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NEW CLINICAL PRACTICE GUIDELINES FOR HER2 TESTING AND INTERPRETATION – SAMPLE COLLECTION INFORMATION FOR PROVIDERS

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Changes in HER2 testing were generated by the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) and published October 7, 2013. These new guidelines on HER2 testing and interpretation are for pathologists, surgeons, oncologists and other clinicians who treat patients with invasive breast cancer.

Marshfield Labs performs HER2 testing on invasive breast cancers by immunohistochemistry (IHC) and by fluorescence in situ hybridization (FISH). Appropriate sample collection and handling is critical for an accurate interpretation of these tests.

The most important changes in the ASCO/CAP guidelines include:

1. The threshold of eligibility for HER2 therapy has been modified for both IHC and FISH testing methods. Criteria for HER2 positive results are now:
   - IHC: 3+ circumferential membrane staining is complete, intense, and within >10% of invasive tumor cells.
   - FISH: HER2/CEP17 ratio ≥2.0 *or* Average HER2 ≥6 copies/cell.
2. HER2 status should be determined on all newly diagnosed invasive primary breast cancers, and also recurrent or metastatic tumors, if tissue samples are available.
3. Testing may be performed by either IHC or FISH. If either test is equivocal, reflex testing using a different method or a new specimen must be performed.
4. Guideline recommendations require that tissues for HER2 testing are fixed in 10% neutral buffered formalin for 6 to 72 hours (previously

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48 hours) with cold ischemic time being ≤1 hour. Exceptions to this process can affect results. With this change, HER2 requirements now conform to estrogen and progesterone testing for handling and fixation. These handling requirements must be documented and made available to testing personnel. This change in fixation time is advantageous for outreach specimens and weekend processing.

5. A result disclaimer is required if the specimen handling falls *outside* of the documented recommended guidelines. Marshfield Labs may use the following comment:

> Per ASCO/CAP guidelines, IHC/FISH test results are valid for non-decalcified paraffin embedded specimens fixed in 10% neutral buffered formalin between 6 and 72 hours. Cold ischemic time should be ≤1 hour. Results from specimens fixed outside these parameters should be interpreted accordingly.

6. IHC Scoring Criteria for HER2 on Breast Cancer has been modified:

IHC	Result	Criteria†
0	Negative	No staining is observed, or membrane staining is incomplete, faint/barely perceptible and within ≤10% of invasive tumor cells.
1+	Negative	Incomplete membrane staining is faint/barely perceptible and within >10% of invasive tumor cells.
2+	Equivocal	Circumferential membrane staining is incomplete and/or weak/moderate and within >10% of invasive tumor cells. Or Complete and circumferential membrane staining is intense and within ≤10% of invasive tumor cells.
3+	Positive	Circumferential membrane staining is complete, intense, and within >10% of invasive tumor cells.
✶	Indeterminate	Technical issues prevent tests from being reported as positive, negative, or equivocal; these may include: • Inadequate specimen handling • Artifacts that make interpretation difficult • Analytic testing failure • Sample has strong membrane staining of normal breast ducts

†Readily appreciated using a low-power objective and observed within a homogeneous and contiguous invasive cell population.

7. FISH Scoring Criteria for HER2 on Breast Cancer has been modified:

Result	Criteria
Positive	HER2/CEP17 ratio is ≥2.0.
Positive	HER2/CEP17 ratio is <2.0 with an average HER2 copy number ≥6.0/cell. .
Equivocal	HER2/CEP17 ratio is <2.0 with an average HER2 copy number ≥4.0 and <6.0/cell. These cases will be reflexed for additional testing
Negative	HER2/CEP17 ratio is <2.0 with an average HER2 copy number <4.0/cell.
NOTE: Tumor heterogeneity, monosomy or polysomy of chromosome 17, and gene deletion may influence the interpretation of the absolute ratio value.	

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TEST INFORMATION

IHC
Test Name: HER-2/NEU Oncoprotein, IHC
Test Code: HER2NEU

FISH
Test Name: HER2, FISH
Test Code: FHER2

Specimen Requirements:
Paraffin embedded tissue block or unstained slides.

Collection Processing Instructions:
Please see Marshfield Labs' Test Reference Manual for detailed instructions.

Breast
1. Cold ischemic time: removal from blood supply to initiation of fixation must be less than or equal to one hour.
2. Fixative type: 10% Neutral Buffered Formalin.
3. Fixative duration requirement: 6 - 72 hours.

The above handling and fixative times must be documented and available to testing personnel, e.g., on report, according to ASCO/CAP guidelines. Delayed, under, or over fixation may affect test results.

Handling of surgical breast specimens obtained at sites other than the Marshfield campus:
Due to changing regulations, Marshfield Labs requires new procedures for breast specimens when cancer is suspected/known. The goal is to expose breast tumor to formalin within one hour of removal.
For breast lumpectomy:
1. Pat the specimen dry
2. Ink the margins. Inking: Unoriented specimens are inked one color. Oriented specimens are inked six colors. Be sure to indicate orientation for each ink color used. Marshfield Labs suggests:

Superior – blue	Medial – red	Anterior/Superficial – yellow
Inferior – green	Lateral – orange	Posterior/Deep - black

3. Dry again and dip in vinegar (helps the ink adhere)
4. Make an incision in the specimen as close as possible to the lesion. Do not cut all the way through the specimen - keep it intact. More than one incision can be made on larger specimens.

Note, the specimen must to be inked before it is incised in order to maintain integrity of the margin. Inking after the margin has been cut is not recommended. Specimen should be immersed in a sufficient volume of 10% neutral buffered formalin (10X volume of specimen) to assure fixation.

Requirements
Completed histology requisition form or Authorization for Test Requests form.
Surgical or cytology pathology report containing pertinent patient history, tissue source, and diagnosis.
One paraffin tissue/cell block with corresponding H&E slide, *or* send 2 (3-4 micron) and 2 (4-5 micron), labeled as such, unstained, air-dried, charged slides with corresponding H&E slide.

QUESTIONS

For additional information, please refer to: Marshfield Labs' Test Reference Manual - HER2NEU.
If questions, phone 800-222-5835 and ask for one of the following individuals:
- For collection and handling information: Faith Bosmans, PA.
- For IHC technical information: Laura Bliven, BS, HT(ASCP), QIHC.
- For IHC interpretive information: Dr. Yeping Sun.
- For FISH technical information: Diane Kronberger MT, CG(ASCP).
- For FISH interpretive information: Dr. Gene Shaw.

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REFERENCES

Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, 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. J Clin Oncol. Oct 7 2013;[Medline].

MICROBIOLOGY UPDATE: NEW TEST FOR *STAPHYLOCOCCUS AUREUS* SCREENING

Thomas Novicki, PhD, DABMM, Clinical Microbiologist

A new culture option has been added to screen for the carriage of both methicillin – susceptible *Staphylococcus aureus* (MSSA) and methicillin – resistant *S. aureus* (MRSA).

This test, Culture, Staphylococcus aureus screen (test code STAUSCR), is not intended for diagnostic purposes; other potential pathogens that may be present will not be identified, nor will antimicrobial susceptibility testing (AST) be routinely performed. It is available for nares swab specimens only at this time. Screening for MSSA as well as MRSA may be useful in some settings. (For a review of screening and decolonization tactics for MRSA & MSSA see J-C. Lucet and B. Regnier, 2010. Clin. Infect. Dis. 51:585.)

This test was developed to complement Marshfield Labs' other staphylococcal screening test, the methicillin resistant *Staph. aureus* (MRSA) screen (test code MRSA) which only identifies and reports the presence of MRSA. In addition, a complete aerobic culture with susceptibilities (test code AERS) may be ordered for diagnostic purposes. (See the Table 1, and also Marshfield Labs' online Test Reference Manual, for more information.)

TEST INFORMATION

Test Name:
Culture, Staphylococcus aureus screen

Test Code: STAUSCR

Synonyms/Keywords:
MRSA, MSSA, Staph

Ordering:

Clinic (Clinical Order Manager):
Culture, Staphylococcus Screen

Hospital (Centricity):
Culture, Staphylococcus Screen

Specimen Requirements:
Nasal/nares swab, BBL Culture Swab Plus, or other Amies/Stuart's transport-based medium

Specimen storage and transport:
Room temperature up to 2 hours; 4°C >2 hours

Minimum:
One swab

Rejection Criteria:
Non-nares body sites; improperly collected or stored/transported specimens; dry swabs

Available:
Daily, with an analytic time of 24 hours

CPT Code:
87081

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Table 1

Test	Code	Microbe(s) Identified	AST Performed	Body Sites
Culture, Staphylococcus aureus Screen	STAUSCR	MRSA, MSSA	Methicillin only	Nares
Culture, Methicillin Resistant Staph. aureus (MRSA) Screen	MRSA	MRSA	Methicillin only	Axilla, groin, nares, skin, throat, urine
Culture, Aerobic with susceptibilities	AERS	Aerobic bacterial pathogens	Standard antimicrobial panel	Any site including nares

QUESTIONS

If questions or for additional information, refer to Marshfield Labs' Test Reference Manual

or

Contact the following at 715-221-6700.

Technical questions:
Mr. Jason Campbell

Interpretive questions:
Dr. Thomas Novicki or Dr. Thomas Fritsche