ASCO® Guidelines

HER2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists

Clinical Practice Guideline Focused Update

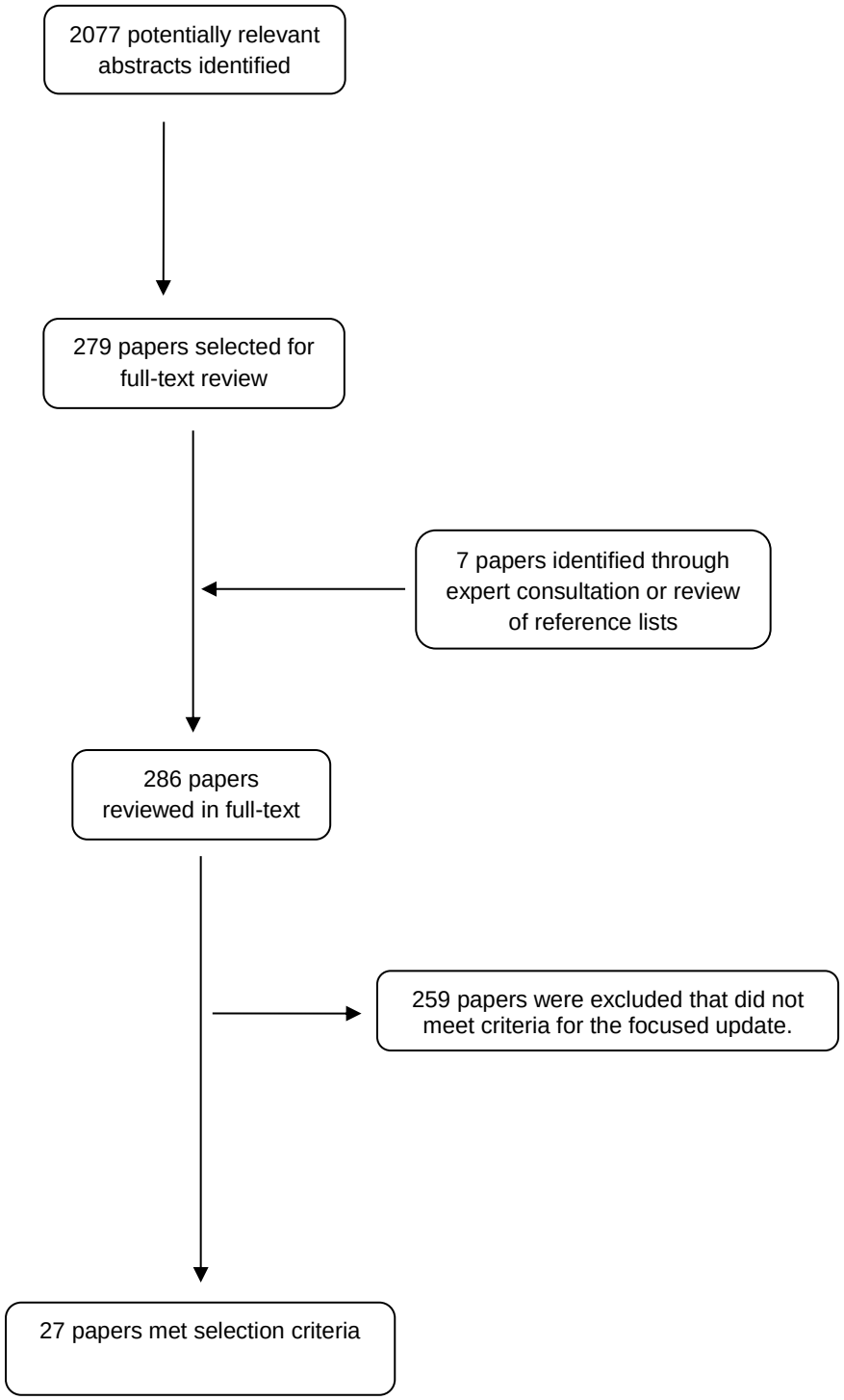
Data Supplement

Table of Contents

Data Supplement 1: Search String Strategy and Dates

(("Immunohistochemistry"[MeSH] OR immunohistochemistry[tiab] OR immunocytochemistry[tiab] OR “IHC”[tiab] OR "In Situ Hybridization, Fluorescence"[MeSH] OR “fluorescence in situ hybridization”[tiab] OR “fluorescence in-situ hybridization”[tiab] OR “FISH”[tiab] OR (chromogenic[tiab] AND hybridization[tiab]) OR “CISH”[tiab] OR ((gold-facilitated[tiab] OR autometallographic[tiab] OR “bright field”[tiab] OR bright-field[tiab]) AND hybridization[tiab]) OR “GOLDFISH”[tiab]) AND (Genes, erbB-2[MeSH] OR Receptor, erbB-2[MeSH] OR “Her-2”[tiab] OR “Her2”[tiab] OR “HER2”[tiab] OR “HER-2”[tiab] OR “erbB-2”[tiab] OR “erbB2”[tiab] OR “epidermal growth factor receptor- 2”[tiab] OR “epidermal growth factor receptor 2”[tiab] OR receptor, epidermal growth factor[mh] OR epidermal growth factor receptor-neu receptor[nm]) AND (Breast neoplasms[MeSH] OR “breast neoplasm*”[tiab] OR “breast cancer*”[tiab] OR “breast tumor*”[tiab] OR “breast tumour*”[tiab])) NOT (animals [mh] NOT human [mh]) AND English[la] AND ("2013/01/01"[PDat]: "2017/05/11"[PDat])

Data Supplement 2: QUOROM Diagram



Data Supplement 3: 2018 Focused Update Clinical Questions

1. What is the most appropriate definition for IHC 2+ (IHC Equivocal)?
2. Must HER2 testing be repeated on a surgical specimen if initially negative test on core biopsy?
3. Should invasive cancers with a HER2/CEP17 ratio ≥2.0 but an average HER2 copy number <4.0 signals/cell be considered ISH Positive?
4. Should invasive cancers with an average HER2 copy number ≥6.0 signals/cell but a HER2/CEP17 ratio <2.0 be considered ISH Positive?
5. What is the appropriate diagnostic work-up for invasive cancers with an average HER2 copy number ≥4.0 but <6.0 signals/cell and a HER2/CEP17 ratio <2.0 and initially deemed to have an equivocal HER2 ISH test result?

Data Supplement 4: 2013 All Clinical Questions

1. What is the optimal testing algorithm for the assessment of HER2 status?
2. What strategies can help ensure optimal performance, interpretation, and reporting of established assays?
    - Testing analytic validation requirements
    - Ongoing competency assessment
    - Reporting requirements
    - Regulatory framework
    - Optimal external quality assurance methods to ensure accuracy in HER2 testing and laboratory accreditation

Data Supplement 5: Research Survey

The HER2 Testing in Breast Cancer Guideline Focused Update Panel convened to address the observed frequency of equivocal cases to update the guideline recommendations**.** Several papers have been published that address these issues; however, they were from laboratories with very different patient populations. These papers could not be compared because of their varied focus points. Without real-world data gathered from diverse, large laboratories, the Steering Committee did not think that the full guideline panel could engage in a meaningful dialogue on this issue. Therefore, the guideline panel requested laboratories to complete a survey with their data before and after implementation of the 2013 guidelines. This survey is available in Appendix A.

After receiving several responses to the survey, the guideline panel held an open session where laboratories who completed the survey could present their data to the guideline panel. This survey and open session allowed for the panel to gain sufficient data about large numbers of patients affected by the guideline's recommendations. In conjunction with the published literature, the data received through the survey and open session helped inform the updated guideline recommendations.

Data Supplement 6: Open Comment Period

An open comment period was held from May 22, 2017 through June 19, 2017 on the CAP Web site www.cap.org. Five draft recommendations, three demographic questions, and one open-ended question were posted for peer review. The majority of respondents were pathologists (71.7%) from an academic/university setting (54.4%) within the United States (84.8%). Oncologists and others including industry personnel, technologists and patient advocates from non-academic settings including Asia and Europe also participated. In addition to the general membership of both ASCO and CAP, an announcement was sent to the following entities deemed to have interest:

- American Cancer Society (ACS)
- American College of Medical Genetics and Genomics (ACMG)
- American Society for Clinical Pathology (ASCP)
- Association of Directors of Anatomic and Surgical Pathology (ADASP)
- Association of Molecular Pathology (AMP)
- Association of Pathology Chairs (APC)
- Breast Cancer Action
- Breast Cancer Trials
- Canadian Association of Pathologists Association - Canadienne des pathologists (CAP-ACP)
- Canadian Partnership Against Cancer (CPAC)
- Cancer Leadership Council (CLC)
- Cancer Research and Prevention Foundation (Prevent Cancer Foundation)
- Center for Disease Control and Prevention (CDC) - Division of Laboratory Programs, Standards, and Services
- Centers for Medicare and Medicaid Services (CMS)
- European Society for Medical Oncology (ESMO)
- Facing our Risk of Cancer Empowered (FORCE)
- Genentech (South San Francisco, CA)
- International Society for Immunohistochemistry and Molecular Morphology (ISIMM)
- Kaiser Permanente / Kaiser Family Foundation

- Living Beyond Breast Cancer
- Metastatic Breast Cancer Network
- METAvivor
- My Cancer Genome
- National Comprehensive Cancer Network (NCCN)
- Society of Surgical Oncology (SSO)
- Society to Improve Diagnosis in Medicine (SIDM)
- United States and Canadian Academy of Pathology (USCAP)
- Women's Empowerment Cancer Advocacy Network (WE CAN)
- Young Survival Coalition

Response categories of "Agree", "Agree with comments" and "Disagree with comments" were captured for every proposed recommendation with 174 written comments received. Three out of the five draft recommendations achieved at least 80% agreement (clinical questions 1, 2 and 3). One draft recommendation did not achieve 70% agreement (clinical question 4) and the following draft recommendation (clinical question 5) had repeat comments stemming from that one which accounted for the 78% agreement rate. The co-chairs reviewed the participant comments and agreed that more clarification and visual algorithms were needed to assist the understanding of the recommendations. After consideration and deliberation two draft recommendations were maintained with the original language (clinical questions 1-2) and three were revised for clarity (clinical questions 3-5).

Data Supplement 7

Data Supplement 7A: Types of Assays for Inclusion

There was insufficient evidence to warrant inclusion of mRNA assays (e.g., using rtPCR) to determine HER2 status in unselected patients. However, the Update Committee endorses the use of FDA-cleared bright-field ISH assays for the following reasons: (a) the test is measuring a parameter (gene amplification) with demonstrable clinical utility to identify patients likely to benefit from HER2-targeted therapies; (b) A consistent body of evidence shows that bright-field ISH has high concordance levels with other ISH methods using fluorescence (FISH) to measure HER2 gene amplification from ring studies, cohort studies, and external quality assessment (EQA) schemes; (c) and, Assays appear reproducible across sites. If a CLIA certified laboratory wishes to use an LDT form of bright-field ISH, the assay must be analytically validated in the laboratory using it and documentation of the clinical validity of the assay must be available.

Data Supplement 7B: Heterogeneity

Any aggregate population of amplified cells comprising >10% of the total tumor cell population on the slide must be separately counted. The number of CEP17 and HER2 signals should be counted in a minimum of 20 nonoverlapping and contiguous invasive cancer cell nuclei in at least 2 tumor areas of each population of tumor cells (unamplified and amplified areas). The HER2/CEP17 ratio should then be calculated for each population of cells individually including the average HER2 signals/cell and ratio of HER2 signals/CEP17 signals, if available. Cases containing amplified and nonamplified areas (using this definition) should be reported as Positive for HER2. The percentage of the total tumor population with amplification should also be reported.

Data Supplement 8: Pre-analytic Issues

- **Time to fixation (cold ischemic time):** Because of the potential importance of biomarkers for determining the most appropriate treatment options for certain patients, there is a need for standardizing pre-analytic variables, with the goal of developing standardized methods of tissue procurement and processing, and documenting how these variables affect the quality of tissue for biomarker testing and molecular analysis. Recent reports have suggested that excessive delay from tissue collection to the initiation of formalin fixation has the potential to adversely impact the analysis of hormone receptor assays and HER2 analysis. [1,2] Khoury et al. [2] analyzed 10 resected breast cancers and suggested that delays to the start of fixation of 60 to 120 minutes may compromise the accurate analysis of ER, PgR and HER2 FISH due to loss of staining or hybridization signal intensity. The implications of these findings are that some tumors with excessive cold ischemic times may be falsely classified as negative for the expression of these important therapeutic targets.

- **Duration of tissue fixation:** A number of recent studies have addressed the issue of prolonged tissue fixation, including two prospective validation studies that compared the results of ER, PR and HER2 studies on tissue fixed for a standard amount of time with tissue from the same samples that underwent prolonged fixation (72 to 96 hours).[3,4] The data from these and other studies suggest that formalin fixation for up to 72 hours does not appear to have any impact on ER, PgR and HER2 reactivity and therefore is an acceptable upper limit of time in routine clinical practice. The immunoreactivity of breast prognostic markers testing for ER, PgR and HER2 may be reduced by very long, extended formalin over-fixation that is not clinically relevant. It is important to measure time to fixative (including sectioning) and time in fixative. It is recommended that the time from removal from the patient to incision of the specimen be as short as possible, ideally within 1 hour.

Data Supplement 9: International Quality Assurance Program links

UK NEQAS http://www.ukneqas.org.uk

CAP http://www.cap.org

Pathology Australia - http://pathologyaustralia.com.au/

European Society of Pathology - https://www.esp-pathology.org/

NordiQC - http://www.nordiqc.org/

References

1. Nkoy FL, Hammond ME, Rees W, et al: Variable specimen handling affects hormone receptor test results in women with breast cancer: a large multihospital retrospective study. Arch Pathol Lab Med 134:606-12, 2010
2. Khoury T, Sait S, Hwang H, et al: Delay to formalin fixation effect on breast biomarkers. Mod Pathol 22:1457-67, 2009
3. Tong LC, Nelson N, Tsourigiannis J, et al: The effect of prolonged fixation on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast cancer: a prospective study. Am J Surg Pathol 35:545-52, 2011
4. Yildiz-Aktas IZ, Dabbs DJ, Cooper KL, et al: The effect of 96-hour formalin fixation on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma. Am J Clin Pathol 137:691-8, 2012

Appendix A. Survey

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Breast Cancer HER2 Testing Survey

Background & Patient Population

Please note questions marked with an * are required.

* 1. Name of Laboratory

* 2. Name of individual completing this questionnaire

This questionnaire should be completed in its entirety twice. The **"Year 1"** reporting period should be a one-year period **prior** to your laboratory's implementation of the ASCO/CAP HER 2 Testing Guideline 2013 Update. The **"Year 2"** reporting period should be a one-year period a**fter** your laboratory had implemented the ASCO/CAP HER 2 Testing Guideline 2013 Update. These questions refer **only to HER2 testing of breast cancer.**

* 3. Indicate the reporting period for which you are completing this questionnaire

- Year 1
- Year 2

* 4. Start date of the one year period for which you are reporting

Date / Time MM / DD / YYYY

* 5. End date of the one year period for which you are reporting:

	MM	DD	YYYY
Date / Time		/	/

Respond to items 6-10 to describe the patient population served by your laboratory during this reporting period.

6. Tested subpopulations. *Indicate the number of patients entering from each of the following subpopulations.*

Tested as part of primary diagnosis (all markers done at the pathology lab of your institution)

Referred for HER2 testing (HER2) is being done by the pathology lab at your institution)

Consultation on previously performed HER2 test (initial HER2 test by IHC or another ISH test already done)

7. Patient age. *Indicate the number of patients in the following age categories.*

Age < 45	
45 ≤ Age < 55	
55 ≤ Age < 65	
65 ≤ Age < 75	
Age ≥ 75	
Age unknown	

8. Node status. *Indicate the number of patients in the following nodal status categories.*

Node positive disease (any number of positive nodes)

Node negative disease (no positive nodes)

Node status unknown

9. Tumor grade (Bloom-Scharff-Richardson schema preferred if available, or similar). *Indicate the number of patients in the following histologic grade categories.*

Grade I	
Grade II	
Grade III	

10. Initial testing result, irrespective of type of test used. Indicate number of cases for which the following test results were observed on the first test.

Positive	
Negative	
Equivocal	
Indeterminate (non-evaluable due to specimen or assay failure)	

* 11. Was IHC performed in your laboratory during this reporting period?

- ◯ Yes
- ◯ No

Breast Cancer HER2 Testing Survey

IHC

1. Indicate the number of HER2 IHC tests that were performed by your laboratory during this reporting period.

Primary HER2 IHC test used (Questions 2-5):

2. Antibody clone

3. Regulatory status of the test used

4. If an FDA-approved or cleared test kit is used, provide the name/company:

5. Is image analysis used for quantification?

- Yes
- No

6. Indicate the number of biopsies of each type for HER2 IHC.

Cytology	
Core	
Excision	

7. Indicate the number of each specimen source for HER2 IHC.

Primary cancer - Total	
Primary Cancer - Breast	
Primary Cancer - Lymph Node	
Metastasis	

8. Initial testing results for cases first tested by IHC. Indicate number of cases initially tested by IHC for which the following test results were observed on the first test.

0	
1+	
2+	
3+	
Indeterminate (non-evaluable due to specimen or assay failure)	

Breast Cancer HER2 Testing Survey

* 1. Was FISH performed in your laboratory during this reporting period?

- ◯ Yes
- ◯ No

Breast Cancer HER2 Testing Survey

FISH

1. Indicate the number of HER2 FISH tests that were performed by your laboratory during this reporting period.

Primary HER2 FISH test used (Questions 2-5):

2. Is single or dual probe used

- ◯ Single
- ◯ Dual

3. Specify probe(s)

4. Regulatory status of the test used

5. If an FDA-approved or cleared test kit is used, provide the name/company:

6. Indicate the number of biopsies of each type for HER2 FISH

Cytology	
Core	
Excision	

7. Indicate the number of each specimen source for HER2 FISH.

Primary cancer	
Breast	
Lymph Node	
Metastasis	

8. Initial testing results for cases first tested by FISH.

How was “positive” defined on this assay as performed in your laboratory during this reporting period?

How was “negative” defined on this assay as performed in your laboratory during this reporting period?

How was “equivocal” defined on this assay as performed in your laboratory during this reporting period?

9. Were there any exceptions to the rules you have defined above?

- ◯ Yes
- ◯ No

If yes, explain:

10. Did you use alternative CEP 17 probes to resolve equivocal cases?

- ◯ Yes
- ◯ No

If yes, explain:

11. Did you use alternative CEP 17 probes for any other reason?

- ◯ Yes
- ◯ No

If yes, explain:

12. Indicate number of cases initially tested by FISH for which the following test results were observed on the first test.

Positive

Negative

Equivocal

Indeterminate (non-evaluable due to specimen or assay failure)

Among all cases initially tested with FISH and producing evaluable results, what numbers were observed in each of the following categories?

13. If DUAL probe FISH was used: (ACN = average HER2 copy number or signals.)

Ratio ≥ 2.0	
Ratio < 2.0	
ACN ≥ 4.0	
ACN < 4.0	
ACN ≥ 6.0	
ACN < 6.0	

14. If SINGLE probe FISH was used:

ACN < 4.0	
4.0 ≥ ACN < 6.0	
ACN ≥ 6.0	

Breast Cancer HER2 Testing Survey

* 1. Was CISH performed in your laboratory during this reporting period?

- ◯ Yes
- ◯ No

Breast Cancer HER2 Testing Survey

CISH

1. Indicate the number of HER2 CISH tests that were performed by your laboratory during this reporting period.

Primary HER2 CISH test used (Questions 2-5):

2. Is single or dual probe used

- ◯ Single
- ◯ Dual

3. Specify probe(s)

4. Regulatory status of the test used

5. If an FDA-approved or cleared test kit is used, provide the name/company:

6. Indicate the number of biopsies of each type for HER2 CISH.

Cytology	
Core	
Excision	

7. Indicate the number of each specimen source for HER2 CISH.

Primary cancer - Total	
Primary cancer - Breast	
Primary cancer - Lymph Node	
Metastasis	

8. Initial testing results for cases first tested by CISH.

How was “positive” defined on this assay as performed in your laboratory during this reporting period?	
How was “negative” defined on this assay as performed in your laboratory during this reporting period?	
How was “equivocal” defined on this assay as performed in your laboratory during this reporting period?	

9. Were there any exceptions to the rules you have defined above?

◯ Yes ◯ No

If yes, explain:

10. Did you use alternative CEP 17 probes to resolve equivocal cases?

◯ Yes ◯ No

If yes, explain:

11. Did you use alternative CEP 17 probes for any other reason?

◯ Yes ◯ No

If yes, explain:

12. Indicate number of cases initially tested by CISH for which the following test results were observed on the first test.

Positive	
Negative	
Equivocal	
Indeterminate (non-evaluable due to specimen or assay failure)	

Among all cases initially tested with CISH and producing evaluable results, what numbers were observed in each of the following categories:

13. If DUAL probe CISH was used: (ACN = average HER2 copy number or signals.)

Ratio ≥ 2.0	
Ratio < 2.0	
ACN ≥ 4.0	
ACN < 4.0	
ACN ≥ 6.0	
ACN < 6.0	

14. If SINGLE probe CISH was used:

ACN < 4.0	
4.0 ≥ ACN < 6.0	
ACN ≥ 6.0	

Considering all HER2 testing of breast cancer performed in your laboratory during this reporting period, for which an **ADDITIONAL HER2 test had to be performed**, respond to questions 1-5 about cases that underwent additional testing.

1. For what number of cases for which you performed two tests was a new specimen obtained for the second test (e.g., a new tumor block, a different specimen like lymph node, core biopsy, or surgical breast specimen) instead of simply using additional sections from the same block used for initial testing?

2. For what numbers of cases did you perform two tests on the same block rather than accessing a new specimen?

3. Indicate the number of re-tested cases for which the following outcomes were observed on the second test, irrespective of testing method used:

Postive	
Negative	
Equivocal	

4. Comment about typical reasons for equivocal results observed on re-tests:

5. If tumor heterogeneity observed, please indicate:

In how many cases was tumor heterogeneity observed	
What general criteria for tumor heterogeneity did you apply	

6. Comment on any interesting cases, with respect to unusual testing results, that you encountered during this reporting period that you feel might be useful for the committee to hear about.

Thank you for completing this survey. To submit your responses, please select "Done" below.

Please remember to make submissions both for Year 1 and Year 2. If you have any questions or additional comments, please feel free to email guidelines@asco.org