ASCO® Guidelines

COLLEGE of AMERICAN PATHOLOGISTS

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: ASCO-CAP Guideline Update				
Topic	**Recommendation**	**Type**	**Evidence Quality**	**Strength**
The recommendations in previous (2013 and 2018) ASCO-CAP HER2 testing guideline updates are affirmed.				
Specimens to be tested	All newly diagnosed patients with breast cancer must have a HER2 test performed. Patients who then develop metastatic disease must have a HER2 test performed in a metastatic site, if tissue sample is available.	-	-	-
Optimal algorithm for HER2 testing	IHC 2+ (equivocal) is invasive breast cancer with weak to moderate complete membrane staining observed in >10% of tumor cells.	EB	H	S
	On the basis of some criteria (including a tumor grade 3), if the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test may be ordered on the excision specimen.	EB	H	S
	If a case has a HER2/CEP17 ratio is ≥2.0 but the average *HER2* signals/cell is <4.0, a definitive diagnosis will be rendered based on additional workup. If not already assessed by the institution/laboratory performing the ISH test, IHC testing for HER2 should be performed using sections from the same tissue sample used for ISH and the slides from both ISH and IHC be reviewed together to guide the selection of areas to score by ISH (local practice considerations will dictate the best procedure to accomplish this concomitant assessment): **a.** If the IHC result is 3+, diagnosis is HER2 positive. **b.** If the IHC result is 2+, recount ISH by having an additional observer, blinded to previous ISH results, count at least 20 cells that includes the area of invasive cancer with IHC 2+ staining: - If reviewing the count by the additional observer changes the result into another ISH category, the result should be adjudicated per internal procedures to define the final category. - If the count remains an average of <4.0 *HER2* signals/cell and *HER2*/CEP17 ratio ≥2.0, the diagnosis is HER2 negative with a comment.[a] **c.** If the IHC result is 0 or 1+, diagnosis is HER2 negative with a comment. [a]	EB	I	S
	If a case has an average of ≥6.0 *HER2* signals/cell with a *HER2*/CEP17 ratio of <2.0, formerly diagnosed as ISH positive for HER2, a definitive diagnosis will be rendered based on additional workup. If not already assessed by the institution/lab performing the ISH test, IHC testing for HER2 should be performed using sections from the same tissue sample used for ISH and the slides from both ISH and	EB	I	S

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: ASCO-CAP Guideline Update				
Topic	**Recommendation**	**Type**	**Evidence Quality**	**Strength**
	IHC be reviewed together to guide the selection of areas to score by ISH (local practice considerations will dictate the best procedure to accomplish this concomitant review): **a.** If the IHC result is 3+, diagnosis is HER2 positive. **b.** If the IHC result is 2+, recount ISH by having an additional observer, blinded to previous ISH results, count at least 20 cells that includes the area of invasion with IHC 2+ staining: - If reviewing the count by the additional observer changes the result into another ISH category, the result should be adjudicated per internal procedures to define the final category. - If the *HER2*/CEP17 ratio remains <2.0 with ≥6.0 *HER2* signals/cell, the diagnosis is HER2 positive. **c.** If the IHC result is 0 or 1+, diagnosis is HER2 negative with comment.[b]			
	If the case has an average *HER2* signals/tumor cell of ≥4.0 and <6.0 and the HER2/CEP17 ratio is <2.0, formerly diagnosed as ISH equivocal for HER2, a definitive diagnosis will be rendered based on additional workup. If not already assessed by the institution/laboratory performing the ISH test, IHC testing for HER2 should be performed using sections from the same tissue sample used for ISH and the slides from both ISH and IHC be reviewed together to guide the selection of areas to score by ISH (local practice considerations will dictate the best procedure to accomplish this concomitant review): **a.** If the IHC result is 3+, diagnosis is HER2 positive. **b.** If the IHC result is 2+, recount ISH by having an additional observer, blinded to previous ISH results, count at least 20 cells that includes the area of invasion with IHC 2+ staining: - If reviewing the count by the additional observer changes the result into another ISH category, the result should be adjudicated per internal procedures to define the final category. - If the count remains an average of ≥4.0 and <6.0 *HER2* signals/cell with *HER2*/CEP17 ratio <2.0, the diagnosis is HER2 negative with a comment.[c] **c.** If the IHC result is 0 or 1+, diagnosis is HER2 negative with a comment.[c]	EB	I	S
ISH rejection criteria	Test is rejected and repeated if: • Controls are not as expected • Observer cannot find and count at least two areas of invasive tumor • > 25% of signals are unscorable due to weak signals • > 10% of signals occur over cytoplasm • Nuclear resolution is poor • Autofluorescence is strong Report HER2 test result as Indeterminate as per parameters described.	-	-	-

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: ASCO-CAP Guideline Update				
Topic	**Recommendation**	**Type**	**Evidence Quality**	**Strength**
ISH interpretation	The pathologist should scan the entire ISH slide prior to counting at least 20 cells or use IHC to define the areas of potential *HER2* amplification. If there is a second population of contiguous cells with increased *HER2* signals/cell and this cell population consists of > 10% of tumor cells on the slide (defined by image analysis or visual estimation of the ISH or IHC slide), a separate counting of at least 20 nonoverlapping cells must also be performed within this cell population and reported.	-	-	-
Acceptable (IHC and ISH) tests	Should preferentially use an FDA-approved IHC, brightfield ISH, or FISH assay.	-	-	-
IHC rejection criteria	Test is rejected and repeated or tested by FISH if: • Controls are not as expected • Artifacts involve most of sample • Sample has strong membrane staining of normal breast ducts (internal controls)	-	-	-
IHC interpretation criteria	Should interpret IHC test using a threshold of more than 10% of tumor cells that must show homogeneous, dark circumferential (chicken wire) pattern to call result 3+, HER2 positive.	-	-	-
Reporting requirements for all assay types	Report must include guideline-detailed elements except for changes to reporting requirement and algorithms defined in this table.	-	-	-
Optimal tissue handling requirements	Time from tissue acquisition to fixation should be as short as possible; samples for HER2 testing are fixed in 10% neutral buffered formalin for 6-72 hours; cytology specimens must be fixed in formalin. Samples should be sliced at 5- to 10-mm intervals after appropriate gross inspection and margins designation and placed in sufficient volume of neutral buffered formalin. Any exceptions to this process must be included in report.	-	-	-
Optimal tissue sectioning requirements	Sections should ideally not be used for HER2 testing if cut > 6 weeks earlier; this may vary with primary fixation or storage conditions	-	-	-
Optimal internal validation procedure	Validation of test must be performed before test is offered	-	-	-

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: ASCO-CAP Guideline Update				
Topic	**Recommendation**	**Type**	**Evidence Quality**	**Strength**
Optimal initial test validation	Laboratories performing these tests should be following all accreditation requirements, one of which is initial testing validation. The laboratory should ensure that initial validation conforms to the published 2010 ASCO-CAP Recommendations for IHC Testing of ER and PgR guideline validation requirements with 20 negative and 20 positive for FDA-approved assays and 40 negative and 40 positive for LDTs. This requirement does not apply to assays that were previously validated in conformance with the 2007 ASCO-CAP HER2 testing guideline, and who are routinely participating in external proficiency testing for HER2 tests, such as the program offered by the CAP.	-	-	-
	Laboratories are responsible for ensuring the reliability and accuracy of their testing results, by compliance with accreditation and proficiency testing requirements for HER2 testing assays. Specific concordance requirements are not required.	-	-	-
Optimal monitoring of test concordance between methods	See text following under "Optimal Laboratory Accreditation" below.	-	-	-
Optimal internal QA procedures	Should review and document external and internal controls with each test and each batch of tests. • Ongoing quality control and equipment maintenance • Initial and ongoing laboratory personnel training and competency assessment • Use of standardized operating procedures including routine use of control materials • Revalidation of procedure if changed • Should perform ongoing competency assessment and document the actions taken as a part of the laboratory record.	-	-	-
Optimal external proficiency assessment	Participation in and successful completion of external proficiency testing program with at least two testing events (mailings) a year • Satisfactory performance requires at least 90% correct responses on graded challenges for either test • Unsatisfactory performance will require laboratory to respond according to accreditation agency program requirements	-	-	-
Optimal laboratory accreditation	Onsite inspection every other year with annual requirement for self-inspection • Reviews laboratory validation, procedures, QA results and processes, results and reports • Unsatisfactory performance results in suspension of laboratory testing for HER2 for that method	-	-	-

Abbreviations. ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; CEP17, chromosome enumeration probe 17; EB, evidence based; ER, estrogen receptor; FDA, US Food and Drug Administration; FISH, fluorescent in situ hybridization; H, high; HER2, human epidermal growth factor receptor 2; I, intermediate; IHC, immunohistochemistry; ISH, in situ hybridization; LDT, laboratory-developed test; PgR, progesterone receptor; QA, quality assurance; S, strong

Notes. [a] Evidence is limited on the efficacy of HER2-targeted therapy in the small subset of cases with a *HER2*/CEP17 ratio ≥ 2.0 and an average *HER2* copy number of < 4.0 per cell. In the first generation of adjuvant trastuzumab trials, patients in this subgroup who were randomly assigned to the trastuzumab arm did not seem to derive an improvement in disease-free or overall survival, but there were too few such cases to draw definitive conclusions. IHC expression for HER2 should be used to complement ISH and define HER2 status. If the IHC result is not 3+ positive, it is recommended that the specimen be considered HER2 negative because of the low *HER2* copy number by ISH and the lack of protein overexpression.

[b] There are insufficient data on the efficacy of HER2‐targeted therapy in cases with a HER2 ratio of < 2.0 in the absence of protein overexpression because such patients were not eligible for the first generation of adjuvant trastuzumab clinical trials. When concurrent IHC results are negative (0 or 1+), it is recommended that the specimen be considered HER2 negative.

[c] It is uncertain whether patients with an average of ≥ 4.0 and < 6.0 *HER2* signals per cell and a *HER2*/CEP17 ratio of < 2.0 benefit from HER2-targeted therapy in the absence of protein overexpression (IHC 3+). If the specimen test result is close to the ISH ratio threshold for positive, there is a higher likelihood that repeat testing will result in different results by chance alone. Therefore, when IHC results are not 3+ positive, it is recommended that the sample be considered HER2 negative without additional testing on the same specimen