On April 1, 2010, PhenoPath introduced several fully validated, 10-color flow cytometry assays, a significant step forward from the 9-color assays we have performed since launching our flow cytometry service in November 2005. The development of a 10-color flow assay on our 3-laser system was facilitated by the introduction of a second, commercially available fluorochrome excited by the violet laser (405 nm) on our Becton-Dickinson LSR II. Having two fluorochromes now excitable by the violet laser, in conjunction with the five fluorochromes excitable by the blue laser and three excitable by the red laser, now allows us to attain “double-digit” flow cytometry for routine leukemia/lymphoma immunophenotyping. Because the basic unit of visual analysis of flow cytometry data is the 2-dimensional histogram or dot-plot, 10-color flow (with 45 unique dot-plots) represents a 25% increase in the amount of data generated compared to 9-color flow (36 unique dot-plots), 61% more data than 8-color flow cytometry (28 unique dot-plots), and 300% more information than 6-color flow cytometry (15 unique dot-plots). Therefore, while the 6- to 8-color flow cytometry now emerging in the diagnostic leukemia/lymphoma marketplace represents a significant advance over the 3- to 4-color flow practiced in the past, 10-color flow cytometry is an objectively superior method to even 8-color flow.

Ten-color flow cytometric analysis of acute myeloid leukemia with monocytic differentiation, involving the bone marrow at greater than 90% of the total leukocytes. Blasts are colored black in the dot-plots above, while promonocyte-like cells are colored lavender.

PhenoPath Laboratories is pleased to announce a joint partnership with Diagnostic Cytogenetics, Inc. (DCI), in which DCI will provide requested cytogenetic testing for specimens sent to PhenoPath for other studies such as flow cytometry. DCI is an outstanding laboratory that offers extremely high-quality testing, very rapid turnaround time, and the same dedication to client service that we strive for at PhenoPath. For Steven Kussick, MD, PhD, PhenoPath’s Associate Medical Director and Director of Hematopathology and Flow Cytometry, the decision to partner with DCI was an easy one. He notes that DCI’s turnaround time for standard cytogenetic studies (karyotyping) is typically 24 hours! Physicians currently using DCI’s services uniformly rave about the consistent quality, outstanding level of service, and exceptional turnaround time.

PhenoPath Laboratories Partners with Diagnostic Cytogenetics Incorporated
The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) have recently reported on the recommendations for immunohistochemical (IHC) testing of estrogen and progesterone receptors in breast cancer, three years after making analogous recommendations for the immunohistochemical (and fluorescence in situ hybridization) testing of HER2 status of breast cancers. While readers are strongly recommended to read the guidelines, this article summarizes the recommendations of the panel.

• While ER and PR are relatively weak prognostic markers, i.e., independent predictors of clinical outcome, they are strong predictors of response to hormonal-based therapies such as tamoxifen. As with HER2, the Panel recognized that there are no “gold standard” IHC assays available for hormone receptor IHC. The Panel agreed that a relevant standard would be any assay whose specific preanalytical and analytical components conformed exactly to assays whose results had been validated against clinical benefit from endocrine therapy.

• Both ER and PR may possess independent predictive power, especially among premenopausal women, and therefore the Panel recommends reporting of results of both ER and PR.

• The optimal cutoff for ER and PR positivity was determined to be 1%, based on a number of published studies. Furthermore, the percentage of positive tumor cells may provide valuable predictive and prognostic information to inform treatment decisions. The Panel recommends for both ER and PR expression by IHC, in positive cases the percentage of cells staining positively should be reported (as assessed by either manual counts or image analysis). The panel further recommends that the intensity of immunostaining (weak, moderate, or strong) also be reported.

• While the Panel agreed that there are no absolute assay exclusions, a new category of “receptor uninterpretable” should be applied if the sample does not conform to the preanalytical specifications of the guidelines (e.g., fixed for less than 6 or more than 72 hours).

• Cautionary statements may be added to negative results occurring in the context of histologic subtypes of tumors almost always estrogen and progesterone receptor positive (e.g., lobular, tubular, mucinous, etc.).

• The CAP Laboratory Accreditation Program will require that every CAP-accredited laboratory performing ER and/or PR IHC testing participate in a proficiency testing program directed to these analytes.

NEW ASCO-CAP GUIDELINES FOR ER/PR ASSESSMENT IN BREAST CANCER RELEASED

Hammond MEH et al. Journal of Clinical Oncology, Vol 28, No 16 (June 1), 2010: pp. 2784-2795

There will be two major initial applications of our 10-color assays: 1) our two standard flow tubes for evaluating myeloid maturation, and of particular importance in the diagnosis of acute myeloid leukemia and other myeloid stem cell neoplasms, are now 10-color assays, replacing the 9-color assays used since November 2005; and 2) our four assays (one each for B and T cells, and two for myeloid cells) utilizing the fluorescent DNA-binding dye DAPI for definitive analytic exclusion of non-viable (DAPI-positive) cells will now include DAPI plus 9 antibodies, as opposed to DAPI plus 8 antibodies previously. Note that we do not routinely use DAPI in our flow assays, because careful forward versus side scatter gating is more than adequate to exclude non-viable cells; the 10-color assays using DAPI are typically reserved for highly degenerated specimens.

Finally, although we have changed the basic methodology of our flow cytometry assays, the meticulous attention to detail that you have come to expect from the PhenoPath flow cytometry assay will not change at all under the new 10-color system.

Coming Next Issue: PhenoPath’s ER and PR IHC assays have been both clinically validated, with outcome data on more than 4,000 patients followed for a median of 12.4 years. Our ER and PR assays have also been validated against biochemical (e.g., DCC) and molecular (RT-PCR) based ER and PR assays. PhenoPath will be offering ER and PR assay validation services, in accordance with the new requirements stipulated in the new guidelines and expanded upon in a recent publication (Fitzgibbons PL et al., Arch Pathol Lab Med 134:930-5, 2010).
VISIT PHENOPATH AT THE FOLLOWING MEETINGS

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<tr>
<td>CIQC/CAP-ACP Seminar in Diagnostic Immunohistochemistry</td>
<td>7/8/10 - 7/9/10</td>
<td>Marriott Château Champlain Hotel, Montreal, Quebec, CANADA</td>
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<td>Presentations</td>
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<td>7/8/10, 3:30-5:30 PM: Allen M. Gown, MD presents, “Current Controversies in ER and HER2 Testing”</td>
<td><a href="http://www.pathologyconference.com">www.pathologyconference.com</a></td>
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ASCP Pathology Update 2010

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<tr>
<td>7/27/10, 1:30-5:30 PM: Allen M. Gown, MD presents, “Immunohistochemistry”</td>
<td><a href="http://www.ascp.org">www.ascp.org</a></td>
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CAP ’10 - The Pathologists’ Meeting

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<td>PhenoPath Booth Exhibit</td>
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XXVIIIth International Congress of the International Academy of Pathology

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<tr>
<td>10/12/10, 3:00-6:30 PM: Allen M. Gown, MD presents, “Symposium 13: Immunohistochemistry: Genogenic Immunohistochemistry”</td>
<td>iap2010.com</td>
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<tr>
<td>10/13/10, 3:00-6:30 PM: Allen M. Gown, MD presents, “Symposium 31: Practical Roles of Pathologists for Molecular Targeted Cancer Therapy: Detection of EGFR Family for Molecular Therapy for Breast Cancers”</td>
<td>iap2010.com</td>
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Clinical Cytometry Companion Meeting @ 2010 ASCP Annual Meeting

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<td>10/27/10, 2:15-5:15 PM: Steven J. Kussick, MD, PhD presents, “Clinical Cytometry Society-Update in Clinical Cytometry”</td>
<td><a href="http://www.ascp.org">www.ascp.org</a></td>
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For up-to-date information, visit our website: www.phenopath.com

Based in Seattle, DCI has been in business since 1981, providing state-of-the-art evaluation for chromosomal abnormalities in neoplasms of bone marrow, blood, and tissue, and in non-neoplastic settings, including prenatal diagnosis and evaluation for congenital chromosomal abnormalities. DCI’s board-certified cytogeneticists are always available for consultation.

Kam Au, Founder of DCI, looks at the partnership as an opportunity for both companies, “PhenoPath has such a wonderful reputation that I am honored to be associated with them. The combination of DCI’s remarkable turnaround time, and PhenoPath’s expertise in hematopathology, is powerful. The idea that our clinicians will be able to receive complete, accurate, comprehensive reports within one or two days is very exciting.” If desired, PhenoPath will be delighted to integrate the DCI cytogenetic findings with the results of our parallel studies at PhenoPath, which typically involve flow cytometry.

Both DCI and PhenoPath have updated requisition forms and are accepting cases from throughout the country for the combined services. If your practice is searching for reference laboratories that offer the latest in testing menus, combined with rapid turnaround time and the highest levels of both quality and customer service, then the DCI-PhenoPath relationship offers you a viable alternative to the larger, less personal labs.
Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is associated with specific chromosomal abnormalities that have prognostic significance. These abnormalities are readily detected by fluorescence in situ hybridization (FISH). PhenoPath Laboratories now offers a panel of these CLL/SLL FISH probes. This FISH panel evaluates for the most common chromosomal abnormalities in CLL/SLL, including, but not limited to, deletions at 11q22.3 (ATM), 13q14.3 (D13S319), 13q34 (LAMP1), as well as trisomy 12. An automated imaging platform (MetaSystems™) facilitates the scanning of probed slides made from fresh specimens, and the data is analyzed by a PhenoPath pathologist. Assessment of CLL/SLL cases for these abnormalities is important for planning appropriate patient therapy.

References:

www.phenopath.com