COLLEGE of AMERICAN PATHOLOGISTS

Frequently Asked Questions

Recommendations for HER2 Testing in Breast Cancer: ASCO – CAP Clinical Practice Guideline Update

Background Questions

Why were changes made to the ASCO/CAP HER2 guideline?

In the intervening years, numerous papers were published and issues raised by individuals and organizations about the original guideline recommendations. The update was created to address concerns, analyze and include the evidence and harmonize the recommendations with those of the ASCO – CAP Guideline Recommendations for Immunohistochemistry Testing of Estrogen and Progesterone Receptors in Breast Cancer (ER/PgR guideline) published in 2010.

What are the changes?

1. Cold ischemic time – For both HER2 and ER/PgR, follow the ER/PgR recommendation that time from tissue removal to initiation of fixation be less than or equal to one hour. Document this time on the accession slip or in the report or both.

2. Handling of specimens obtained remotely – For both HER2 and ER/PgR, follow the ER/PgR recommendation that specimens obtained remotely using non-biopsy procedures be bisected through the tumor on removal. Record on the accession slip the time of removal, fixative type and time placed in fixative.

3. Fixation duration in neutral buffered formalin should be 6-72 hours and conformance to these guidelines should be documented in the report or on the accession slip.

4. All breast cancer metastases or recurrences should also be tested for HER2.

5. Core samples, preferred for ER and PgR testing, are now acceptable for HER2 testing as well. In situations where the core shows artifacts or the results are negative in a patient with a high grade tumor, the test should be repeated on a resection specimen.

6. Algorithms for defining what should be called HER2 positive, HER2 equivocal, HER2 negative and indeterminate have been refined and clarified for both IHC and ISH. Testing by analytically validated (hopefully also FDA-approved) brightfield in situ hybridization methods are now acceptable. See Figures 1- 3 in the guideline for algorithms defining specifics.

7. Interpretation guidelines for both IHC and ISH have been clarified.

8. Concordance requirements for IHC and ISH have been changed. Concordance between these assay types should be 95% but it is the responsibility of the laboratory director of each lab to define the level of concordance in his/her laboratory and monitor it in order to provide accurate testing.

9. Validation for both new HER2 tests and modified HER2 testing has been changed to agree with published validation requirements for ER and PgR testing.

General Questions

Why were the HER2 and ER/PgR testing guidelines produced?

Laboratory assays for HER2 and Estrogen Receptor (ER) and Progesterone Receptor (PgR) are essential in selecting patients for anti-HER2 and hormonal therapy, yet inaccuracies in testing pose a significant problem in ensuring that patients are treated appropriately. The CAP and the American Society of Clinical Oncology (ASCO) collaborated in producing guidelines to improve testing accuracy and reduce the substantial risks associated with false positive and false negative results.

How can I get the ASCO/CAP HER2 and ER/PgR guidelines?

The guidelines were published jointly in the *Journal of Clinical Oncology* and *Archives of Pathology & Laboratory Medicine.*

- The HER2 guideline update can be found on the accompanying web page.
- The ER/PgR testing guidelines can be found here: http://www.archivesofpathology.org/doi/pdf/10.1043/1543-2165-134.6.907

What PT material does the CAP offer for HER2 testing?

HER2 by Immunohistochemistry (IHC) Survey (HER2)
The HER2 Survey is an IHC Survey that provides 20 challenges, two tissue microarray slides consisting of 10 cores each, twice per year. Enrollment in the HER2 Survey will satisfy LAP requirements for participation in a CAP-accepted PT program for HER2 by IHC.

HER2 by Fluorescence in situ Hybridization (FISH) Survey (CYH)
CYH is a FISH Survey that provides 10 challenges, twice per year. Enrollment in the CYH will satisfy the LAP requirement for participation in a CAP-accepted PT program for HER2 by FISH, interpretation and hybridization onsite activity. Laboratories that do interpretation only must perform alternative assessment.

HER2 by Brightfield in situ Hybridization Survey (ISH2)
The ISH2 Survey is a CISH (chromogenic in situ hybridization) and SISH (silver in situ hybridization) Survey that provides 10 challenges, twice a year. Enrollment in the ISH2 Survey will satisfy alternative assessment requirements for ISH.

ER and PgR by Immunohistochemistry (PM2)
The PM2 Survey is an IHC Survey that provides 20 challenges, two tissue microarray slides each consisting of 10 cores, twice per year. Enrollment in PM2 is required for CAP-accredited laboratories beginning in 2011.

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Fixation and Processing Questions for HER2 and ER/PgR

How long should breast specimens be fixed before tissue processing begins?

Breast specimens that will be subject to ER/PgR and HER2 testing should be fixed in neutral buffered formalin for a minimum of six hours and a maximum of 72 hours. This fixation time begins when the specimen is initially placed in formalin (not when the specimen is sectioned during gross examination) and ends when the cassettes are no longer in formalin. This is not an absolute exclusion criterion. For specimens fixed longer than 72 hours for HER2 or ER and PgR in which negative test results are obtained, the report should state that prolonged fixation could be a possible cause for the negative result, and alternative testing methods should be considered (e.g. FISH for HER2; gene expression assay for ER). For HER2 testing, labs should also consider confirming by FISH any specimen fixed longer than 72 hours that is not Score 3 by IHC.

Do I need to include the actual fixation time on the report?

No. For all cases in which the fixation time is within the recommended interval specified in the ASCO/CAP guidelines for HER2 and ER/PgR testing (6 to 72 hours for ER and PgR and HER2), laboratories can append a standard statement to their reports that fixation time was in compliance with ASCO/CAP guidelines. However, laboratories will be required to put a disclaimer in any report in which the fixation time is outside those parameters. In addition, for cases with fixation times outside the recommended intervals in which a negative test result is obtained, the report should state that prolonged fixation could be a possible cause for the negative result and alternative testing methods should be considered (e.g. FISH for HER2; gene expression assay for ER). For HER2 testing, labs should also consider confirming by FISH any specimen fixed longer than 72 hours that is not Score 3 by IHC. It is also acceptable to test another sample from the same patient for these factors in these situations rather than using alternative testing methods on the same sample.

The guidelines recommend slicing breast specimens at 5 to 10 mm intervals before fixing in formalin. Should specimens be refrigerated without fixative until this can be done?

No. Refrigeration delays fixation, which has a detrimental effect on immunostaining. The testing guidelines require that specimens that will be subject to HER2, ER, or PgR testing be placed in formalin less than one hour after the tumor is removed from the patient; any further delay in fixation is now considered unacceptable.

In addition to placing in fixative as soon as possible, the guidelines also recommend slicing the specimen at regular intervals to ensure adequate fixation throughout. Since most cases also require assessment of specimen margins, institutions must develop procedures to ensure proper handling of breast excision specimens. As with any other intraoperative consultation, a pathologist (or other appropriately trained person under the direct supervision of a pathologist) must be available to handle these specimens.

Is shorter fixation (i.e. less than 6 hours) acceptable for needle biopsies due to their smaller size?

No. The original HER2 Testing Guidelines specified a minimum one-hour formalin fixation time for needle biopsies, but included a caveat that longer fixation is strongly recommended for these specimens. While formalin penetrates tissues at the rate of about 1mm/hour, penetration is not

the same as fixation and the biochemical cross-linking that represents formalin fixation requires more time. Published studies have documented that a minimum of 6-8 hours formalin fixation is needed to obtain consistent IHC assay results for ER; fixation for less than this time has been shown to cause false negative ER staining. Because of the adverse effects of underfixation, which cannot be overcome by antigen retrieval, testing on specimens fixed for less than 6 hours is no longer acceptable. Cases in which tissues have been fixed less than 6 hours should be reported as 'Estrogen Receptor Uninterpretable' with an explanatory comment.

Do the guidelines exclude testing of cytology specimens (fluids and aspirates) that have been fixed in 95% ethanol rather than formalin?

No. Fixatives other than formalin are not precluded by the guidelines. For tissue specimens, laboratories that choose to use a fixative other than neutral buffered formalin must validate that fixative's performance against the results of testing of the same samples fixed in neutral buffered formalin and tested with the identical assay. Since cytology specimens are not ordinarily fixed in formalin such concordance studies are not practical, but labs performing testing on such specimens must document that they validated their methods and achieved acceptable concordance, perhaps by comparing staining of alcohol fixed cytology specimens with subsequently excised routinely processed, formalin-fixed, surgical pathology specimens.

Would using a rapid processor be acceptable?

The effect of rapid tissue processing protocols on predictive marker testing is unknown. Before offering such testing using any alternative method, the lab must validate that method by comparing it with testing done by standard methods (i.e. the lab must test the same samples processed routinely and processed by the alternative method, and demonstrate 95% concordance for positive and negative results). Validation of reagents or equipment by vendors or manufacturers does not represent an acceptable substitute for validation done by each laboratory.

The HER2 Testing Guidelines state that "samples fixed in formalin should be routinely processed into paraffin and cut onto glass slides within 48 hours." Does this mean that sectioning onto glass slides must be done within 48 hours?

No, the 48-hour limit referred only to the former upper limit of formalin fixation. Once the tissue is processed and paraffin-embedded, there is no specified time frame for subsequent sectioning and testing; however, the interval should be as short as possible as antigen preservation is better within the block than on glass.

The Guidelines state that sections should not be used for IHC testing if cut more than 6 weeks earlier. Does this mean that stains should be done within 6 weeks of paraffin embedding or within 6 weeks of sectioning onto glass slides?

The latter is correct. There is no requirement that HER2 stains be done within 6 weeks of embedding, but labs should avoid doing HER2 stains on sections that were cut more than 6 weeks earlier. This also applies to positive control sections; labs should avoid using control slides that have been stored for prolonged periods after sectioning.

Laboratory Accreditation and Proficiency Testing Questions

Does the CAP address HER2 and ER/PgR testing in the Laboratory Accreditation Program (LAP) checklists?

Yes, checklist requirements regarding HER2 assay validation, specimen fixation, proficiency testing, and use of the ASCO/CAP scoring criteria for reporting results are included in the Anatomic Pathology (ANP), Cytogenetics (CYG), and Molecular Pathology (MOL) checklists.

These checklists are available to CAP accredited laboratories through e-LAB Solutions or can be purchased by non-CAP accredited laboratories.

Is participation in proficiency testing (PT) required for all sites that do HER2 testing?

Yes. In order to be compliant with the ASCO/CAP HER2 guidelines, any laboratory that reports results of such testing must participate in an accepted PT program (see exception below). The CAP Accreditation Program requires participation in a CAP-accepted PT program.

Exception: Laboratories that interpret and report the results of HER2 testing by FISH in which the hybridization is performed at an outside laboratory should not enroll in proficiency testing for that assay due to prohibitions on proficiency testing referral by CMS; such laboratories must perform alternative assessment. This exception does not apply to laboratories that interpret and report the results of HER2 testing by immunohistochemistry when staining is done at an outside facility.

The ASCO/CAP guidelines for HER2 testing apply only to breast carcinoma. HER2 testing on other tumor types (e.g. gastric carcinoma) is not covered by these guidelines at the current time.

Is participation in proficiency testing (PT) required for all sites that do ER and/or PgR testing?

In order to be compliant with the ASCO/CAP ER/PgR guidelines, any laboratory that reports results of such testing on primary breast cancers must participate in a PT program (see exception below). The College's Laboratory Accreditation Program (LAP) requires participation in a CAP-accepted PT program.

Exception: Laboratories that do ER and/or PgR staining only on tissues other than primary breast cancers (e.g. other tumor types such as meningioma; for lineage determination only), are not required to enroll in proficiency testing that is specific for those analytes. Laboratories that send all primary breast cancers out to another laboratory for both staining and interpretation are not required to enroll in PT.

What PT material does the CAP offer?

HER2 by Immunohistochemistry (IHC) Survey (HER2)
The HER2 Survey is an IHC Survey that provides 28 challenges, two tissue microarray slides consisting of 14 cores each, twice per year. Enrollment in the HER2 Survey will satisfy LAP requirements for participation in a CAP-accepted PT program for HER2 by IHC.

HER2 by Fluorescence in situ Hybridization (FISH) Survey (CYH)
CYH is a FISH Survey that provides 10 challenges, twice per year. Enrollment in the CYH will satisfy the LAP requirement for participation in a CAP-accepted PT program for HER2 by FISH, interpretation and hybridization onsite activity. Laboratories that do interpretation only must perform alternative assessment.

HER2 by Brightfield in situ Hybridization Survey (ISH2)
The ISH2 Survey is a CISH and SISH Survey that provides 10 challenges, twice a year. Enrollment in the ISH2 Survey will satisfy alternative assessment requirements for ISH.

ER and PgR by Immunohistochemistry (PM2)
The PM2 Survey is an IHC Survey that provides 20 challenges, two tissue microarray slides each consisting of 10 cores, twice per year. Enrollment in PM2 is required for CAP-accredited laboratories.

What PT does the CAP Laboratory Accreditation Program require?

At this time, CAP is the only accepted PT provider for HER2, ER and PgR.

We report HER2, ER and PgR using an automated image analysis system. What requirements apply to us?

Image analysis can be an effective tool for improving interpretation consistency; however, the pathologist is responsible for ensuring that the result provided by image analysis reflects measurement of invasive carcinoma only. The pathologist must document that he or she has reviewed either the stained patient test slides or the images and ensured that the appropriate area was scored.

Image analysis equipment, just as other laboratory equipment, must be calibrated and subjected to regular maintenance and internal quality control evaluation. Image analysis procedures must be validated before implementation. See the CAP guideline, Validating Whole Slide Imaging for Diagnostic Purposes in Pathology for more information.

Laboratories that do HER2 or ER/PgR staining by IHC and use in-house image analysis for interpretation and reporting are required to enroll in an IHC-based PT program and report the results following the usual testing and reporting methods used.

Laboratories that interpret and report the results of HER2 or ER/PgR testing by IHC in which staining and image analysis are performed at an outside laboratory are required to enroll in PT but must ensure that they only receive back the stained PT slide or an image of the stained PT slide. The laboratory must ensure that the outside laboratory does not send back any quantitative image analysis data as that would constitute PT Referral by CMS which can have serious consequences. As noted above, image analysis is a useful tool, but pathologists should also be able to manually score the slide without the use of quantitative image analysis.

All labs participating in PT must provide results for all PT challenges regardless of specific methods of testing used. If the PT program includes manual scoring of virtual slides or images (in addition to actual tissue challenges), every lab must provide manual scoring results for these challenges even if they normally only interpret glass slides or report results by quantitative image analysis.

We do not do IHC staining, but interpret and report HER2 and ER/PgR slides that are stained by an outside facility. Are we still required to enroll in PT?

Yes. Laboratories that interpret HER2, ER, or PgR slides stained by another facility must enroll in a CAP-accepted PT program and report the results of their interpretation. Since CAP is currently the only accepted HER2/ER/PgR PT provider, such labs must enroll in CAP's HER2 and/or PM2 Surveys. You must send the unstained Survey slides to the outside facility for immunohistochemical staining, and report the results of your interpretation of the stained slides.

We send HER2 and ER/PgR materials to an outside facility for IHC staining and image analysis and provide interpretation in house. Are we required to enroll in PT?

Yes. All laboratories that perform and/or interpret HER2 or ER/PgR testing are required to enroll in a CAP-accepted PT program (see exception below). Laboratories that send materials to another facility for staining by IHC and image analysis are required to enroll in an appropriate IHC-based PT program. For the tissue challenges in the HER2 and PM2 Surveys, the laboratory should send the slides to the outside facility for staining only; do not request quantitative image analysis at the outside facility even if this is routinely done for patient testing. Doing so could be considered PT Referral and result in severe sanctions by CMS. You must report the results of manual scoring for these PT slides. The PT Referral prohibition does not apply to staining and image analysis that are both performed in house.

Exception: Laboratories that do such testing only on tissues other than primary breast cancers (e.g. other tumor types such as meningioma; for lineage determination only) are not required to enroll in proficiency testing that is specific for those analytes. Laboratories that send all primary breast cancers to another facility for both staining and interpretation are not required to enroll in PT.

In the CAP's HER2 and PM2 Programs, all results of PT challenges are reported using manual scoring. There is currently no separate reporting by quantitative image analysis. All laboratories must provide results using the scoring systems outlined in the PT kit instructions and stained tissue challenges, even those that normally report results using a quantitative image analysis system provided by an outside laboratory.

Our HER2 and ER/PgR cases are sent to an outside laboratory for testing and interpretation, but we include their results in our pathology reports. Are we required to enroll in PT?

No. Proficiency testing only applies to laboratories that perform and/or interpret the assays, not to those that simply report the results that are performed and interpreted by an outside laboratory. Labs must enroll in PT if they provide a professional interpretation, even if they are using an outside laboratory for staining and/or image analysis.

Is the laboratory required to submit results from each pathologist during every PT event?

Only the results of the laboratory are reported to the PT provider. The laboratory is not required to provide responses from each pathologist for every PT challenge; however, these challenges must be integrated into the routine laboratory workload and analyzed using the same personnel and systems as for patient samples. Thus, if multiple pathologists routinely report HER2 or ER/PgR

results in your lab, PT challenges must be done by a rotation that allows all pathologists to participate in scoring these challenges.

What are the new requirements for ongoing competency assessment for pathologists?

Pathologists should perform ongoing competency assessment as a part of every laboratory's internal quality assessment program. The competency of the laboratory professionals and pathologists interpreting assays must be continuously addressed as required under CLIA (for US laboratories).

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