Phenomena™

THE NEWSLETTER OF PHENOPATH LABORATORIES

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Don't Procrastinate: Time to Validate!

Recently published guidelines from the American Society of Clinical Oncologists (ASCO) and the College of American Pathologists (CAP) mandate that all laboratories performing HER2 testing of breast cancer specimens validate their IHC assay. The following are frequently asked questions (FAQs) regarding this validation. We welcome any additional questions you might have. Please contact PhenoPath Laboratories should you wish our assistance with this validation process.

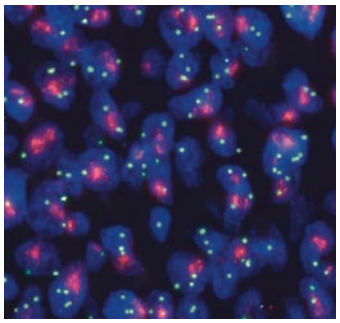
Q: *Our laboratory already runs HER2 IHC, and we follow a standard immunostaining protocol and scoring system. Do we still have to validate our HER2 IHC?*
Yes. Regardless of how long a laboratory has been performing HER2 IHC, and regardless of the test volume, the new ASCO-CAP Guidelines issued in January 2007 require that all laboratories performing HER2 IHC testing validate their HER2 IHC assay.

Q: *Is there something unique about HER2 IHC validation compared with other IHC antibodies?*
All antibodies employed in diagnostic immunohistochemistry need to be validated, as do all pathology tests. In the case of HER2 IHC, however, the ASCO-CAP Guidelines recommend validation of HER2 IHC through the use of parallel testing by another previously validated method.

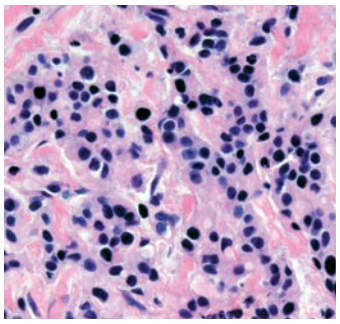
Q: *Can I do the validation in my own laboratory?*
Yes, but only if your laboratory performs HER2 testing by more than one method (e.g., IHC and FISH). If your laboratory only performs HER2 by IHC, you will need to perform the HER2 validation in conjunction with another laboratory that performs an alternative HER2 detection technique, e.g., FISH.

Q: *Is there a "gold standard" for HER2 testing? Is IHC or FISH preferred?*
No, there is no "gold standard" for HER2 testing, which is why the Guidelines call only for concordance between positive and negative results using two methodologies (e.g., IHC and FISH).

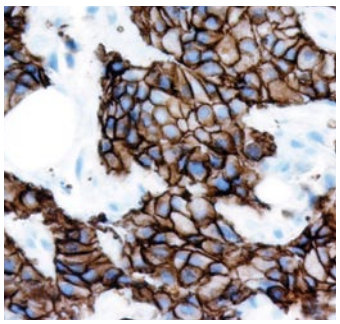
Q: *How many cases are required for validation? How often do we need to validate the assay?*
It has been determined that a validation set should be composed of 25-100 cases. Note that these 25-100 cases need to be either positive (3+) or negative (0, 1+), i.e., no cases scored as 2+ (equivocal) should be included in the validation set. Re-validation needs to be performed annually.



HER2 FISH



H&E



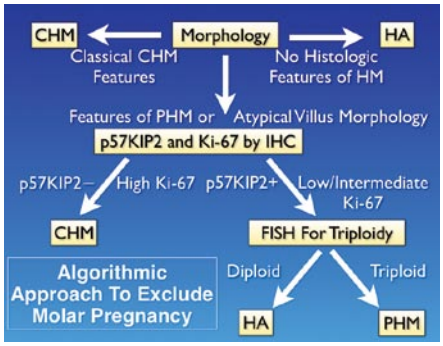
HER2 IHC

Q: *Does my tissue need to be formalin fixed?*
It is highly recommended that neutral buffered formalin (NBF) be employed as the fixative. If a laboratory chooses to employ an alternative fixative, according to the ASCO-CAP Guidelines, that laboratory is required to perform a separate validation on a set of tumors in which portions of each have been fixed separately in NBF and the alternative fixative, with 95% concordance of positive and negative results.

Q: *What about length of fixation requirements?*
The ASCO-CAP Guidelines suggest that tissue for HER2 testing should be fixed for greater than 6 but less than 48 hours. (However, this is not an absolute exclusion criterion, and tissues fixed shorter or longer than this may be employed as long as a disclaimer appears on the report of negative cases that tissues fixed outside the guidelines might yield false negative results.) It is highly recommended, however, that laboratories follow these new fixation time requirements.

Q: *Why should we use PhenoPath Laboratories for our HER2 validation?*
The pathologists and technical staff at PhenoPath Laboratories have nationally recognized experience and expertise in HER2 testing. PhenoPath has consistently demonstrated the high quality of its HER2 IHC by publishing concordance studies of HER2 IHC and FISH data in peer-reviewed publications, and presenting these data at national pathology and oncology meetings.

Q: *What are the most recent HER2 IHC and FISH concordance data at PhenoPath Laboratories?*
Based on an analysis of 6604 cases, in which parallel IHC and FISH HER2 studies were performed between 2003 and 2006, 1904/1919 (99.2%) of those showing IHC results of 0 or 1+ proved to be non-amplified by FISH, and 529/562 (94.7%) of those cases showing IHC results of 3+ proved to be amplified. These data were presented at the March 2007 USCAP Meeting in San Diego and have been submitted for publication.



Molar Pregnancies

There is significant interobserver and intraobserver variability in the diagnosis of hydatidiform mole, particularly those arising in the first trimester. Since distinguishing partial (PHM) and complete (CHM) hydatidiform moles from hydropic spontaneous abortions (SAs) can impact patient care, as at least 25% of complete moles have the potential to develop choriocarcinoma and persistent gestational trophoblastic disease, the use of ancillary studies, including p57KIP2, Ki-67 immunohistochemistry, and interphase FISH may be helpful in supplementing morphology in the diagnosis of hydatidiform moles.

p57KIP2 is a cyclin-dependent kinase inhibitor that is paternally imprinted and maternally expressed such that CHMs which are paternally derived show loss of expression of p57KIP2. p57KIP2 is only expressed if a maternally derived gene is present, and thus, both PHMs and hydropic SAs express p57KIP2. CHMs also show a high Ki-67-defined proliferative rate within cytotrophoblasts which is not seen in PHMs or hydropic SAs. Interphase FISH can be helpful in identifying triploidy that is the hallmark of PHMs, but not a typical feature of CHMs or hydropic SAs.

In a recent oral presentation at USCAP (March 2007), we retrospectively examined 191 cases (including 53 PHMs, 60 CHMs, and 78 SAs) that were submitted for routine evaluation to exclude hydatidiform mole. Loss of p57KIP2 and high Ki-67-defined proliferative index were noted in 59 of the 60 CHMs, whereas PHMs and SAs displayed no loss of expression of p57KIP2 and had a significantly lower Ki-67-defined proliferative rate. As expected, an increased percentage of cells containing three chromosome 17 signals by FISH, indicative of triploidy, was noted in 29 of 29 PHMs, as compared to CHMs and SAs which were mostly diploid. Thus, p57KIP2 and Ki-67 immunohistochemistry, as well as interphase FISH, are useful in the diagnosis of hydatidiform mole, and are particularly helpful in cases in which the morphology alone may not be definitive.

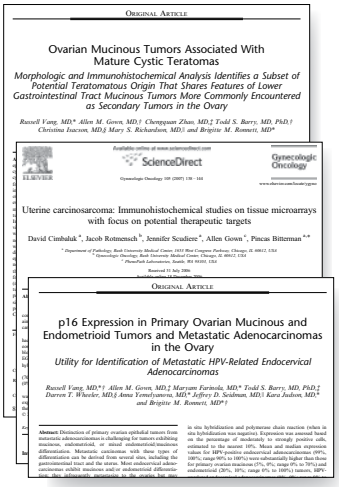
Shipping Specimens to PhenoPath in PhenoBoxes

PhenoPath Laboratories provides prepaid and pre-addressed Fed-Ex overnight airbills and PhenoBoxes for our clients to send specimens to our laboratory.

We request that you combine multiple specimens in one PhenoBox to form one shipment whenever possible, especially when shipping on the same day. Your staff only has to fill out one airbill, saving time and effort. This also helps conserve material, always a plus when "thinking green."

Please ensure that each specimen is properly labeled and that a PhenoPath test request form is included for each specimen. Our skilled specimen processing staff will inventory each shipment before accessioning, to ensure that each case is handled accurately and efficiently.

If you have any questions regarding specimen shipping, please contact us at (206) 374-9000.



Original Article

Ovarian Mucinous Tumors Associated With Mature Cystic Teratomas

Morphologic and Immunohistochemical Analysis Identifies a Subset of Potential Teratomatous Origin That Shares Features of Lower Gastrointestinal Tract Mucinous Tumors More Commonly Encountered as Secondary Tumors in the Ovary

Russell Vang, MD,* Allen M. Gown, MD,† Chengquan Zhao, MD,‡ Todd S. Barry, MD, PhD,† Christina Isacson, MD,‡ Mary S. Richardson, MD,† and Brigitte M. Ronnett, MD*

ScienceDirect

Gynecologic Oncology

Uterine carcinosarcoma: Immunohistochemical studies on tissue microarrays with focus on potential therapeutic targets

David Cimbaluk *, Jacob Rotmensch b, Jennifer Scudiere *, Allen Gown *, Pincas Bitterman a,*

Original Article

p16 Expression in Primary Ovarian Mucinous and Endometrioid Tumors and Metastatic Adenocarcinomas in the Ovary

Utility for Identification of Metastatic HPV-Related Endocervical Adenocarcinomas

Russell Vang, MD,*† Allen M. Gown, MD,‡ Maryam Farinola, MD,* Todd S. Barry, MD, PhD,‡ Darren T. Wheeler, MD,‡ Anna Yemelyanova, MD,* Jeffrey D. Seidman, MD,§ Kara Judson, MD,* and Brigitte M. Ronnett, MD*†

Recent PhenoPath Publications in Gynecologic Pathology

PhenoPath Laboratories' pathologists, working in collaboration with colleagues at the Johns Hopkins University School of Medicine and Rush Medical Center, have published three papers in the past few months on immunohistochemical studies involving tumors of the gynecologic tract. In the study by Vang et al. (*Am J Surg Pathol* 31:854-69, 2007), an interesting subset of ovarian tumors associated with mature cystic teratoma was found to display immunohistochemical features of lower GI-type mucinous carcinomas. In the study by Vang et al. (*Am J Surg Pathol* 31:653-63, 2007), p16 expression was found to be a highly specific and sensitive marker of HPV-related endocervical adenocarcinomas metastatic to the ovary among the primary ovarian tumors and metastatic adenocarcinomas from other sites that are in the differential diagnosis of ovarian tumors having mucinous and/or endometrioid/endometrioid-like differentiation. Finally, in the paper by Cimbaluk et al. (*Gynecol Oncol* 105:138-44, 2007) immunohistochemical studies of potential therapeutic targets in uterine carcinosarcoma are presented, including HER2, c-kit, EGFR, vascular endothelial growth factor (VEGF), and cyclo-oxygenase-2 (COX-2), with strong expression of VEGF found in both mesenchymal and epithelial components.

PhenoPath 2007-2008 Fellow

PhenoPeople Profile

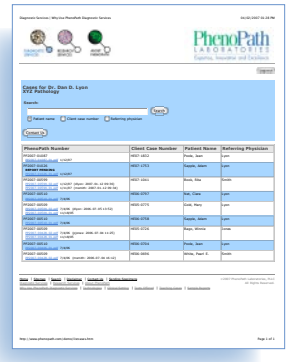
Geoffrey S. Baird, M.D., Ph.D.

PhenoPath Laboratories is pleased to welcome Dr. Geoffrey Baird as the 2007-2008 PhenoPath Fellow. Geoff received a B.S. in Chemistry from Stanford University in 1995, then went on to earn a Ph.D. in the Biomedical Sciences Program in 2001 and an M.D. in 2003, both from the University of California, San Diego. During his studies, he was honored with the Pharmacology Education and Research Foundation 2003 Medical Student Award. He completed his residency in Anatomic and Clinical Pathology at the University of Washington in June, 2007.

Geoff has an extensive background in research and development in several laboratories, and has applied for and received multiple patents as well as co-authored numerous publications. Geoff's current research interests include quantitative immunohistochemistry, fluorescence technology, and immunoassay development.

Geoff is married to Denise, an attorney, and has two boys ages four and two. He spends much of his free time with his family exploring the Pacific Northwest and has strong interests in sports and outdoor activities.

Have you heard about **PhenoNet**, PhenoPath's new electronic reporting system?



Contact your service representative at 888-927-4366 or visit www. phenopath.com.

PhenoPath Laboratories Needs Your NPI

The Centers for Medicare and Medicaid Services (CMS) recently established the National Plan and Provider Enumeration System (NPPES) to distribute and maintain National Provider Identifiers (NPIs). HIPAA regulations now require the use of standard unique identifiers for health care providers to aid in the exchange of electronic health information.

The date for NPPES compliance was May 23, 2007. If you have not yet provided PhenoPath Laboratories with your NPI information, we urge you to do so now. Without your NPI, we are required to bill your facility directly for laboratory services for Medicare and Medicaid patients, as well as for some third-party payers that require your NPI number.

Please go to www.phenopath.com for more information on how to obtain/submit your NPI.

VISIT US AT THE FOLLOWING MEETINGS:

For up-to-date information, visit our website www.phenopath.com.

Diagnostic Pathology Update 2007 (USCAP)
July 14 - 20, 2007, Banff Park Lodge, Banff, Alberta, Canada
Dr. Allen M. Gown is a featured guest speaker at the Diagnostic Pathology Update 2007 in Banff Park Lodge, Banff, Alberta, Canada on July 16, 2007. He will present a talk from 7:30 - 9:30 pm entitled "Special Topic: Immunohistochemistry." www.uscap.org

2007 Breast Cancer Symposium
September 7 - 8, 2007, San Francisco Marriott, San Francisco, CA
An abstract featuring studies performed at PhenoPath Laboratories will be presented at the 2007 Breast Cancer Symposium in San Francisco, CA from September 7-8, 2007. www.breastcasymposium.org

22nd Annual Clinical Cytometry Meeting and Course
October 5 - 8, 2007, Hyatt Regency Washington on Capitol Hill, Washington, DC
Dr. Steven J. Kussick is a featured guest speaker at the 22nd Annual Clinical Cytometry Meeting, in Washingon, DC on Monday, October 8, 2007. He will present a talk from 11:45 am to 1:15 pm entitled, *"Flow Cytometry in the Diagnosis of Myelodysplastic Syndromes and Myeloproliferative Disorders."* www.cytometry.org

Nassau County Society of Pathologists Meeting
October 20, 2007, North Shore University Hospital, Manhasset, NY
Dr. Allen M. Gown and Dr. Clive Taylor are featured guest speakers at the Nassau County Society of Pathologists Meeting at the North Shore University Hospital in Manhasset, NY on October 20, 2007. They will present several talks regarding *"Advances in Diagnostic Immunohistochemistry for the Practicing Surgical Pathologist."* www.naspath.org

Arizona Society of Pathologists' 2007 Fall Meeting
October 27-28, 2007, Hilton Scottsdale Resort, Scottsdale, AZ
Dr. Todd S. Barry is a featured guest speaker at the Arizona Society of Pathologists Meeting in Scottsdale, AZ on Sunday, October 28, 2007. He will present the following talks:
8:30-9:15 am: Update and Troubleshooting in Immunohistochemistry
9:15-10:00 am: Case Studies in Immunohistochemistry
10:30-11:15 am: Use of FISH in Diagnostic Pathology www.azmedassn.org

California Society of Pathologists
December 5 - 8, 2007, Palace Hotel, San Francisco, CA
Visit us at the California Society of Pathologists Meeting at the Palace Hotel in San Francisco, CA from December 5-8, 2007. PhenoPath's Esoteric Pathology Consultant, David Sklenar, will be manning the exhibit booth. www.calpath.org

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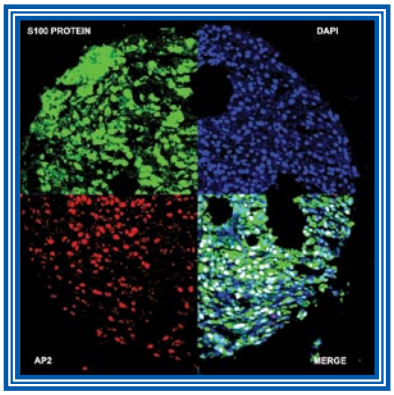
Featuring Dr. David L. Rimm

At Our Summer Quarterly Conference



Dr. David L. Rimm, of Yale University School of Medicine in New Haven, CT, will present "Biomarker Discovery by Quantitative and Multiplexed Analysis of Tissue Microarrays" at the Quarterly Pathology/ Immunohistochemistry Conference on *Thursday, August 2, 2007*. The format of the conference is a social hour commencing at 6:30 p.m. followed by Dr. Rimm's lecture at 7:30 p.m. A light catered dinner will be served during the social hour.

Dr. Rimm is currently a Professor in the Department of Pathology at the Yale University School of Medicine, the Director of the Yale Pathology Tissue Services and Tissue Microarray Facility, and the Director of Medical Studies. He received M.D. and Ph.D. degrees from Johns Hopkins School of Medicine, and subsequently completed residency training in pathology at Yale, followed by a cytopathology fellowship at the Medical College of Virginia. Dr. Rimm is board certified in anatomic pathology and cytopathology. He is an author on more than 150 peer-reviewed articles, has been issued 6 patents, and is a scientific founder of HistoRx, a digital pathology company.

Dr. Rimm's research interests focus on in situ quantitative assessment of tissue biomarkers, including predicting outcomes and response to therapies in breast, lung and colon cancer, and melanoma. A subset of his group studies the cell and molecular biology of cell adhesion and growth factor receptors, and a third subset works on spectral-spatial analysis of cytologic specimens.

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