

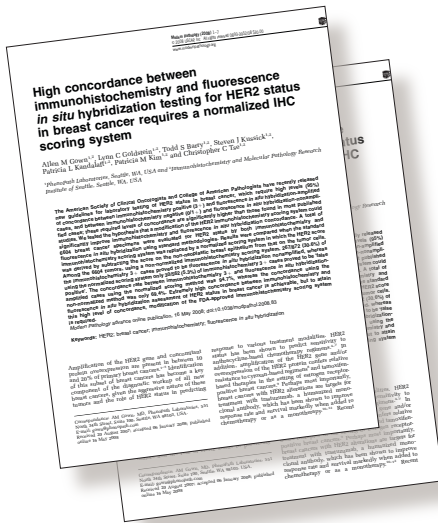


phenomena

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Test Validation

Why PhenoPath Surpasses Others



*First of an Ongoing Series of Articles
by Doug Sites
Director of Sales & Marketing*

Today, dozens of laboratories are vying for your business, and the one assumption every sales representative makes is that quality is a given and the sales process should skip over this question without any discussion. In fact, reps today are taught to start conversations with, "Doctor, I think we can agree, that in today's laboratory, new technology and equipment has made quality a moot point." We beg to differ. Quality continues to be one of the top three items noted by pathologists when asked to list the most important things that determine to whom they send their specimens, yet it is the one point rarely explored. The best marketing scam over the last several years has been the "24-hour turn-around-time game", pushed at the clinician's office to force questionable and false beliefs that faster is better. When labs shifted the paradigm from quality results to 24-hour turn-around-time, we walked away from the idea that occasionally, it is more important to get the right answer first versus the first answer fast.

At PhenoPath, our quality starts with the validation process we have incorporated for every test we bring to market. Unlike some labs that may bring on a new clone for a test they are currently offering and simply run two or three cases to validate their new antibody, PhenoPath runs these clones through a battery of tests to confirm the ability of the antibody to recognize the target antigen in normal and diseased tissues where it is reasonably expected to localize, and to determine the sensitivity and specificity of the antibody. A PhenoPath pathologist is assigned to oversee each new test to assure consistency in the process and document that all benchmarks have been met, and once testing is completed, a team of pathologists and technologists then reviews results before the test is signed off. In the case of breast marker studies such as HER2 and ER, PhenoPath pathologists and staff have taken validation to an even higher level, in which thousands of breast cancer specimens have been analyzed and results published in the **Journal of Clinical Oncology** and **Modern Pathology**, respectively. In the case of ER, the availability of outcome data has permitted *clinical validation* of this IHC test in predicting outcome and response to hormonal therapy. In the case of HER2 IHC, PhenoPath is the only major laboratory that has published its concordance data with HER2 FISH on over 8,000 cases.

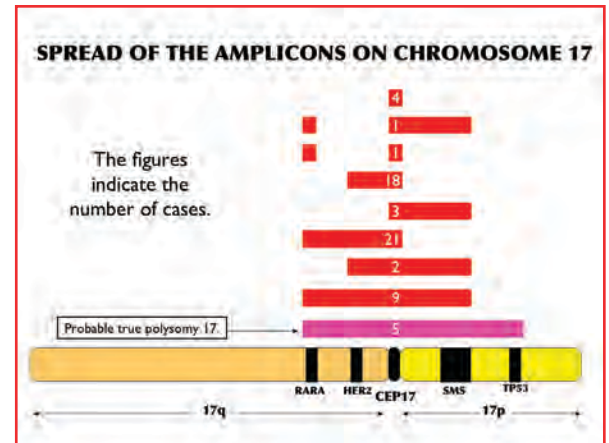
As a clinician, you want to give your clients and their patients the best results available, and the topic of quality testing should be a key discussion. If you want to have a competitive advantage over the labs that are calling directly on your client base, a conversation on right results versus fast results need to occur sooner rather than later. And for those laboratories looking to sell you their services, ask how they validate their assays, and find out if they have a *clinically validated* ER IHC or if they have *published* their HER2 IHC and FISH concordance data. At PhenoPath, what you will find will make you more confident in the results you are receiving and reporting.

New Tests at PhenoPath

SMS/RARA

The *SMS/RARA* FISH assay improves accuracy of HER2 gene status determination in patients with both negative/equivocal HER2 FISH results and increased CEP17 signals.

Detection of HER2 gene amplification by fluorescence in situ hybridization (FISH) is one of the key tests predicting clinical response of breast cancer to trastuzumab (Herceptin™). According to the CAP/ASCO guidelines for HER2 testing (Arch Pathol Lab Med—Vol 131, January 2007), the tumor is considered positive for HER2 gene amplification when the HER2/CEP17 ratio is greater than 2.2, negative if less than 1.8, and equivocal if the ratio is between 1.8 and 2.2. The need to use CEP17 as reference for HER2 evaluation is supported by the observation that an increased HER2 gene copy number, as a result of chromosome 17 polysomy, may not bear the same clinical significance as HER2 gene amplification. However, response to trastuzumab has been observed in some cases with polysomy 17. In a study performed at PhenoPath Laboratories, and presented at the 2008 San Antonio Breast Cancer Symposium, 64 cases of breast cancer which showed elevated chromosome 17 copy number were further analyzed for copy numbers of SMS and RARA, genes which reside on the short and long arms, respectively, of chromosome 17, and which are telomeric to the centromere/HER2 loci. Results of this study suggest that greater than half of all cases categorized by CEP17 analysis alone as polysomic might actually be eusomic, but with the amplicon including HER2 and CEP17. The accompanying diagram shows the location and size of amplicons as suggested by these FISH studies incorporating four different loci on chromosome 17. As a result of these studies, we recommend performing additional FISH studies for other chromosome 17 gene loci for all HER2 FISH negative/equivocal cases with increased CEP17 signals to improve accuracy of HER2 gene status determination.

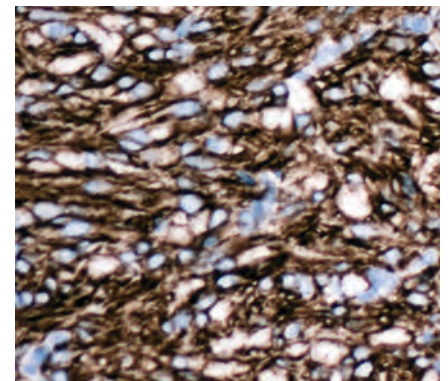
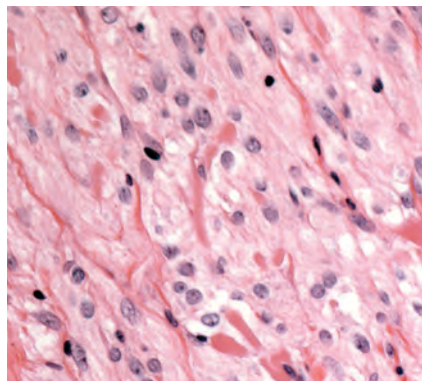


Ref: Tse CH, Gown AM, Goldstein LC, Kandalafi PL, Baird G, Kussick SL, Barry TS. *Cancer Res* 69 (Suppl 2):1079, 2009

DOG1

DOG1 is a better marker for GISTs than is *c-kit* (CD117), and may improve identification of patients eligible for small molecule inhibitor drugs such as imatinib (Gleevec™).

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the GI tract. Approximately 85% of these tumors possess activating mutations of either KIT or PDGFRA, which promote tumor growth, but which also serve as targets for small molecule inhibitor drugs such as imatinib (Gleevec™) and sunitinib (Sutent™). GISTs can manifest considerable histologic overlap with other mesenchymal processes, such as smooth muscle and nerve sheath tumors, and the use of immunohistochemistry (IHC) to identify the expression of *c-kit* (CD117) and/or CD34 can be helpful in the positive identification of these tumors. Nonetheless, between 5 and 15% of GISTs demonstrate either weak or negative immunostaining with antibodies to *c-kit* (CD117), with many of the latter corresponding to those possessing PDGFRA mutations, and those with epithelioid histologic features. DOG1 is a protein that was found by expression profiling to be selectively expressed in GISTs, and antibodies to this protein have recently become available. In a recent study published by Espinosa et al, DOG1 expression was found in 87% of GISTs, but the sensitivity of DOG1 was particularly high (79%) in the subset of GISTs with PDGFRA mutations, in which the corresponding sensitivity of *c-kit* was only 9%. The specificity of DOG1 was quite high, too, with only a tiny fraction of leiomyosarcomas and synovial sarcomas, among more than 900 soft tissue tumors examined, showing positivity. We have confirmed the high sensitivity and specificity of DOG1 in extensive validation studies performed at PhenoPath, and are pleased to offer this additional new test for the analysis of tumors in which GIST features prominently in the differential diagnosis.



H&E stained section (left) of gastrointestinal stromal tumor with epithelioid features showing strong, uniform expression of DOG1 (right)

Ref: Espinosa I, et al. *Am J Surg Pathol* 32:210-218, 2008

www.phenopath.com

Recent Publications by PhenoPathologists

ABERRANT EXPRESSION OF NEUROENDOCRINE MARKERS IN RHABDOMYOSARCOMA

In a study performed in conjunction with Dr. Andrew Folpe at the Mayo Clinic and investigators at the Children's Hospital and Baylor College of Medicine in Houston, a surprisingly high incidence of expression of neuroendocrine markers (synaptophysin, chromogranin A) was found in an analysis of 44 alveolar rhabdomyosarcomas. Thirty-two percent of cases (12 of 37) expressed at least one of the neuroendocrine markers, suggesting that aberrant expression of neuroendocrine markers is relatively common in alveolar rhabdomyosarcoma. These findings have significant implications for the diagnosis of alveolar rhabdomyosarcoma, particularly in adults and in the head and neck locations. These data, along with the cytokeratin expression documented in a comparable subset of these alveolar rhabdomyosarcomas in this study, suggests the presence of true epithelial and/or neuroendocrine differentiation in a subset of alveolar rhabdomyosarcomas. These findings underscore the importance of employing a panel of IHC markers, including desmin and myogenin, as well as molecular studies, in the diagnosis of primitive round cell neoplasms in all age groups and in all locations.

Bahrani A, Gown AM, Baird GS, Hicks MJ, Folpe AL. Aberrant expression of epithelial and neuroendocrine markers in alveolar rhabdomyosarcoma: a potentially serious diagnostic pitfall. *Mod Pathol* 21:795-806, 2008



ANALYSIS OF SPINDLE CELL LESIONS OF THE URINARY BLADDER

In a study performed in part at PhenoPath Laboratories, and in collaboration with Dr. Mahul Amin and colleagues at Cedars-Sinai Medical Center in Los Angeles, as well as investigators at The Mayo Clinic and Massachusetts General Hospital, the immunohistochemical profiles of spindle cell lesions of the urinary bladder (e.g., sarcomatoid urothelial carcinoma, leiomyosarcoma, and pseudosarcomatous myofibroblastic proliferation) were compared, using a panel of antibodies including those to various cytokeratins, smooth muscle actins, p63, and ALK. Results of the study demonstrated that when combined with previously characterized markers, antibodies to p63 and cytokeratin 5/6, as well as the 34βE12 antibody to high molecular weight cytokeratins, together provide useful discriminatory power.

Westfall DE, Folpe AL, Paner GP, Oliva E, Goldstein L, Alsabeh R, Gown AM, Amin MB. Utility of a comprehensive immunohistochemical panel in the differential diagnosis of spindle cell lesions of the urinary bladder. *Am J Surg Pathol* 33:99-105, 2009



VISIT US AT THE FOLLOWING MEETINGS:

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

Oregon Pathologists Association Meeting

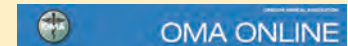
September 11-12, 2009, St. Vincent Hospital, Portland, OR

Presentations:

September 11, 2009, 7:30 PM: Allen M. Gown, MD presents, "Seeing the World on a Bicycle"

September 12, 2009, 9:00 AM to Noon: Allen M. Gown, MD presents, "Applications of Immunohistochemistry to Problems in Surgical Pathology"

www.theoma.org



CAP '09: The Pathologists' Meeting

October 11-14, 2009, Washington, DC

www.cap.org



College of American Pathologists presents: The Sounds of Success: Virtual Management College

October 13, 2009, Audioconference

Presentation:

October 13, 2009, Noon: Allen M. Gown, MD presents, "Antibody & Test Validation in IHC"

www.cap.org



24th Annual Clinical Cytometry Society Meeting & Course

October 16-21, 2009, Hyatt Regency Jacksonville Riverfront, Jacksonville, FL

Presentation:

October 17, 2009, 11:45 - 1:15 PM: Steven J. Kussick, MD, PhD presents, "Myelodysplastic Syndromes"

www.cytometry.org



California Society of Pathologists 62nd Annual Convention: Seminars in Pathology

December 2-5, 2009, Hyatt Regency San Francisco, Embarcadero Ctr., San Francisco, CA

www.calpath.org



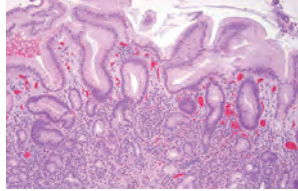
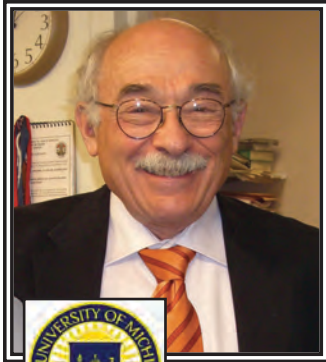
San Antonio Breast Cancer Symposium

December 9-13, 2009, Henry B. Gonzalez Convention Center, San Antonio, TX

sabcs.org



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Featuring *Dr. Henry Appelman* At Our Fall Conference

Henry Appelman, M.D., of the University of Michigan, Ann Arbor, MI, will present “Gastritides, Diseases That Need To Be Destroyed Before They Destroy Us” at the PhenoPath Fall Conference on *Thursday, September 10, 2009*. The format of the conference is a social hour commencing at 6:30 p.m., followed by Dr. Appelman’s lecture at 7:30 p.m. A light catered dinner will be served during the social hour.

Henry D. Appelman, M.D., the M. R. Abell Professor of Surgical Pathology at the University of Michigan, did his undergraduate and medical school training and his pathology residency at the University of Michigan, where he was tutored by amazing pathologists who served as role models, including A. James French, his original chairman and a power in national pathology circles, and Murray R. (Gus) Abell, one of

the most accomplished tissue diagnosticians of his generation. Dr. Appelman was introduced to his subspecialty of gastrointestinal pathology while assigned to the Armed Forces Institute of Pathology from 1966-68, under the tutelage of Dr. Elson B. Helwig, one of the giants of surgical pathology. Gastrointestinal pathology was mostly limited to surgical resections when he started in the business, but he and fiberoptic endoscopy and endoscopic biopsies grew up and evolved together, so he learned modern GI pathology on the go, as did all of his contemporaries. He was forced to deal with liver pathology, and had to teach himself how to do it.

Dr. Appelman has authored or co-authored over 120 papers (which he modestly states were mostly written by much more accomplished scientists who graciously allowed his name to be included somewhere in the middle of the author list) and numerous chapters, and he has edited or co-authored four books, including the Fascicle on Tumors of the Esophagus and Stomach for the Armed Forces Institute of Pathology with the late and great Dr. Klaus Lewin. His publications include analyses of gastric mesenchymal tumors, including glomus tumors and stromal tumors, the colitic dysplasia-carcinoma sequence, acute infectious colitis, anorectal prolapse lesions, Barrett’s and cardiac carcinomas, the morphology of end-stage achalasia, appendiceal chronic inflammations and neoplasms, the chronic diarrheal colitides, superficial Crohn’s disease, changes in distribution of inflammation in ulcerative colitis, and lymphocytic and sloughing esophagitis. His publications also include diseases of the liver, especially post-transplant disorders.

Dr. Appelman is a dedicated educator, known for his teaching excellence, enthusiasm and sense of humor. He received a Distinguished Service Award from the Commission on Continuing Education of the American Society of Clinical Pathologists (ASCP) in 1999, and the 2006 H. P. Smith Award for Distinguished Pathology Educator from ASCP. He served as president of the United States and Canadian Academy of Pathology and the World Organization for Specialized Studies on Diseases of the Esophagus (OESO) headquartered in Paris.

We’re looking forward to Dr. Appelman’s visit and we are sure you will enjoy his unique and entertaining style of teaching.