New ASCO-CAP HER2 Testing Guidelines
To Have Dramatic Impact on HER2 Breast Cancer Testing in 2007

Significant changes in HER2 IHC and FISH testing in breast cancer are taking effect in 2007, based on new testing guidelines issued jointly by the College of American Pathologists (CAP) and the American Society of Clinical Oncologists (ASCO) that were published simultaneously in the respective journals of these societies, *Archives of Pathology and Laboratory Medicine* (131:18-43, 2007) and the *Journal of Clinical Oncology* (25:118-145, 2007.)

These new guidelines were developed in an attempt to ensure optimal performance of HER2 testing in all laboratories, and to maximize test accuracy. The guidelines will have implications for breast cancer specimen handling/fixation and HER2 testing and reporting, as well as quality assurance. PhenoPath has already implemented changes mandated by these guidelines, and is prepared to work with other laboratories to assist them in attaining compliance. PhenoPath has documented our extremely high concordance between HER2 IHC and FISH in a paper to be presented at the USCAP Meeting in March, 2007 (see article page 3).

Some highlights of the guidelines are as follows:

- All laboratories performing HER2 testing (FISH or IHC) must validate their assays by demonstrating 95% concordance of both positive and negative tests with the alternate method (IHC or FISH).
- Tissues must be fixed in formalin for > 6 hours, but < 48 hours. Laboratories have the option of validating their assay for tissues outside of these fixation times.
- New scoring criteria for IHC: 3+ requires intense membranous signal on > 30% (no longer 10%) of the invasive tumor cells, and 2+ requires either non-uniform or weak signal in at least 10% of tumor cells, or intense signal in < 30% of tumor cells.
- 2+ IHC results must be categorized as “equivocal” and require secondary testing by FISH.
- FISH-identified HER2/CEP-17 ratios of 1.8-2.2 must be categorized as “equivocal” and require review by a second pathologist as well as secondary testing by IHC.
- All laboratories performing HER2 testing must enroll in CAP proficiency testing and demonstrate at least 90% accuracy.

PhenoPath is working to set up a special program to assist pathologists around the United States whose laboratories wish to meet these new ASCO-CAP standards, but need an external laboratory to perform FISH studies to validate their HER2 IHC. Please call PhenoPath at (206) 374-9000 or visit our website (www.phenopath.com) for additional information about our external HER2 testing validation services.

Landmark Study on Estrogen Receptor IHC Testing
Published by PhenoPath Pathologists In *Journal of Clinical Oncology*

A study published in the December 20, 2006 issue of the *Journal of Clinical Oncology* (24:5637-5644, 2006) reports that the use of the rabbit monoclonal SP1 anti-estrogen receptor (ER) antibody for identifying ER in breast cancer specimens is significantly more sensitive and specific than the 1D5 mouse monoclonal antibody currently used widely in the United States and around the world. The study was conducted by PhenoPath Laboratories, in conjunction with the Genetic Pathology Evaluation Centre and British Columbia Cancer Agency in Vancouver, BC.

This tissue microarray-based study of 4,150 patients with 12.4 year median follow-up, compared the performance of the SP1 and 1D5 antibodies in identifying estrogen receptor-positive breast cancers, and determining the predictive and prognostic significance. The SP1 antibody identified significantly more cases as positive compared with 1D5, and SP1 better predicted those patients who would respond to treatment that targeted the ER receptor (tamoxifen) than did 1D5. Importantly, about 8% of the patients were characterized as ER-negative with the older 1D5 reagent (false-negative results), but were positive with the new SP1 antibody, and this group of patients clearly demonstrated improved survival, similar to patients with ER-positive cancers. Extrapolating from this study, as many as 8% of newly diagnosed breast cancer patients may be misclassified as ER-negative using the older testing methodologies and, therefore, inappropriately denied ER-targeted therapy such as tamoxifen. The finding of this study may result in a reassessment of current ER testing methodologies. In an editorial response to the study in the same issue of the *Journal of Clinical Oncology*, Dr. Mitch Dowsett of the Royal Marsden Hospital in London, England, stated "My own laboratory will not switch immediately, but the data are sufficient for us to seriously consider performing our own evaluation of it against the 6F11 antibody."
Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a common B cell neoplasm, accounting for ~5% of B cell lymphomas/leukemias. It is the most frequent chronic leukemia in the Western hemisphere and affects mainly elderly patients. Patients with CLL/SLL often have widely variable clinical courses following diagnosis, suggesting that CLL/SLL is a heterogeneous disease with overall survival ranging from months to decades. Accurately identifying those individuals with aggressive forms of CLL/SLL is clinically important, as these patients may benefit from early therapeutic intervention. Patients with more indolent CLL/SLL can likely be monitored closely without treatment, until symptoms develop that require therapy.

Over the past several years, numerous biomarkers of aggressive CLL/SLL disease have been described, including absence of somatic mutation of the IgH chain gene, expression of CD38 or ZAP-70, and presence of certain cytogenetic alterations, particularly deletions of the p53 and ATM genes. Evaluation of these biomarkers can be technically challenging, and in some instances routine clinical assays are not available. In addition, classic cytogenetic analysis may be hampered by the lack of growth of CLL/SLL cells in culture. Fluorescence in situ hybridization (FISH) provides a rapid and sensitive technique for reliably identifying common genetic alterations in CLL/SLL, including trisomy 12 (16%), deletion of the p53 gene on chromosome 17 (7%), deletion of the ATM gene on chromosome 11 (18%), and deletion at chromosome 13q14 (55%). Importantly, routine FISH analysis using a panel of five probes can provide important prognostic information, as median survival varies widely depending upon the genetic alteration identified: p53 (32 months), ATM deletion (79 months), normal FISH (111 months), trisomy 12 (112 months), and 13q14 deletion (133 months).

**HER2 IHC/FISH Concordance Studies at PhenoPath Laboratories**

Dr. Allen Gown of PhenoPath Laboratories will be presenting PhenoPath’s HER2 FISH and IHC concordance data at a platform presentation at the upcoming US-Canadian Academy of Pathology meeting in San Diego in March 2007 (see details on page 3). Based on an analysis of 8,353 cases on which both HER2 FISH and IHC studies were performed between 2000 and 2006, a concordance rate of 94% was found between IHC 3+ and FISH amplification, and 98.2% between IHC negative (0 or 1) and FISH non-amplification. To attain such high concordance, the study demonstrates the critical importance of normalizing IHC HER2 scoring by subtracting the signal on non-neoplastic breast epithelium from the score on the adjacent tumor. These follow-up concordance studies expand upon data previously published by PhenoPath pathologists in JAMA in 2004 (Yaziji H et al., JAMA 291:1972-7, 2004).
**USCAP Annual Meeting**


PhenoPath Laboratories is actively participating in the 2007 Annual Meeting of the United States and Canadian Academy of Pathology in San Diego, CA, from March 24 – 30, 2007. Our pathologists are giving multiple talks in addition to presenting several abstracts highlighting our ongoing clinical research studies, as detailed below. PhenoPath will also be exhibiting at the meeting from March 26 – 28, showcasing new immunohistochemistry and molecular tests, along with our 9-color flow cytometry services.

**Saturday, March 24, 2007**

7:00-10:00 PM: Allen M. Gown, MD, of PhenoPath Laboratories is presenting at the **CAP Companion Society Education Program Meeting, Best Practices in Contemporary Diagnostic Immunohistochemistry.** Dr. Gown’s talk is entitled “Best Practice Panels for Carcinoma of Unknown Primary.”

**Monday, March 26, 2007**


**Tuesday, March 27, 2007**


12:00-1:00 PM: Angelo Paolo Dei Tos, MD, of Hospital of Treviso, Italy and Allen M. Gown, MD, of PhenoPath Laboratories present at an Educational Event sponsored by Lab Vision – Thermo Fisher Scientific. Dr. Gown’s talk is entitled, "IHC Markers in Breast Cancer: Methodology Matters." Location: Edward B/C/D Room.

**Wednesday, March 28, 2007**

8:00 AM to 5:30 PM: Marc Ladanyi, MD, of Memorial Sloan-Kettering Cancer Center, New York and Allen M. Gown, MD, of PhenoPath Laboratories are Co-Directors of the Long Course, **Targeted Therapy of Cancer: New Roles for Pathologists.** This course focuses on how targeted therapies of cancer are impacting the practice of surgical pathology. Each talk provides an overview of the biology behind the specific targeted therapies, indications for their use, their clinical impact, the eligibility criteria (as defined by histology, or by detection of the target by IHC or molecular assays), specimen requirements and how testing is performed, histologic aspects of response assessment, molecular monitoring of disease, and biologic and histopathologic aspects of secondary resistance, as relevant for each cancer setting. Dr. Gown is a featured speaker and will present:

- An overview of the current status of ER/PR determination and hormonal therapy
- Summary of HER2 biology and clinical correlations
- Pros and cons and pitfalls of IHC and FISH in the determination of HER2 status, and accepted practice for HER2 status determination

**Friday, March 30, 2007**

1:00 to 4:30 PM: Neal Goldstein, MD, Department of Anatomic Pathology, William Beaumont Hospital and Todd Barry, MD, PhD, of PhenoPath Laboratories present the Short Course, **“Update and Troubleshooting Immunohistochemistry for Pathologists.”** The format of this course is a series of short lectures pertaining to automated IHC including interpreting IHC in small needle core biopsies, advantages and disadvantages of recent commercial antibodies, and troubleshooting for pathologists regarding the most common causes (and our list of most likely solutions) of suboptimal automated IHC stains. This course differs from past IHC courses in that it does not use a case model approach or cover set antibody panels for specific diagnostic differential situations. This course is designed for practicing and in-training anatomic pathologists.

See our website for other presentations by our pathologists this quarter in addition to the USCAP Meeting.

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**Teaching Cases on the Web**

Check out our latest teaching case on our website (www.phenopath.com), which uses flow cytometry, in situ hybridization, and morphology to diagnose acute promyelocytic leukemia, microgranular variant, with a t(15;17). Previous teaching cases are still available, including a case that uses in situ hybridization studies to show loss of 1p36 and 19q13 as an aid in the diagnosis of oligodendroglioma. Watch for new cases announced on our homepage.
Dr. David Y. Mason, of the University of Oxford in England, will present “New Immunohistological Markers of Human Haematopoietic Tissue” at the Quarterly Pathology/Immunohistochemistry Conference on Monday, February 26, 2007. The format of the conference is a social hour commencing at 6:30 p.m. followed by Dr. Mason’s lecture at 7:30 p.m. A light catered dinner will be served during the social hour.

Dr. Mason is currently Professor of Cellular Pathology, and Director of the Leukaemia Research Fund Immunodiagnostics Unit (Nuffield Department of Clinical Laboratory Sciences, University of Oxford).

His unit specializes in the study of lymphoid neoplasms using mainly microscopy-based techniques with particular emphasis on the production and identification of antibodies reactive with denatured leukocyte-associated proteins, since these reagents can be used for the immunohistological analysis of routine tissue biopsies.

His research focuses on two main areas of human lymphoma: 1) Improvement in laboratory diagnosis and assessment; and 2) The study of underlying genetic “causes” of these neoplasms. Much of Dr. Mason’s work is in the “translational” field, and has resulted in the production and commercial distribution of monoclonal antibodies, a number of which are of central importance to the routine diagnosis of human lymphoma (e.g., antibodies to CD45, BCL2, ALK).

The Winter Quarterly Conference will be co-sponsored by Dako.