ASCO–CAP HER2 Test Guideline Recommendations

Summary of Guideline 2007 and 2013 Recommendations

Topic	2007 Recommendation	2013 Recommendation
Specimens to be tested	All primary breast cancer specimens and metastases should have at least one HER2 test performed	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	Positive for HER2 is either IHC HER2 3+ (defined as uniform intense membrane staining of > 30% of invasive tumor cells) or 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	Must report **HER2 test result as positive for HER2 if:** [a,b] ● IHC 3+ based on circumferential membrane staining that is complete, intense [c,d] ● ISH positive based on: Single-probe average *HER2* copy number ≥6.0 signals/cell.[c,e] Dual-probe *HER2*/CEP17 ratio ≥2.0;[c,e] with an average *HER2* copy number ≥4.0 signals/cell Dual-probe *HER2*/CEP17 ratio ≥2.0;[c,e] with an average *HER2* copy number <4.0 signals/cell[b] Dual-probe *HER2*/CEP17 ratio < 2.0;[c,e] with an average *HER2* copy number ≥6.0 signals/cell
	Equivocal for HER2 is defined as: IHC 2+ or 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	Must report **HER2 test result as equivocal** and order reflex test (same specimen using the alternative test) **or** new test (new specimen, if available, using same or alternative test) if: [a,b] ● IHC 2+ based on circumferential membrane staining that is incomplete and/or weak/moderate[f] and within >10% of the invasive tumor cells;[d] *or* complete and circumferential membrane staining that is intense and within ≤10% of the invasive tumor cells [d] ● ISH equivocal based on: Single-probe ISH average *HER2* copy number ≥4.0 and <6.0 signals/cell[e,f] Dual-probe *HER2*/CEP17 ratio <2.0 with an average *HER2* copy number ≥4.0 and <6.0 signals/cell[e,f]

Abbreviations: HER2, human epidermal growth factor receptor; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; ISH, in situ hybridization; QA, quality assurance; NBF, neutral buffered formalin; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists

Wolff AC, Hammond ME, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update.

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	Negative for HER2 is defined as: • IHC HER2 0: no staining • IHC HER2 1+: Weak incomplete membrane staining in any proportion of tumor cells or weak, complete membrane staining in <10% of cells • 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	Must report a **HER2 test result as negative** if a single test (or both tests) performed show:[a,b] • IHC 1+ as defined by incomplete membrane staining that is faint/barely perceptible and within >10% of the invasive tumor cells[d] • IHC 0 as defined by no staining observed[d] or membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of the invasive tumor cells[d] • ISH negative based on: Single-probe average *HER2* copy number <4.0 signals/cell Dual-probe *HER2*/CEP17 ratio <2.0 with an average *HER2* copy number <4.0 signals/cell
	Indeterminate for HER2	Must report **HER2 test result as indeterminate** if technical issues prevent one or both tests (IHC and ISH) from being reported as positive, negative, or equivocal. Conditions may include: • Inadequate specimen handling • Artifacts (crush or edge artifacts) that make interpretation difficult • Analytic testing failure Another specimen should be requested for testing to determine HER2 status. Reason for indeterminate testing should be noted in a comment in the report.
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	Same and report **HER2 test result as Indeterminate** as per parameters described above.

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ISH interpretation	Interpretation performed by counting at least 20 cells; a pathologist must confirm that counting involved invasive tumor criteria followed	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 cells with increased *HER2* signals/cell and this cell population consists of more than 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. For brightfield ISH, counting requires comparison between patterns in normal breast and tumor cells because artifactual patterns may be seen that are difficult to interpret. If tumor cell pattern is neither normal nor clearly amplified, test should be submitted for expert opinion.
Acceptable [IHC and ISH] tests[g]		Should preferentially use an FDA-approved IHC, brightfield ISH, or FISH assay.[g,h]
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)	Same
IHC interpretation criteria	Positive HER2 result requires homogeneous, dark circumferential (chicken wire) pattern in > 30% of invasive tumor. Interpreters have method to maintain consistency and competency	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	Same except for changes to reporting requirement and algorithms defined in this table. (Data Supplements 9 and 10)
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 6–48 hours; cytology specimens must be fixed in formalin. Samples should be sliced at 5-mm to10-mm intervals after appropriate gross inspection and margins designation and placed in sufficient volume of neutral buffered formalin	Duration of fixation has been changed **from 6–48 hours to 6–72 hours.** Any exceptions to this process must be included in report.

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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	Same
Optimal internal validation procedure	Validation of test must be done before test is offered	Same Data Supplement 12 lists examples of various external quality assurance schemes.
Optimal initial test validation	Initial test validation requires 25–100 samples tested by alternative validated method in the same laboratory or by validated method in another laboratory	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 (Data Supplement 12).
	Proof of initial testing validation in which positive and negative HER2 categories are 90% concordant with alternative validated method or same validated method for HER2	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. (Data Supplement 11)
Optimal monitoring of test concordance between methods	Concordance testing must be done prior to initiation of testing, optimally as the form of testing validation. If concordance is below 95% for any testing category, that category of test result of either FISH or IHC must be automatically flexed to alternative method before final interpretation.	See text following under "Optimal Laboratory Accreditation"
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	Same.
	Initial and ongoing laboratory personnel training and competency assessment	Same.
	Use of standardized operating procedures including routine use of control materials	Same.
	Revalidation of procedure if changed	Same.
	Ongoing competency assessment and education of pathologists	Should perform ongoing competency assessment and document the actions taken as a part of the laboratory record.

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Optimal external proficiency assessment	Participation in and successful completion of external proficiency testing program with at least two testing events (mailings) a year	Same.
	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	Same.
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	Same (Data Supplement 11).

Notes

[a] If a reflex test (same specimen/same tissue) ordered after an initial equivocal HER2 test result does not render a positive or negative HER2 test result, the pathologist should review histopathologic features, confer if possible with the oncologist regarding additional HER2 testing, and document it in the pathology report. The pathologist may pursue additional HER2 testing without conferring with the oncologist. This should be accomplished using: (1) the alternative test (IHC and ISH) on the same specimen, (2) either test on another block (same specimen), or (3) either test on another specimen (eg, core biopsy, surgical resection, lymph node, and/or metastatic site). Because the decision to recommend HER2-targeted therapy requires a HER2-positive test result, additional HER2 testing should be attempted in equivocal specimens to attempt to obtain a positive or negative HER2 test result and most accurately determine the HER2 status of the tumor specimen.

[b] See Data Supplement 2E for additional information on rare scenarios.

[c] Observed in a homogeneous and contiguous population and within >10% of the invasive tumor cells.

[d] Readily appreciated using a low-power objective.

[e] By counting at least 20 cells within the area.

[f] Observed in a homogeneous and contiguous population.

[g] Alternatively, a laboratory accredited by the CAP or another accrediting entity may choose to use an LDT, in which case its analytical performance must be documented in the same clinical laboratory that will use the assay, and documentation of analytical validity of the assay must be available.

[g] A list of HER2 assays approved by the FDA as in vitro companion diagnostic devices to aid in the assessment of patients for whom trastuzumab treatment is being considered can be found in the Medical Devices section of the US FDA website (http://www. access data.fda.gov/scripts/cdrh/devicesatfda/index.cfm?start_search=1&search_term=HER2&approval_date_from=&approval_date_to=07/14/2013&sort=approvaldatedesc&pagenum=10; last checked July 14, 2013). The product package insert for trastuzumab and pertuzumab prepared by the FDA indicates that "HER2 testing should be performed using FDA-approved tests by laboratories with ßdemonstrated proficiency".

9/2013