



Topic: ER/PgR Testing Guidelines Recent Changes to the HER2 and ER/PgR Guidelines

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QUESTIONS REGARDING RECENT CHANGES TO THE HER2 AND ER/PgR GUIDELINES

Why were changes made to the ASCO/CAP HER2 and ER/PgR guidelines?

These changes were made so that cancer specimens will be handled in a uniform manner for ER, PgR and HER2 testing in breast cancer specimens. Since the HER2 guideline was published in 2007, new evidence is available and the recommendations in the guidelines need to be reconciled.

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.

What are the changes made to minimum fixation times?

The minimum fixation time for HER2 has been clarified and we recommend that samples for HER2 testing be fixed a minimum of 6 hours. The original statement that smaller samples can be fixed for less than 6 hours is not supported by the literature. We recommend that sample for HER2 testing be fixed a minimum of 6 hours regardless of sample size.

What about changes to maximum fixation times? The HER2 fixation time of 6-48 hours is not consistent with that of the ER/PgR fixation time of 6-72 hours.

We are unable to find evidence to support increasing the HER2 fixation time and therefore recommendations for fixation times in neutral buffered formalin are unchanged (6-48 hours for HER2 and 6-72 hours for ER/PgR). The data about the stability of ER and PgR at intervals of 48-72 hours suggest that changing this interval for HER2 testing will not result in adverse testing results. However, there is a lack of specific published studies for HER2 IHC that included specimens with low levels of HER2 expression that would be more vulnerable to fixation time changes.

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IMMUNOHISTOCHEMISTRY FOR HER2 ON GASTRIC AND GASTROESOPHAGEAL JUNCTION CARCINOMAS

Do the ASCO/CAP guidelines for HER2 immunohistochemistry apply to gastric cancer?

No. The ASCO/CAP guidelines for breast cancer do not apply to HER2 IHC as performed on gastric cancer.

Are the grading criteria for HER2 IHC on gastric carcinoma the same as those for breast carcinoma?

No. The criteria for interpreting HER2 IHC on gastric carcinoma differ significantly from the breast cancer criteria in three significant ways. Firstly, in contrast to the grading scheme in breast cancer, the gastric carcinoma interpretation criteria use 10% tumor cell staining as a cutoff to distinguish negative from 1+. In gastric carcinoma, the distinction between 1+, 2+, and 3+ depend on the intensity of staining presuming that more than 10% of tumor cells show HER2 expression (see table below). Secondly, in strongly staining cases, Her2 3+ gastric cancers only show expression along the basolateral or lateral cell membranes; apical membranes do not stain. Thus, the criteria for 2+ and 3+ staining in gastric cancer require only lateral or basolateral staining, unlike the breast cancer criteria which require complete, circumferential staining. Thirdly, the criteria for HER2 overexpression differ when interpreting biopsy and resection specimens due to increased heterogeneity of HER2 expression in gastric and gastroesophageal junction carcinomas (see table below).

Surgical Specimen Staining Pattern	Biopsy Specimen Staining Pattern	R2 Overexpression Assessment
No reactivity or membranous reactivity in <10% of tumor cells	No reactivity in any tumor cell	Negative
Faint or barely perceptible membranous reactivity in 10% or more of tumor cells; cells are reactive only in part of their membrane	Tumor cell cluster* with faint or barely perceptible membranous reactivity irrespective of percentage of tumor cells stained	Negative
Weak to moderate complete, basolateral or lateral membranous reactivity in 10% or more of tumor cells	Tumor cell cluster* with weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained	Equivocal



Strong complete, basolateral or lateral membranous reactivity in 10% or more of tumor cells

Tumor cell cluster* with strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumor cells stained

Positive

From Bang et al, Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of Her2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomized controlled trial. *Lancet* 28:376, 2010.

*"Tumor cell cluster" is defined as a cluster of 5 or more tumor cells (Ruschoff et al, HER2 diagnostic in gastric cancer –&38211; guideline validation and development of standardized immunohistochemical testing. *Virchows Arch* 457:299-307. 2010.)

Do the LAP questions that specifically apply to HER2 immunohistochemistry on breast cancer apply to gastric cancer?

No. However, general questions pertaining to IHC assays that provide independent predictive information do apply to HER2 IHC performed on gastric cancer (see ANP.22969 and ANP.22970).

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 PT Referral prohibitions; 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.

Do I need to separately validate my HER2 IHC assay for gastric cancer?

Yes. While the assay conditions between HER2 IHC performed on breast and gastric carcinomas need not differ, the interpretation criteria differ significantly (see above). As such, we recommend that a small revalidation be performed to assure that the correlation between IHC results and those of FISH are adequate. The required level of correlation and the number of cases that should be included in the revalidation is best determined by the immunohistochemistry laboratory director. However, 90-95% concordance between FISH and IHC is recommended.

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 an 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 College introduced this new program (2008) that will satisfy biannual PT requirements in 2010.

Does a proficiency testing product exist for HER2 IHC on gastric cancer?

No, not at the moment.

What are the published benchmarks for HER2 expression in gastric cancer?

Depending on the series, the prevalence of HER2 expression in gastric cancer seems to be 15-25%. The level of expression of intestinal type gastric cancer seems to be much higher (~32%) compared with diffuse-type gastric cancer (~6%). Also, tumors that are primarily located at the gastroesophageal junction seem to have higher HER2 positive rates compared to tumors that occur in the rest of the stomach (33% versus 21%).



REFERENCES

- Hofmann M et al, Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathol* 52:797. 2008.
- Bang Y et al, Pathologic features of advanced gastric cancer (GC): relationship to human epidermal growth factor receptor 2 (HER2) positivity in the global screening programme of the ToGA trial. *J Clin Oncol* 25:15s (suppl; abstr 4556).

Are the recommendations for HER2 FISH testing in gastric cancer similar to those of breast cancer?

Although there is some evidence that HER2 IHC may, in some circumstances, be more predictive than FISH, the current recommendations for HER2 FISH testing in gastric cancer are the same as those for breast cancer. That is, FISH testing should be performed when the IHC is equivocal (2+).

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 CAP/ASCO HER2 and ER/PgR guidelines?

The guidelines were published jointly in the *Journal of Clinical Oncology* and [Archives of Pathology & Laboratory Medicine](#).

- HER2 testing guidelines: *Arch Pathol Lab Med*. 2007;131:18-43.
- ER/PgR testing guidelines: *Arch Pathol Lab Med*. 2010;(in press).

The guidelines may also be found on the CAP and [ASCO](#) websites.

LABORATORY ACCREDITATION AND PROFICIENCY TESTING QUESTIONS

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

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.

Checklist requirements for ER/PgR testing are available in the June 2010 edition of the Anatomic Pathology (ANP) Accreditation Checklist.

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

When will we be required to be compliant with these guidelines?

Laboratories were required to implement the HER2 guidelines by year-end 2007 and successful proficiency testing performance was required in 2008. Laboratories must begin to implement the ER/PgR guidelines as soon as possible and enroll in a CAP-accepted proficiency testing program by 2011.

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

Yes. In order to be compliant with the CAP/ASCO 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 CAP/ASCO 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 beginning in 2011 and will monitor performance beginning in 2012.

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 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 College introduced this program which can be used to 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.

What PT does the CAP Laboratory Accreditation Program require?

At this time, CAP is the only accepted PT provider for HER2, ER and PgR. Participation in HER2 PT was required beginning in 2008, and participation in ER/PgR PT is required beginning in 2011.

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.

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.



TESTING VALIDATION QUESTIONS

The HER2 and ER/PgR testing guidelines include requirements for ongoing validation. To comply with this requirement, must we regularly repeat the initial test validation study (i.e. annually or semiannually)?

No, provided that there has been no significant change to the test system. Ongoing validation of a predictive marker assay refers to regular (periodic) assessment of the assay to ensure that its analytic sensitivity has not drifted. This does not require repeating the entire procedure used for initial test validation, but at a minimum should include documentation of successful performance on semi-annual proficiency testing challenges. Additional useful means of regularly monitoring assay performance include tracking positive and negative rates (trend analysis), correlating with tumor grade and concordance with other testing modalities (FISH, gene expression assay, etc).

Note: The assay validation procedure must be repeated whenever there is a significant change to the test system, such as a change in the primary antibody clone or the introduction of new antigen retrieval or detection system.

If we originally validated our HER2, ER and PgR assays when we brought these tests online do we need to revalidate them to be compliant with the CAP/ASCO Guidelines?

No, if your laboratory originally validated these tests with the appropriate number and range of challenges and has retained this documentation your laboratory is not required to revalidate these tests.

Can we use the CAP HER2, ER and PgR proficiency samples that provide data from validated assays for use with our HER2, ER and PgR initial test validation?

Yes, external validated materials may be used for initial test validations for ER and PgR provided they contain appropriate number and range of challenges.

Are there any unique validation materials available to perform validation/verification studies for HER2, ER and PgR?

Not at this time. The College is investigating if alternate material can be developed for this purpose.

REFERENCES

- Willmore-Payne C, Metzger K, Layfield L. Effects of fixative and fixation protocols on assessment of Her-2/neu oncogene amplification status by fluorescence in situ hybridization. *Appl Immunohistochem Mol Morphol*. 2007;15:84-87.
- Penault-Llorca F, et al. Optimization of immunohistochemical detection of ERBB2 in human breast cancer: impact of fixation. *J Pathol*. 1994;173:65-75.

Is there a listing of reference laboratories performing validated ER and PgR assays that are willing to perform cross laboratory validation studies?

Yes, the CAP offers an online service for laboratories that are willing to perform validation studies for external clients. However, the College is not in the position to certify the validity of these services.

Can the semi-annual proficiency testing challenges be used to fulfill the requirement for competency assessment of pathologists and laboratory professionals interpreting assays?

Yes. The tissue microarray slides provided in the PT challenges can be used to assess competency after PT results have been submitted to the PT provider. While the official results reported to the PT provider must reflect the method of reporting done for routine clinical cases (i.e. ordinarily a single pathologist), labs can then have all pathologists who interpret and report HER2 and ER/PgR tests independently review the stained slides. These results can be tracked and compared with those of all Survey participants to assess pathologist competency. The Laboratory Director will be responsible for reviewing the results of this assessment and determining when action is needed. This assessment must also be available at the time of on-site inspection.



Is validation required if we incorporate a new tissue processor or new step in the process if the manufacturer claims that it will not alter our results?

Yes. Every lab must validate its assays before reporting patient results regardless of method used and regardless of any validation done by a reagent or equipment manufacturer. A vendor's published validation study is not a substitute for a lab validating its own assay procedures.

Our laboratory is considering switching to a different fixative. Will it be necessary to repeat the validation study?

Yes. Using non-formalin solutions may impact HER2 testing. Alcohol-based fixatives have been shown to generate false-positive IHC staining (Penault-Llorca), and many studies have shown reduced or absent FISH results after alcoholic fixation (Willmore-Payne). Given these facts, labs are required to validate that solutions such as these have no detrimental impact on testing.

REFERENCES

- Willmore-Payne C, Metzger K, Layfield L. Effects of fixative and fixation protocols on assessment of Her-2/neu oncogene amplification status by fluorescence in situ hybridization. *Appl Immunohistochem Mol Morphol*. 2007;15:84-87.
- Penault-Llorca F, et al. Optimization of immunohistochemical detection of ERBB2 in human breast cancer: impact of fixation. *J Pathol*. 1994;173:65-75.

How many cases should be included in the ER/PgR validation study?

The CAP/ASCO guideline recommends that initial test validation should include a minimum of 40 cases (20 positive and 20 negative cases) for FDA approved/cleared tests; laboratories should consider using higher numbers of test cases if a Laboratory Developed or Laboratory Modified Test is to be validated. Validation should be performed by comparing the laboratory's results with another assay that has been appropriately validated as per the ASCO/CAP ER/PgR Guidelines. Acceptable concordance levels are 90% for positive results and 95% for negative results. If significant changes are made to the testing methods (e.g. antibody clones, antigen retrieval protocol or detection system), revalidation is required.

What does the CAP recommend for initially validating immunohistochemistry assays other than HER, ER or PgR assays?

The performance characteristics of each assay in the immunohistochemistry laboratory must be appropriately validated before being placed into clinical use. The initial goal is to establish the optimal antibody titration, detection system, and antigen retrieval protocol. Once optimized, a panel of tissues must be tested to determine the assay's sensitivity and specificity. The scope of the validation is at the discretion of the laboratory director and will vary with the antibody. For a well-characterized antibody with a limited spectrum of antigenic targets, like chromogranin or prostate specific antigen, the validation can be limited. A panel of 10 positive and 10 negative neoplasms would be sufficient in this setting. For an antibody that is not well characterized and/or has a wide range of reported reactivity, a more extensive validation is necessary. The number of tissues tested should in this circumstance be large enough to determine whether the staining profile matches that previously described.

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2. Hsi ED. A practical approach for evaluating new antibodies in the clinical immunohistochemistry laboratory. *Arch Pathol Lab Med*. 2001;125:289-294.

FIXATION AND PROCESSING QUESTIONS

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 48 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 48 hours for HER2 and longer than 72 hours for 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 48 hours that is not Score 3 by IHC.

**Note: This fixation interval (6-48 hours) is based on the original HER2 Testing Guidelines. While the 2010 ER and PgR Testing Guidelines allow for a 6-72 hour fixation interval, the fixation interval for HER2 has not been changed. CAP and ASCO have reviewed the scientific literature and found insufficient evidence to revise the fixation interval for HER2 so that it is aligned with ER and PgR.*

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 CAP/ASCO guidelines for HER2 and ER/PgR testing (6 to 48 hours for HER2; 6 to 72 hours for ER and PgR), laboratories can append a standard statement to their reports that fixation time was in compliance with CAP/ASCO 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 48 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. (See [suggested reporting template](#), PDF, 16 K).

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 upper limit of formalin fixation. Once the tissue is processed and paraffin-embedded, there is no specified time frame for subsequent sectioning and testing.

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.

ER/PgR ASSAY AND REPORTING QUESTIONS

Are the antibodies listed in Table 3 of the guidelines the only acceptable antibodies that labs may use?

No. The guidelines note that it is acceptable to use other antibodies provided they are 90&337; concordant with a clinically validated assay for the ER- and PgR-positive category and 95% concordant with a clinically validated assay for the ER- and PgR-negative category. The laboratory director must determine whether the laboratory-selected antibody has been clinically validated in scientifically valid published studies. If there are insufficient studies, the laboratory director has the option of validating the new antibody against those already shown to be acceptable.

What are the appropriate controls for ER and PgR IHC?

The ER and PgR IHC assays require ongoing evaluation of both external and internal controls to ensure assay quality. Appropriate external control tissues might include breast carcinomas with known high levels of ER and PgR expression. Additionally, tumors that show low or intermediate levels of expression are also required to more sensitively assess subtle drift in assay performance. A single external control that shows only strong ER/PgR expression is not sufficient. Other acceptable external controls include cell lines with known hormone receptor content that ranges from a high level of expression to negative and normal endometrial tissue. Both batch controls and on-slide external controls are acceptable.

Ongoing evaluation of the normal breast epithelial elements' receptor expression serves as an internal control. Normal breast epithelial cells vary in the intensity of hormone receptor expression from high to low levels of expression. If the normal cells show only rare or weak staining, this increases the possibility that the assay lacks sensitivity.

Internal control tissues also serve as a helpful negative control since myoepithelial cells are uniformly negative for hormone receptors.

Is there a recommended reporting template for ER and PgR IHC?

The ER/PgR guidelines mandate uniform reporting parameters and criteria for hormone receptor interpretation. There is a [suggested reporting checklist](#) (PDF, 16 K). This format is not mandatory. However, inclusion of all elements in some format is required.