CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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HER2 Testing (Breast Tissue) by FISH, 2018 ASCO/CAP HER2 Result/Interpretation Guideline Updates
--Resulting Update--

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Testing Schedule:

Set up Mondays and Wednesdays, dayshift. Resulted Tuesdays and Thursdays, dayshift. The testing schedule may be adjusted week to week to maximize workflow efficiencies. Expected Turn-Around-Time (TAT) is 2-5 days from receipt of specimens at CPAL.

Specimen (IMPORTANT):

1. One H&E stained slide, cover slipped with ***invasive tumor area circled by a pathologist***.
2. Additionally, a HER2 IHC slide from the same tissue block may be sent.
3. Three unstained (no cover slip) serial sections of:
    a. ***formalin-fixed, paraffin-embedded breast tissue <u>within 10 cuts</u> of the H&E/IHC companion slide(s)***
    b. on positively-charged organo-silane slides
    c. 4-6 microns thick
4. **<u>Blocks will not be accepted</u>**.
5. Specimens for which no desired sampling area (tumor) is indicated will be returned to the client so that the proper region of interest can be indicated and resubmitted to CPAL.
6. Specimens should be transported and stored at room temperature.

Results Reported:

1. The results will be reviewed and interpreted by the submitting pathologist using the SoloWeb application. The "Results" section of the HER2 report may be completed with one of the following selections:
    a. HER2 Group 1: Ratio ≥ 2.0 and average HER2 copy number ≥ 4.0 signals/cell.
    b. HER2 Group 2: Ratio ≥ 2.0 and average HER2 copy number < 4.0 signals/cell.
    c. HER2 Group 3: Ratio < 2.0 and average HER2 copy number ≥ 6.0 signals/cell
    d. HER2 Group 4: Ratio < 2.0 and average HER2 copy number ≥ 4.0 and < 6.0 signals/cell
    e. HER2 Group 5: Ratio < 2.0 and average HER2 copy number < 4.0 signals/cell
    f. HER2 Indeterminate: Technical issues prevented this case from being reported. See case notes for details. This result requires another specimen be tested to determine HER2 status.
    g. DCIS: No invasive tumor identified and results are based on in situ carcinoma.

2. Interpretation of HER2 FISH Testing (patient's HER2 status): Based on recommendations in the 2018 ASCO/ CAP guidelines. (Arch Pathol Lab Med; doi: 10.5858/arpa.2018-0902-SA), the determination of a patient's HER2 status should be based on several factors including the HER2/CEP17 ratio, the average HER2 copy number per cell, and HER2 IHC results. The pathologist should also correlate all the clinical and histopathologic information to determine if the HER2 result is concordant with those findings. If discordance is noted, repeat testing and/or further investigation and discussion with the clinician is warranted to resolve the issue. The "FISH Interpretation" section of the HER2 FISH report will be completed with one of the following selections:
    a. Positive, HER2 Group 1: (ratio ≥ 2.0, ≥ 4.0 signals/cell) Results show a HER2/CEP17 ratio ≥ 2.0 and an average HER2 copy number ≥ 4.0 copies/cell. This is a POSITIVE result according to 2018 ASCO/CAP guidelines. (Arch Pathol Lab Med; doi: 10.5858/arpa.2018-0902-SA).
    b. HER2 Group 2: (ratio ≥ 2.0, < 4.0 signals/cell). Results show *possible* HER2 amplification with a HER2/CEP 17 ratio ≥ 2.0. According to 2018 ASCO/CAP Guidelines, a definitive diagnosis must be rendered based on additional work-up using IHC testing performed on the same tissue section as FISH. (Arch Pathol Lab Med; doi: 10.5858/arpa.2018-0902-SA).
    c. HER2 Group 3: (ratio < 2.0, ≥ 6.0 signals/cell). Results show *possible* HER2 amplification with a HER2/CEP 17 ratio ≥ 2.0. According to 2018 ASCO/CAP Guidelines, a definitive diagnosis must be rendered based on additional work-up using IHC testing performed on the same tissue section as FISH. (Arch Pathol Lab Med; doi: 10.5858/arpa.2018-0902-SA).
    d. HER2 Group 4: (ratio < 2.0, ≥ 4.0 and < 6.0 signals/cell). Results formerly classified as FISH equivocal for HER2. According to 2018 ASCO/CAP Guidelines, a definitive diagnosis must be rendered based on additional work-up using IHC testing performed on the same tissue section as FISH. Scoring was repeated by a second technologist.

e. Negative, HER2 Group 5: (ratio < 2.0, < 4.0 signals/cell). Results show no evidence of HER2 amplification, displaying a HER2/CEP 17 ratio of < 2.0 with an average HER2 copy number <4.0 signals/cell. This is a NEGATIVE result according to 2018 ASCO/CAP guidelines. (Arch Pathol Lab Med; doi: 10.5858/arpa.2018-0902-SA).
f. Indeterminate: The FISH assay resulted in poor signal quality and/or poorly defined DAPI cells. Analysis could not be performed. This is an INDETERMINATE result according to 2018 ASCO/CAP guidelines. (Arch Pathol Lab Med; doi: 10.5858/arpa.2018-0902-SA) and requires testing of another specimen to determine HER2 status.
g. Genomic Heterogeneity: Upon scanning of the slide, clustered genomic heterogeneity is noted in > 10% of tumor cells. Overall results are reported based on the amplified cluster(s) of cells. See case notes for specific ratio and average HER2 copy number/cell for each area (amplified and non-amplified), as recommended in 2018 ASCO/ CAP guidelines. (Arch Pathol Lab Med; doi: 10.5858/arpa.2018-0902-SA)
h. DCIS only: No invasive tumor is identified and results are based on in situ carcinoma. The clinical significance of HER2 positive status of in situ breast cancer is uncertain at this time. Current ASCO/CAP guidelines recommend HER2 testing only for invasive carcinoma. Clinical correlation is recommended.

Clinical Use:

To detect amplification of the HER-2*/neu* gene in patients with invasive breast cancer. Results are used to assess eligibility for trastuzumab (Herceptin) or lapatinib (Tykerb) therapy. Results may be used as an adjunct to existing clinical/pathologic data currently used as prognostic factors in Stage II, node-positive breast cancer patients. Results may also be used as an aid to predict disease-free and overall survival in this same cohort of patients whom have been treated with adjuvant CAF chemotherapy.

Method:

Fluorescent In Situ Hybridization (FISH)
This test utilizes the IVD dual-color PathVysion HER2 DNA Probe Kit (Abbott Molecular, Inc.). The specific probes used are LSI HER-2/*neu* Spectrum Orange and CEP 17 Spectrum Green. Breast tissue is fixed in formalin for >6 and <72 hours. Sections of this paraffin-embedded tissue are cut (4-6 microns) and mounted on positively charged glass slides. Using the pathologist-circled target area of the H&E slide as a reference, the target area on the unstained slide is marked by diamond-tip etcher on the back of the slide. The probe set is hybridized to the target area and 50 interphase nuclei are analyzed (BioView Duet Image Analysis System with manual scoring) with the results expressed as the average ratio of HER2 signals as compared to CEP17 signals. *Final interpretation and sign out is performed by the ordering pathologist using the interactive web based analysis available through the CPAL laboratory.*

Clinical Background:

The HER2 proto-oncogene is one of a family of 4 related growth factor receptor genes. Its amplification can lead to tumor development through enhanced cell proliferation, survival, motility and adhesion. Amplification is observed in approximately 20% of invasive breast cancers and is associated with a more aggressive disease course.

HER2 status is used to determine eligibility for trastuzumab (Herceptin) therapy. Patients with HER2 amplification are suitable candidates for trastuzumab therapy, as this monoclonal antibody is directed against the extracellular domain of HER2.

HER2 status may also be helpful when considering other types of therapy. HER2 amplified tumors have been associated with increased sensitivity to anthracycline and CAF (cyclophosmamide/doxorubicin/5-fluorouracil) chemotherapy, but decreased benefit from CMF regimens (C/methotrexate/F). HER2 amplified patients may also tend to be less sensitive to tamoxifen, but the data on this is still conflicting.

Electronic HER2 Case Review, Interpretation and Sign Out:

HER2 cases may be reviewed from the comfort of the pathologist's office. HER2 cases referred to CPAL are processed and analyzed. Selected areas of tissue are scored and a case is prepared for pathologist review. When each case is ready for review, an e mail notification is sent to the ordering pathologist indicating that the case is ready for final review, interpretation and sign out via an easy to use web based connection (SoloWeb). Please contact the laboratory for assistance in setting up and utilizing this system.

Limitations of Procedure:

1. Optimum tissue fixation should be between 6 and 72 hours in 10% NBF. Other types of fixatives should not be used.

2. The patient's HER-2 status should be interpreted in conjunction with other clinical and pathologic prognostic data (tumor size, lymph node status, ER/PR, etc).

3. FISH assay results may not be informative if the specimen quality and/or specimen slide preparation is inadequate.

References:

1. PathVysion HER-2 DNA Probe Kit package insert, March 2017, Abbott Molecular Inc., Des Plaines, IL

2. Romond EH, Perez EA, Bryant J, et al: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005 Oct 20;353(16):1673-1684.

3. Wolff AC, Hammond ME, Schwartz JN, et al: Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer, American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update, Arch Pathol Lab Med; doi: 10.5858/arpa.2018-0902-SA)

4. Gonzales-Angulo AN, Hortobagyi GN, Esteva FJ. Adjuvant therapy with trastuzumab for HER-2/neu–positive breast cancer. Oncologist. 2006;11:857-867.

5. Dressler LG, Berry DA, Broadwater G, et al. Comparison of HER2 status by fluorescence in situ hybridization and immunohistochemistry to predict benefit from dose escalation of adjuvant doxorubicin-based therapy in node-positive breast cancer patients. J Clin Oncol. 2005;23:4287-4297.