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

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HER2 Testing (Breast Tissue) by FISH

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Effective Date:

Phased roll out beginning April 2013

Intended 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.

Testing Schedule:

Typically set up Mondays and Wednesdays and resulted Tuesdays and Thursdays. The testing schedule will be adjusted week to week to maximize workflow efficiencies. Expected Turn-Around-Time (TAT) is 2-4 days from receipt of specimens at CPAL.

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 <48 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.*

Specimen (IMPORTANT):

One H&E stained, cover slipped with ***invasive tumor area circled by a pathologist***. Three unstained (no cover slip) serial sections of ***formalin-fixed, paraffin-embedded breast tissue*** on slides. **Blocks will not be accepted**. 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.

Specimens should be transported and stored at room temperature.

Reference Ranges:

Ratio of HER2 (red): CEP17 control (green) as follows:

Ratio <1.8	Negative for HER2 amplification
Ratio 1.8 to 2.2	Equivocal*
Ratio >2.2	Positive for HER2 amplification

*verified by a second observer, counting additional cells.

CPAL follows the testing and reporting recommendations of the *American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) Panel* and *NCCN*.

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.

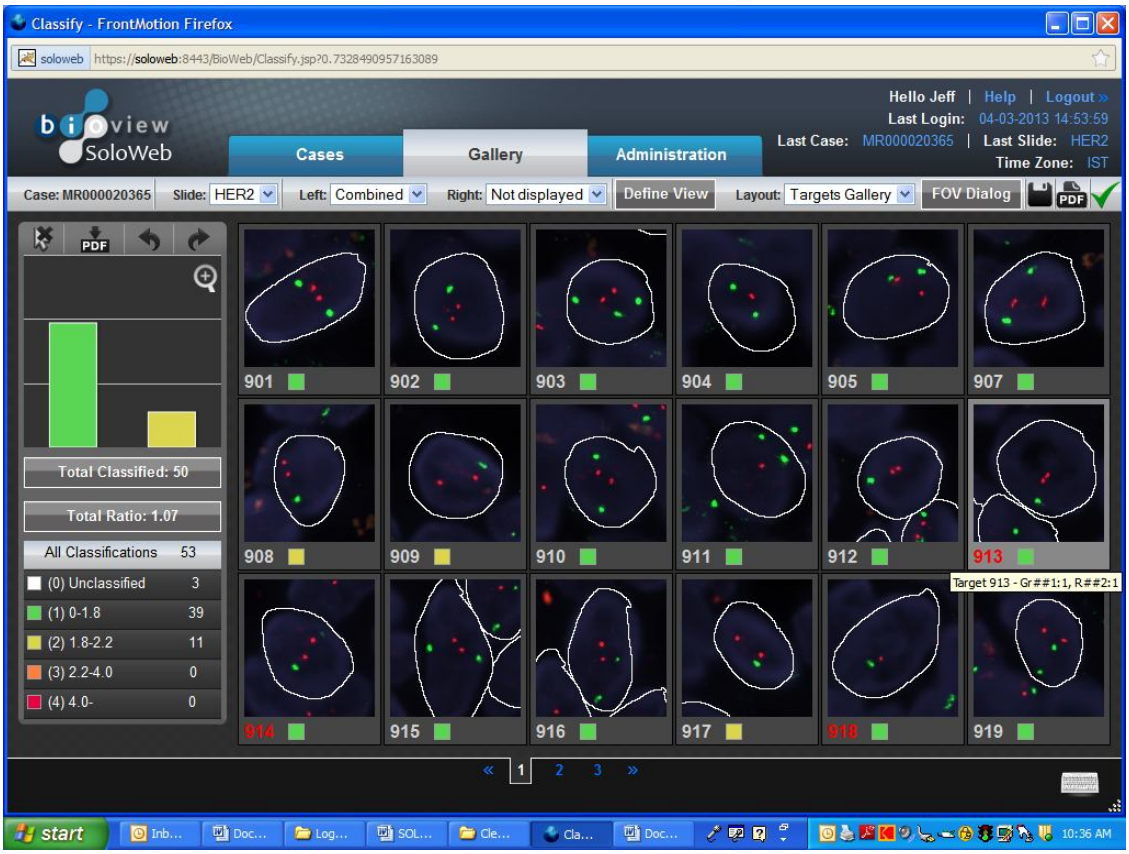
HER2 status is used to determine eligibility for trastuzumab (Herceptin) therapy. Patients with HER2 amplification are suitable candidates for trastuzumab therapy, as this monoclonal antibody is directed against the extracellular domain of HER2.

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

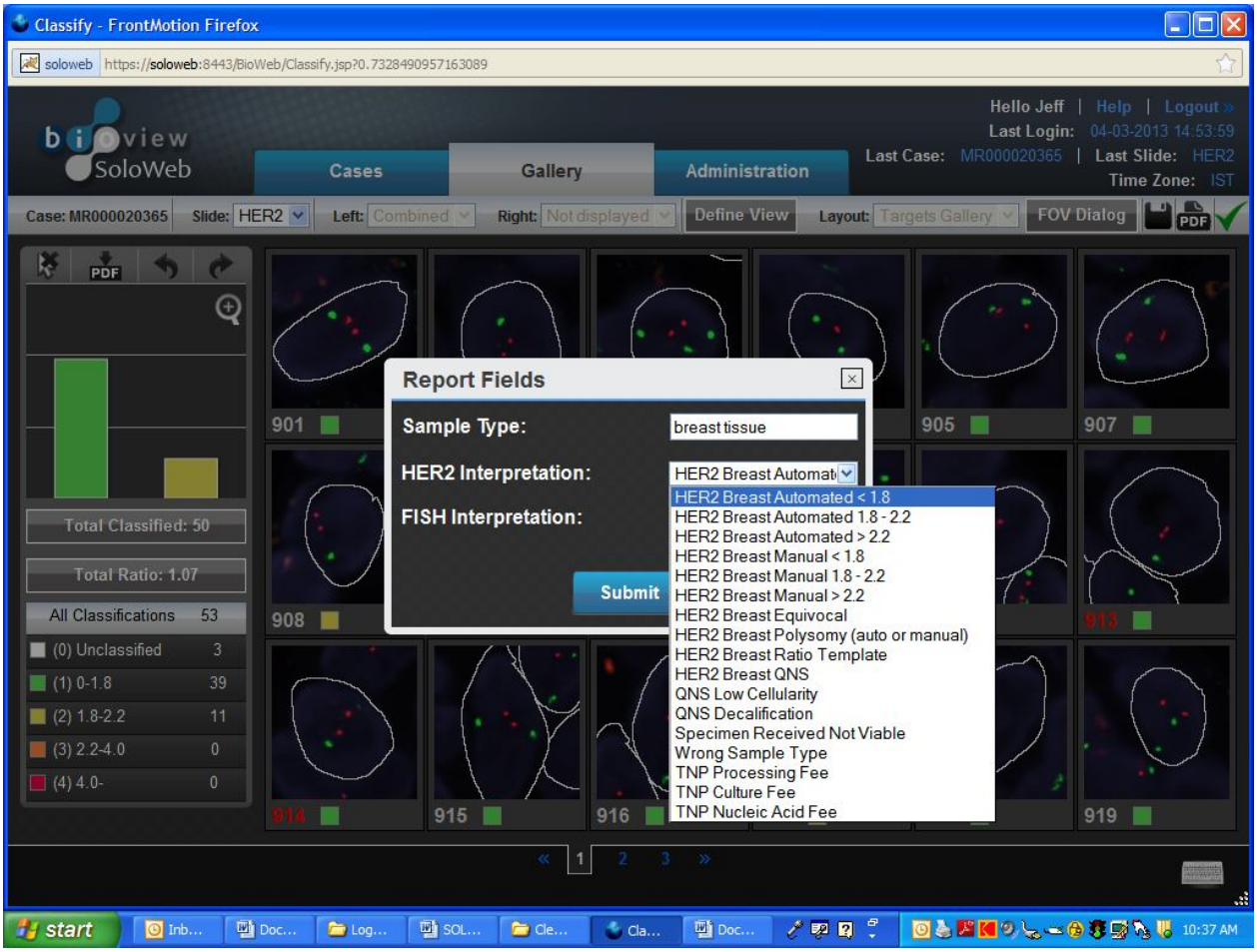
benefit from CMF regimens (C/methotrexate/F). HER2 amplified patients may also tend to be less sensitive to tamoxifen, but the data on this is still conflicting.

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. The selected areas of tissue are reviewed 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 very user friendly system.



Example screen shot #1 of SoloWeb interactive case review and sign out.



Example screen shot #2 of SoloWeb interactive case review and sign out.

Limitations of Procedure:

Optimum tissue fixation should be between 6 and 48 hours in 10% NBF. Other types of fixatives should not be used.

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).

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

References:

PathVysion HER-2 DNA Probe Kit package insert, Jan 2010, Abbott Molecular Inc., Des Plaines, IL

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.

Wolff AC, Hammond ME, Schwartz JN, et al: American Society of Clinical Oncology/College of American Pathologists Guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med. 2007 Jan;131(1):18-43.

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

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.

See Also **www.CPALmolecular.com**