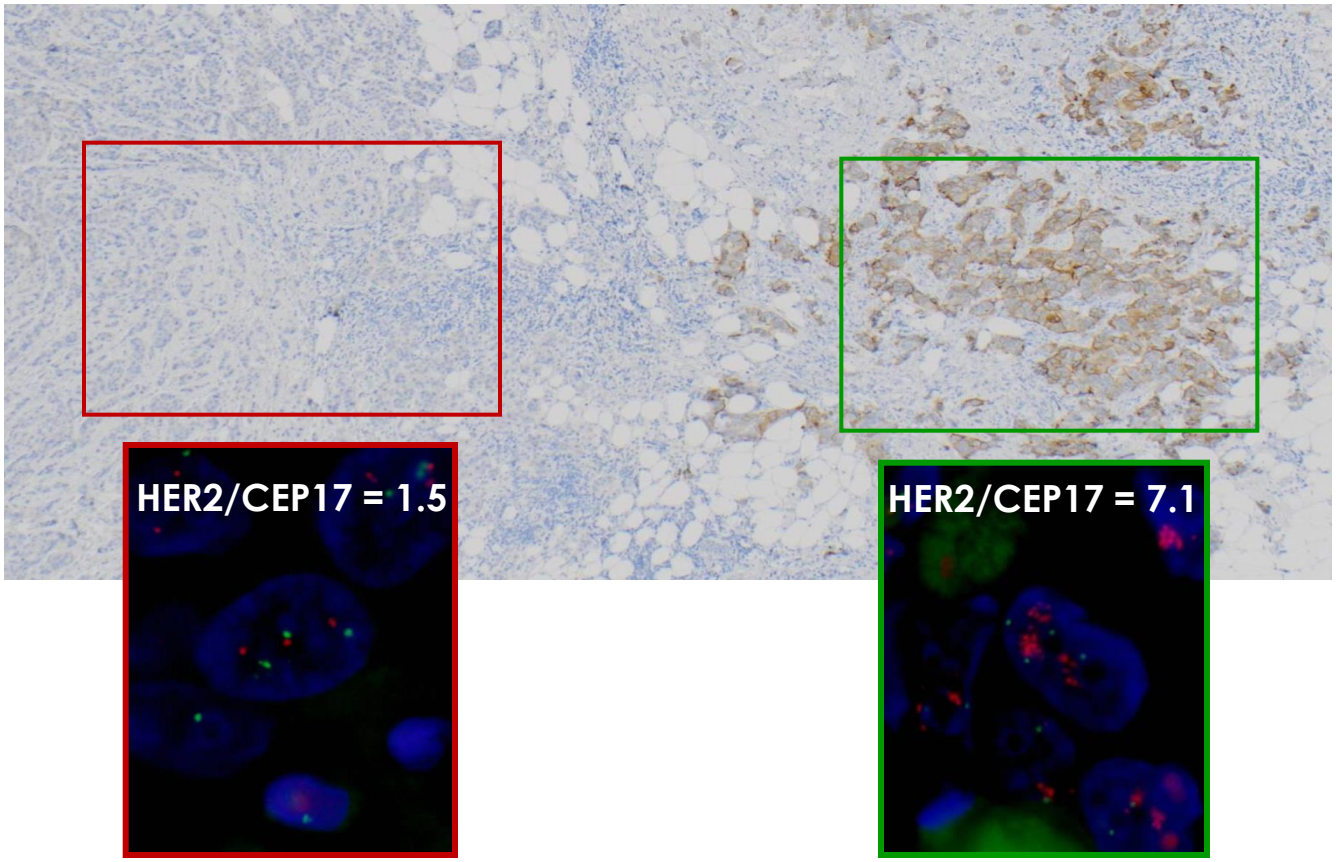
If a reflex test on a HER2 **Equivocal** result does not render a (+) or (-) HER2 result, must review clinical and pathologic features of case and should confer with the oncologist about additional testing.

2013 HER2 Testing Changes to Address Intratumoral Heterogeneity

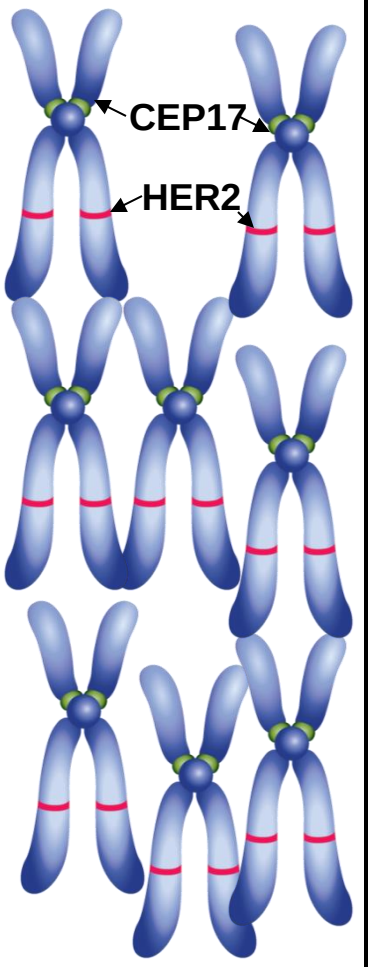
- **Intratumoral heterogeneity for HER2 can be seen in breast cancer by IHC and ISH.**
  - The fields selected for evaluation will determine whether or not the tumor is reported as ISH amplified.
  - Can lead to discordant results for HER2 analysis
    - Between IHC and ISH, cores vs excision, between blocks
  - Easier to detect with IHC (can be used to target ISH analysis)
- **Clinical significance of heterogeneity remains unclear however:**
  - Patients with HER2 IHC 3+ (10-30%) and ISH ratio (2-2.2) appear to benefit from treatment with HER2-targeted therapy.
  - It is important to carefully review all the pathologic features (grade, proliferative index, ER/PR &HER2 results) for such cases.

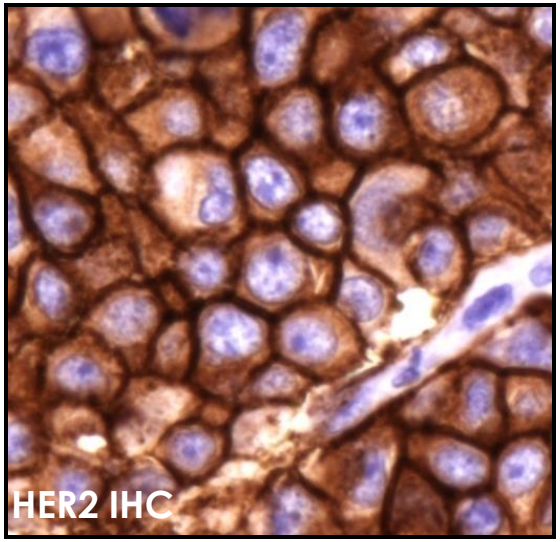
Hanna, et al., *Mod Pathol*. 2014 Jan;27(1):4-18.

2013 HER2 Guideline Definition, HER2 Positive: Heterogeneity



Chromosomal Abnormalities involving CEP17 (Aneusomy)





- **Polysomy 17 = increased copy number of HER2 & CEP17 signals**
  - Most frequently defined as average CEP17# >3 by ISH
  - HER2/CEP17 <2 (not amplified)
- **aCGH studies have shown true chromosome 17-polysomy is rare**
- **CEP17 copy number >3.0 in ISH is frequently related to gain or amplification of the centromeric region**
  - Typically high grade tumor and HER2 IHC is (2+) or (3+)
  - HER2/CEP17 ratio < 2 may be misleading in such cases

Hanna, et al., *Mod Pathol*. 2014 Jan;27(1):4-18, Tse, et al., *J Clin Oncol*. 2011 Nov 1;29(31):4168-74 .

2013 HER2 Testing Recommendations for Specimens with Aneusomy

- **Co-amplification of CEP17 region is observed in many ISH assays with increased HER2 and CEP17 copy #**
  - May lead to a HER2/CEP17 ratio < 2.0 suggesting lack of HER2 amplification and discordant IHC/ISH results
  - If the *HER2* copy number is ≥6, the HER2 test result must be reported as **Positive** regardless of the HER2/CEP17 ratio
  - HER2 amplification defined by ratio criterion (≥2), HER2 copy# criterion(≥6) or both
  - HER2 testing can be repeated with alternate chromosome reference probe to help to demonstrate an amplified ratio

Getting the Right Answer

Critical evaluation of every assay result

Do the ER, PgR and HER2 results fit with clinical profile for the patient?

Repeat Testing for ER/PgR Negative Results

- Postmenopausal patient
- Grade 1 tumor
- Low proliferative index
- Tubular histology
- Mucinous histology
- Classic lobular histology
- No internal positive control

Repeat Testing HER2-Negative Results IHC or ISH

- 50 years of age or less
- ER negative
- ER positive & PR low/negative
- High proliferative index (Ki-67 > 20%)
- Grade II or III
- Peritumoral lymphatic invasion

Discordance Between HER2 Result and Patient Profile

- A new HER2 test **should be** considered following a HER2 negative result, if the tumor has a high nuclear grade or Nottingham score.

- A new HER2 test **should not** be ordered if the following histopathologic findings occur and the initial HER2 test was negative:
  - Histologic grade 1 carcinoma of the following types:
    - Infiltrating ductal or lobular carcinoma, grade 1, ER and PgR positive
    - Tubular (at least 90% pure)
    - Mucinous (at least 90% pure)
    - Cribriform (at least 90% pure)
    - Adenoid cystic carcinoma (90% pure) and often triple negative

Troubleshooting Discrepant or Difficult to Interpret Cases

1. **Gather more information about the case**
   - Specimen handling & fixation information
   - Processing information
   - Interpretation information

2. **Determine the source of the issue**
   - **A. Technical problem**
     - Pre-analytical tissue requirements not met
     - Improper assay procedure
   - **B. Interpretation problem**
     - Artifacts present
     - Improper use of criterion
     - Lack of training
   - **C. Unusual tumor biology**

3. **Consult with the medical oncologist about the patient**

4. **Mitigate the problem**

Key Messages

- **Guidelines are living document which change**
  - From user feedback
  - From new publications and data
- **Iteration of guidelines leads to greater clarity**
- **Algorithm changes in the HER2 testing guideline update will provide better safety for patients**
  - Positive patients will be found and treated
  - Equivocal patients will have further work done to better define their HER2 status
  - Negative patients will be spared unnecessary treatment
  - Scrutiny of cases by physicians will find patients with unusual situations and generate discussion between Pathologists and Medical Oncologists