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.

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.
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 paternally 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.
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.

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**VISIT US AT THE FOLLOWING MEETINGS:**

*For up-to-date information, visit our website [www.phenopath.com](http://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](http://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.breastcancer.org](http://www.breastcancer.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 Washington, 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](http://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](http://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](http://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](http://www.calpath.org)
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.