Phenomena™

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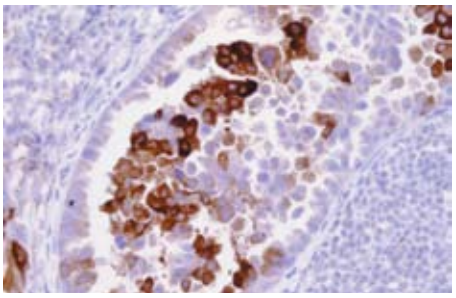
BIGGER is Not Necessarily Better

Carcinomas of Unknown Primary – Selecting The Optimal Antibody Panel

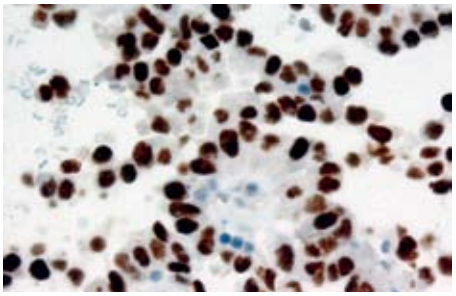
Determining the primary site of carcinomas presenting as metastases using immunohistochemistry can provide important information to a patient's oncologist, by pointing to one, or a limited number, of probable primary sites, and eliminating others. It is important, however, in the interest of providing the most relevant information, for the IHC testing in this clinicopathologic setting to be focused and intelligent. This means selecting a panel of antibodies that is most useful in a given clinical setting, and not running a 'generic' panel of antibodies on all carcinomas of unknown primary. The latter is wasteful of resources, and can result in the performance of unnecessary antibody tests. For example, in trying to determine whether a tumor in the lung of an elderly female with a history of breast cancer represents a metastasis from that prior tumor or a new primary lung carcinoma, cytokeratin 7 and 20 studies would not be particularly useful, as both of those tumors would be expected to show a cytokeratin 7+/cytokeratin- immunophenotype. Similarly, antibodies to CEA would not be particularly helpful in this clinicopathologic setting. The most useful antibodies would be markers of primary breast cancer (GCDFP-15 and mammaglobin) as well as lung cancer (TTF-1, surfactant ApoA). Were the patient's prior breast tumor ER positive, this might be another marker of breast cancer in this case, although one must be aware that up to 10% of primary lung carcinomas can be demonstrated to be ER-positive. Dr. Larry Weiss and colleagues recently found that up to 18% of primary lung adenocarcinomas can express ER [1], although in our more recent studies on lung adenocarcinomas we find a frequency of 9.6%.

This example also demonstrates the importance of employing antibodies to maximize sensitivity. If the clinical question is to determine if a metastatic carcinoma in the lung represents a new lung primary or a breast cancer metastasis, in the setting of a history of an ER-negative breast cancer, it is important to employ, in the panel of antibodies, markers that have adequate sensitivity to address the clinical problem at hand. In this setting, employing only antibodies to the gross cystic disease fluid protein-15 (GCDFP-15) as the marker of metastatic breast cancer may be inadequate, given that the sensitivity of GCDFP-15 is in the range of 50-55% [2]. At PhenoPath Laboratories we also run antibodies to mammaglobin, a breast-restricted protein which has a sensitivity for breast cancer of slightly less than 50% (Sasaki et al., Mod Pathol 20:2008-15, 2007); our studies have shown that the combined sensitivity of the two markers is 70%, demonstrating the importance of using both when entertaining breast as a possible primary site [2].

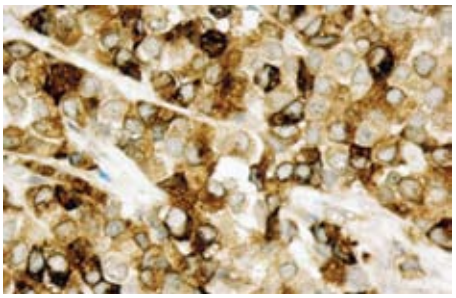
In cases where the differential diagnosis is more open ended, such as the identification of the primary site of a carcinoma presenting in the liver in a patient with no history of carcinoma, a larger panel of antibodies will be required, but again the choice of antibodies must be dictated by the tumor histology as well as the clinicopathologic findings. It is possible to employ a panel of antibodies that is both parsimonious as well as powerful, and examples of this will be presented in future issues of ***Phenomena***.



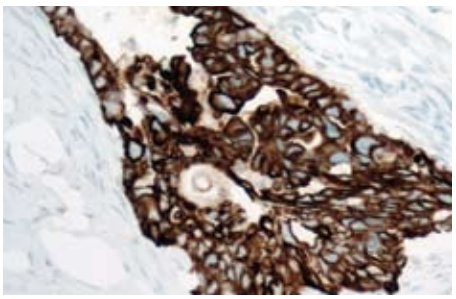
Surfactant ApoA



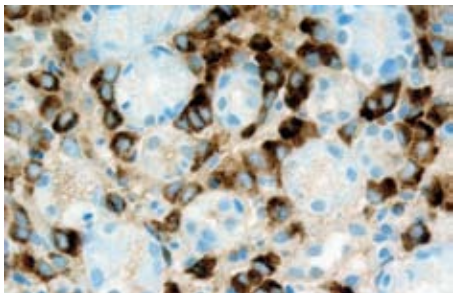
TTF-1



Mammagloblin



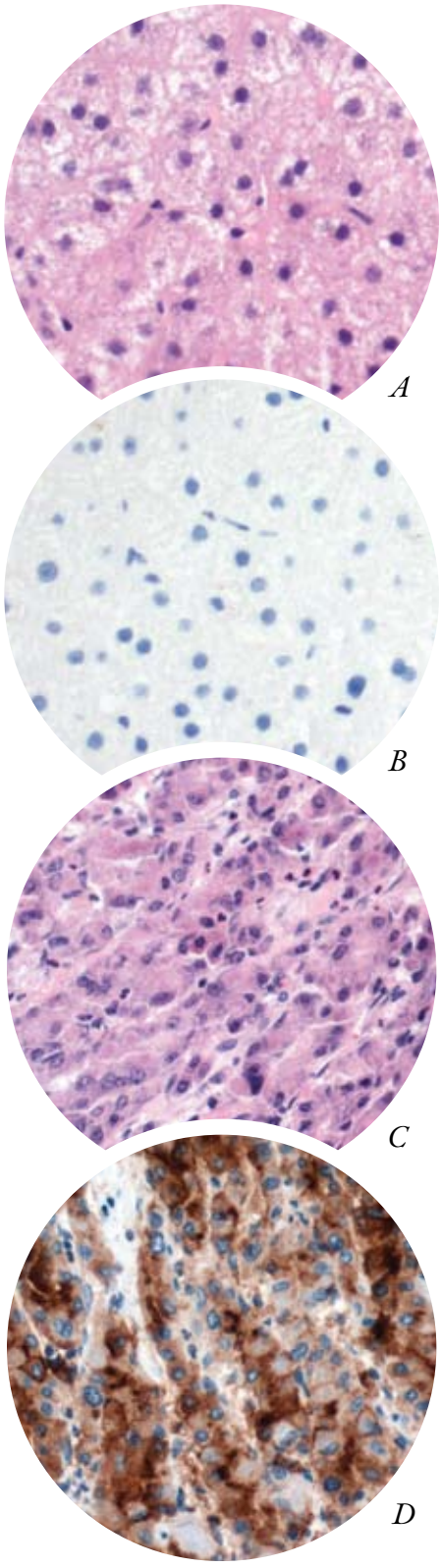
Cytokeratin 7



GCDFP-15

1. Lau, S.K., P.G. Chu, and L.M. Weiss, *Immunohistochemical expression of estrogen receptor in pulmonary adenocarcinoma.* Appl Immunohistochem Mol Morphol, 2006. 14(1): p. 83-7.
2. Tse, C.H., et al., *Improved detection of breast carcinoma using mammaglobin and gross cystic disease fluid protein-15 by immunohistochemistry*. Mod Pathol, 2006. 19(S1): p. 44A.

GLYPICAN-3



A

B

C

D

Glypicans are one of two principal members of the family of proteins known as membrane-bound heparan sulfate proteoglycans (HSPGs). Glypicans have a common structure of a "core protein" anchored to the cell membrane through a glycosylphosphatidyl inositol anchor, and through their ability to regulate the activity of growth and survival factors, glypicans have been shown to play a critical role in developmental morphogenesis as well as in tumor progression. Expression of one glypican, GPC3, has been shown to be a serum and tissue oncofetal marker of hepatocellular carcinoma (HCC) [1]. Initial studies by Yamauchi and colleagues [2] documenting the ability of GPC3 expression, as assessed by immunohistochemistry, to identify hepatocellular carcinoma, were confirmed and expanded by Wang et al and Libbrect et al [2, 3] in which GPC3 expression was demonstrated to distinguish HCCs (positive) from cirrhotic nodules, dysplastic nodules, and focal nodular hyperplasia-like nodules (negative). Most recently, Coston and colleagues [4], demonstrated that GPC3 manifested a sensitivity and specificity of 85% and 97% for HCC, and demonstrated how GPC3 expression, along with the "complete" pattern of vascularization noted with antibodies to CD34, can reliably distinguish HCC from benign liver nodules. Studies at PhenoPath Laboratories corroborate these published results.

The images to the left demonstrate GPC3 immunohistochemistry in a recent case studied here. Note complete absence of GPC3 expression in the cirrhotic nodule (*A,B*), but strong uniform expression of GPC3 in the HCC that developed in the context of this cirrhosis (*C,D*).

1. Capurro, M., et al., *Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma.* Gastroenterology, 2003. 125(1): p. 89-97.
2. Yamauchi, N., et al., *The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma.* Mod Pathol, 2005. 18(12): p. 1591-8.
3. Libbrecht, L., et al., *Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules.* Am J Surg Pathol, 2006. 30(11): p. 1405-11.
4. Coston, W.M., et al., *Distinction of hepatocellular carcinoma from benign hepatic mimickers using Glypican-3 and CD34 immunohistochemistry.* Am J Surg Pathol, 2008. 32(3): p. 433-44.

PhenoPeople PROFILE

Gregg Whiteker

PhenoPath Laboratories is pleased to feature the newest addition to the PhenoFamily, Services Sales Representative Gregg Whiteker.

Gregg joined PhenoPath Laboratories on March 1st of this year. Gregg will call on clients and prospective clients in Tennessee, Kentucky, Georgia, Alabama and Mississippi. He hopes to introduce new clients to the PhenoPath culture of quality and service and show how PhenoPath can help expand and grow their practices.

Most recently, Gregg served as Regional Sales Specialist at LithoLink, a kidney stone disease management firm located in Chicago. Gregg was also employed as a Sales Representative for American Esoteric Laboratories, and as a representative of Genzyme Genetics for a number of years; he has extensive background in anatomic pathology and esoteric testing.

Gregg graduated from the University of Kentucky with a Bachelor's of Business Administration. He proudly notes that his alma mater has "the winningest men's basketball program in the history of the game," the Kentucky Wildcats!

In his spare time, Gregg enjoys sketching and computer graphic arts. He also has a strong interest in music and has been known to perform at karaoke events. Gregg is excited to announce his recent marriage to Kim, his high school sweetheart. Kim is a massage therapist; she has two children, Emily and Zack, whom Gregg looks forward to joining as a family.

PhenoPath Laboratories is delighted to welcome Gregg as the newest member of our PhenoFamily.

VISIT US AT THE FOLLOWING MEETINGS:

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

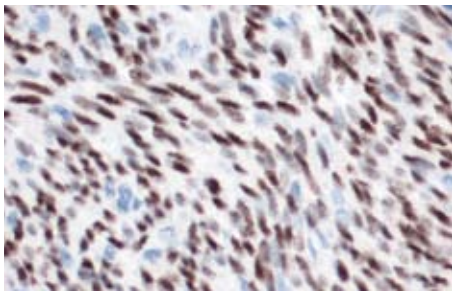
Breast Pathology:
Current Concepts and Controversies
June 2 – 3, 2008, The Fairmont Copley Plaza Hotel, Boston, MA
Allen M. Gown, MD is a featured speaker and will present two talks entitled "Current Status of ER/PR Testing" and "Current Status of HER2 Testing" on June 2, 2008.
cme.med.harvard.edu

PSA Pathology Business Conference:
Good News, There is a Future in Pathology
June 4 – 5, 2008, Charleston Marriott, Charleston, SC
Allen M. Gown, MD is a featured speaker and will present a talk entitled "Impact of ASCO/CAP Guidelines on HER2 Testing in Breast Cancer" on June 4, 2008.
PhenoPath will be represented at an exhibit booth.
www.psapath.com

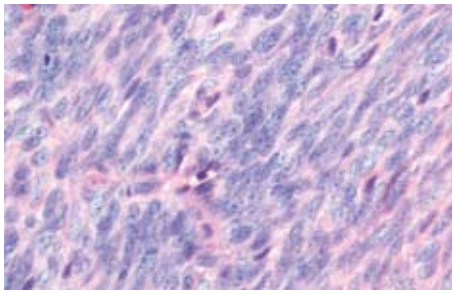
USCAP Diagnostic Pathology Update:
July 12 – 18, 2008, Hyatt Regency Maui, Lahaina, HI
Allen M. Gown, MD is a featured speaker and will present a talk entitled "Special Topic: Immunohistochemistry" on July 16, 2008.
www.uscap.org

TLE1 A Novel Immunohistochemical Marker Of Synovial Sarcoma

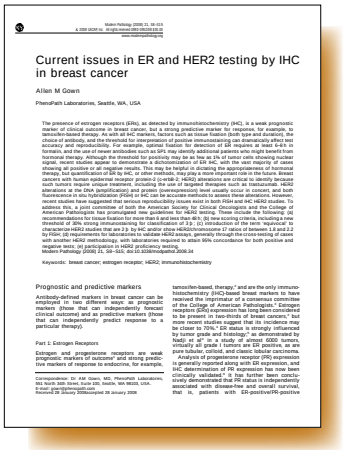
Synovial sarcoma is a soft tissue tumor that is defined by the presence of a t(X;18). While this translocation can be identified by fluorescence in situ hybridization (FISH) studies on deparaffinized formalin-fixed tissue, a novel immunohistochemical marker, a protein related to the Wnt pathway called TLE1, has been recently identified as a very useful diagnostic marker to assist in the identification of synovial sarcoma (Terry J et al., Am J Surg Pathol 31:240-6, 2007). In the latter study, high levels of expression of TLE1 were reported in 97% of molecularly confirmed cases of synovial sarcoma; this high level of sensitivity was confirmed in validation studies performed at PhenoPath Laboratories (see images on right). As also corroborated by studies here, lower levels of positive immunostaining were seen in other tumors (e.g., nerve sheath tumors, solitary fibrous tumors), but of the 35 different bone and soft tissue types studied, only synovial sarcomas displayed high levels of immunostaining. Thus, while TLE1 immunostaining will not replace FISH studies for identifying the t(X;18), the high negative predictive value can assist in determining which cases should be forwarded for subsequent FISH analysis.



H&E



TLE1



Current issues in ER and HER2 testing by IHC in breast cancer

Allan M Gown

PhenoPath Laboratories, Seattle, WA, USA

A summary of the current issues in ***ER*** and ***HER2*** testing by immunohistochemistry in breast cancer written by PhenoPath's Dr. Gown (which was the subject of Dr. Gown's presentation at the 2007 Long Course at the annual meeting of the United States and Canadian Academy of Pathology) appeared in a special supplement to Modern Pathology just published in May 2008 (*Mod Pathol 2008;21:S8-S15*).

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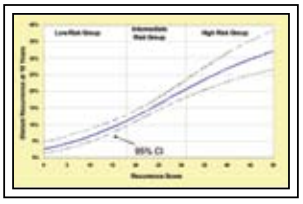
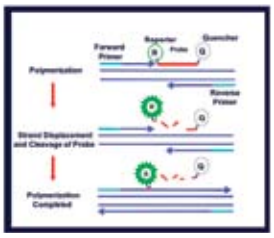
Dr. Steven Shak Was Featured At Our Spring Quarterly Conference





Steven Shak, M.D., of Genomic Health, Inc., in Redwood City, CA, presented "Making Personalized Medicine a Reality: Lessons from Trastuzumab and Oncotype DX®" at the Quarterly Pathology/Immunohistochemistry Conference on Thursday, ***May 8, 2008***.

Dr. Shak is the Chief Medical Officer of Genomic Health, Inc. He has a B.A. in Chemistry from Amherst College, an M.D. degree from New York University School of Medicine, and completed his postgraduate training in medicine and research at Bellevue Hospital in New York City and the University of California, San Francisco. Dr. Shak has previously held faculty positions at the New York University School of Medicine and Bellevue Hospital.

Dr. Shak's research interests are focused on translating innovations in biotechnology and molecular biology into clinical practice. He has worked in the fields of inflammation, pulmonary disease, and oncology. He has closely collaborated with pathologists throughout his career.

Dr. Shak has worked with oncology clinical research groups in the U.S. to use new molecular diagnostic methods and rigorous clinical studies to develop the Oncotype DX® breast cancer assay. As Senior Director and Staff Clinical Scientist at Genentech, Inc., he led the clinical team that gained approval for trastuzumab (Herceptin®), a targeted biologic treatment for metastatic breast cancer. He also initiated the cancer clinical trials of the anti-angiogenesis agent, bevacizumab (Avastin®).

Dr. Shak has received numerous awards and honors for his contributions to medicine and patient care. He is currently on the Board of Directors of The Children's Cause for Cancer Advocacy (a pediatric cancer advocacy organization), and The Cystic Fibrosis Foundation.

✂ *Clip and Save*

Optimal Handling of Specimens

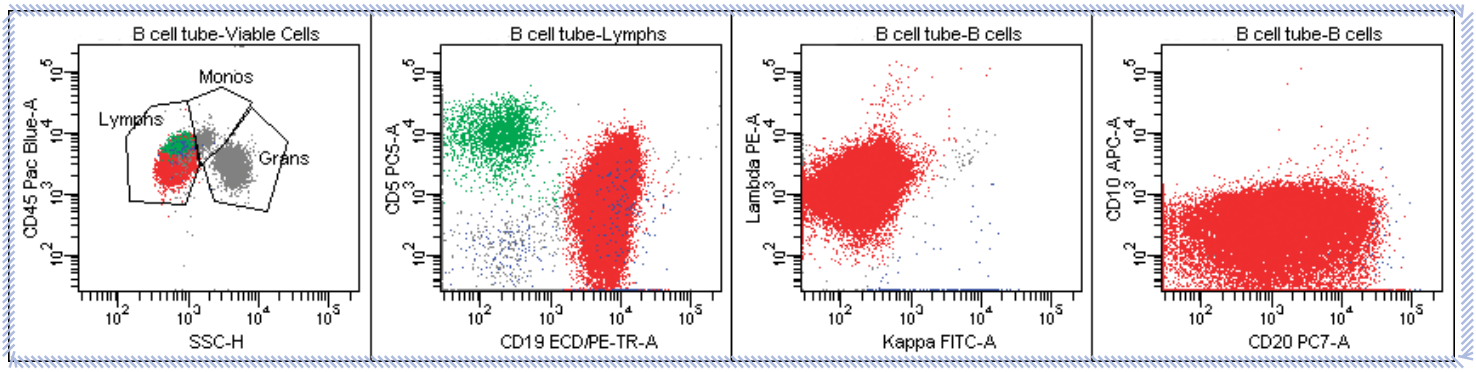
Evaluated for Lymphoma and/or Plasma Cell Dyscrasia

Summary:

The rapid advancement in our understanding of lymphomas and plasma cell dyscrasias (PCDs) has generated an ever-widening set of diagnostic and prognostic tests applicable to these disorders. Therefore, careful planning by the pathologist is essential at the initiation of the work-up for lymphoma or PCD, to ensure optimal patient care.

The following principles should be applied to all lymphoma and PCD workups:

1. When fresh tissue, blood, bone marrow, or body fluid is available, a portion should be sent for flow cytometric evaluation, to look for a new malignancy or recurrence of a previous malignancy after therapy, because of the tremendous sensitivity of flow cytometry for identifying malignant hematopoietic populations.
2. Well-prepared, Romanowsky-stained, touch imprints should be made from all fresh biopsies being evaluated for lymphoma or PCD, including bone/bone marrow core biopsies, to facilitate optimal cytologic evaluation.
3. For the portions of specimens alloted for fixation and paraffin embedding, overnight formalin fixation of 2-3 mm thick tissue portions is STRONGLY RECOMMENDED to optimize both the histologic evaluation and any ancillary immunohistochemical or molecular studies performed on the block. ADEQUATE FIXATION IS CRITICAL.
4. Immunophenotypic information must ALWAYS be correlated with the morphologic findings, and in many cases with the relevant molecular findings, to arrive at a definitive diagnosis using the WHO classification system.
5. For new diagnoses of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and plasma cell myeloma, concurrent cytogenetic and/or FISH evaluation should be performed to look for prognostically relevant chromosomal abnormalities.



Flow cytometric findings in CLL/SLL

Introduction:

Multiple testing modalities are employed in the diagnosis of lymphoma and plasma cell dyscrasia (PCD) in the modern hematopathology laboratory. The 2000 WHO classification of tumors of the hematopoietic and lymphoid tissues [1] bases the diagnoses of lymphoma and PCD on a combination of morphologic, immunophenotypic, and, in some cases, molecular findings. For a significant minority of these tumors, molecular findings from polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH) studies are also important for diagnosis and/or establishing prognosis. In a subset of cases, particularly lymphomas, the differential diagnosis may include infectious etiologies, necessitating microbiological evaluation. Therefore, careful handling of specimens from patients in whom these diagnoses are being considered is IMPERATIVE to maximize the yield of diagnostic information. This article reviews the current diagnostic modalities used in the evaluation for these malignancies, and describes the optimal utilization of these modalities for patient care.

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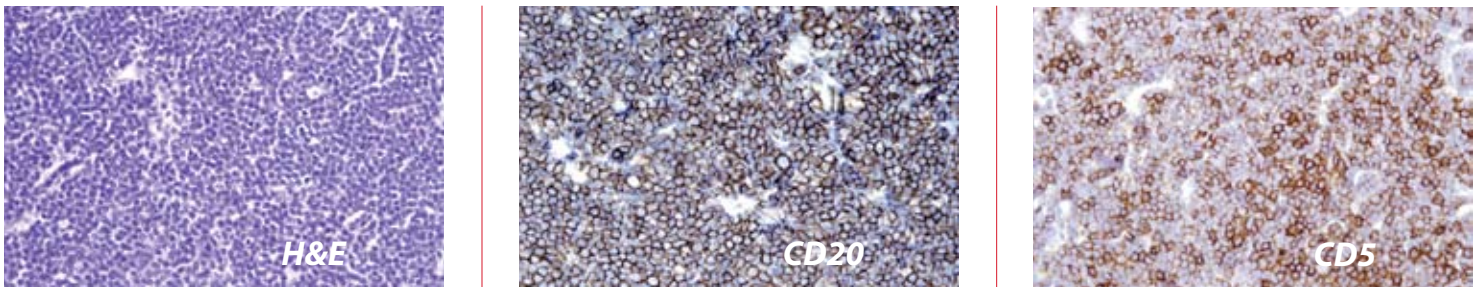
Optimizing Morphologic Evaluation:

Careful morphologic evaluation of lesional tissue remains a mainstay of the diagnosis of lymphoma and PCD. Because the individual cells in benign and malignant proliferations of lymphocytes and/or plasma cells may look very similar, histologic evaluation of the overall tissue architecture, in combination with cytologic evaluation, is often required to distinguish benign from neoplastic processes.

Histologic evaluation: Optimal histologic evaluation of tissue architecture REQUIRES adequate tissue fixation; unfortunately, a significant minority of the outside blocks evaluated in our hematopathology reference practice show suboptimal fixation. For optimal fixation, lymph nodes or other soft tissue biopsies should be serially sectioned at 2 to 3 mm intervals, typically perpendicular to the long axis of the lymph node, and fixed OVERNIGHT prior to processing. If there is an urgent need for histologic evaluation within 24 hours of the biopsy, a 2 mm pilot section may be submitted for processing the same day that the biopsy is obtained. Although careful fixation of the entire node may lead to a one-day delay in overall turnaround time in some cases, the improved quality of the histology more than makes up for such delay. In terms of fixatives, we STRONGLY favor neutral buffered formalin (NBF), since adequate NBF fixation produces excellent histology, and preserves antigens and nucleic acids for immunohistochemical and molecular studies, respectively. Some hematopathologists prefer B5 fixation because of the greater detail B5 imparts to nuclear chromatin, but we believe that the issues of B5 waste disposal, the deleterious effects on molecular testing, and the compromised immunohistochemical staining of some antigens greatly outweigh the marginal improvement in the visualization of the chromatin. Similarly, we do not believe that other commercial fixatives (Prefer™, B-Plus™, etc.) have any advantages over formalin fixation. For biopsies containing bone, a RAPID DECALCIFICATION method should be used to minimize damage to tissue morphology and antigenicity. With the increased application of FISH to bone-based biopsies, decalcification techniques that preserve DNA quality are clearly optimal. A relatively recent publication describes an ultrasonic technique in an EDTA-based buffer that offers improved nucleic acid preservation compared to acid-based decalcification [2].



Immunohistochemical findings in CLL/SLL

Cytologic evaluation: Touch imprints from excisional or core biopsies, including core biopsies of bone or bone marrow, generally provide cytologic detail superior to paraffin sections, and should be made routinely when fresh tissue is available. When touch imprints are prepared, care should be taken to avoid both excessively thick deposition of cells and excessive peripheral blood contamination. In our laboratory, touch imprints are visualized with a modified Wright stain, but any high-quality Romanowsky stain will suffice. We do NOT use Papanicolaou stains to visualize hematolymphoid cells.

The section on technical factors in the preparation and evaluation of lymph node biopsies in Neoplastic Hematopathology, 2nd ed [3] provides a useful reference on the handling of lymphoid tissue for optimum morphologic evaluation.

Optimizing Immunophenotypic Evaluation:

The two major methods for immunophenotyping lymphocytes and plasma cells are flow cytometric (FC) evaluation of fresh tissue, and immunohistochemical (IHC) evaluation of paraffin-embedded tissue. For a detailed review of immunophenotyping in hematopathologic diagnosis, see the section on immunophenotypic markers in Neoplastic Hematopathology, 2nd ed [4]. Because the great majority of useful antibodies in hematopathology have been optimized for formalin-fixed paraffin-embedded tissue, there is currently almost no need for frozen section immunoperoxidase studies. FC and IHC should be considered complementary studies, in that each offers diagnostic advantages and disadvantages.

The major advantages of FC, compared to IHC, include:

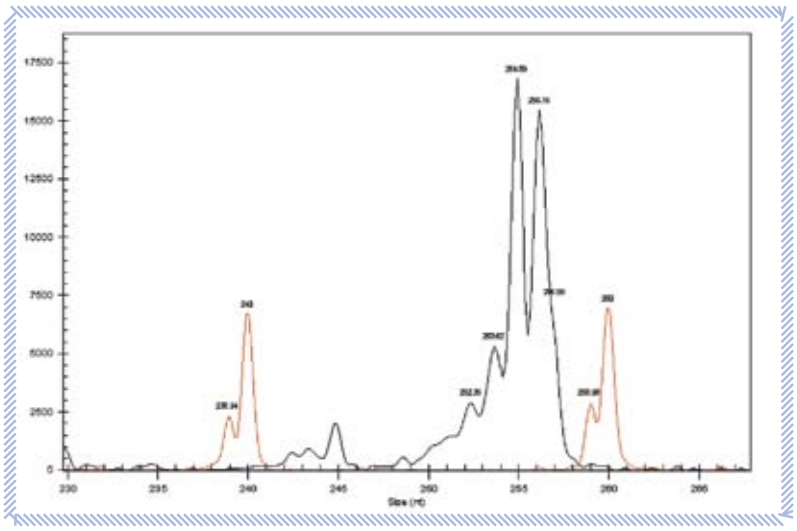
1. **Multiparametric nature.** This allows the simultaneous evaluation of cell size and cytoplasmic complexity, along with 3 to 10 antigens depending on the methodology, as opposed to the single antigen staining characteristic of IHC. This increase in phenotypic information per cell makes FC particularly useful for evaluating small specimens, such as fine needle aspirations or needle core biopsies.
2. **Superior sensitivity for detecting low-level/partial involvement by a neoplastic cell population, compared to histology and IHC.** This superior sensitivity derives from both the multiparametric nature of FC and the fact that a very large number of cells (e.g., 500,000 to 1,000,000) can be readily evaluated in a single flow cytometric assay.
3. **Rapid turnaround time.** A complete FC assay, from tissue processing to report generation, can typically be performed in several hours. This rapidity facilitates clinical decision-making in emergent cases (e.g., superior vena cava syndrome or spinal cord involvement), and also allows for the rapid reflexing to additional diagnostic studies (e.g., FISH evaluation for a c-MYC gene rearrangement in suspected cases of Burkitt lymphoma).

Because of the tremendous diagnostic power of FC, we strongly believe that a portion of ALL SAMPLES obtained to rule out lymphoma and/or PCD should be sent for FC. Moreover, in a patient with a well-characterized lymphoma, FC evaluation of a needle core biopsy or FNA may be adequate to document recurrent disease, without the need for a more invasive procedure. Two useful references on FC testing in neoplastic hematopathology include Gorczyca W [5] and Nguyen DT, et al. [6]

The major advantages of IHC, compared to FC, include:

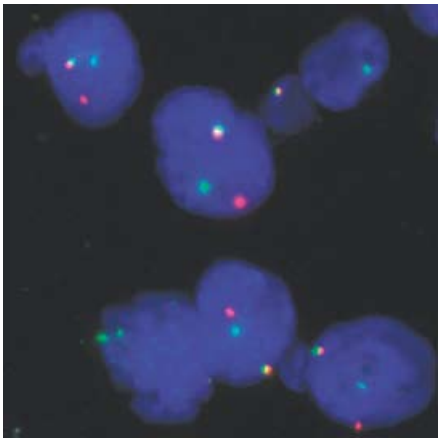
1. **The preservation of tissue architecture/histologic context in IHC slides.**

2. **Several critical antigens in lymphoma diagnosis are not evaluable by routine flow cytometry, and must be evaluated by IHC.** Such antigens include cyclin D1 in the diagnosis of mantle cell lymphoma, and bcl-6 and MUM-1 in the subclassification of diffuse large B cell lymphoma. Therefore, while FC may be more sensitive than IHC in confirming the malignant nature of the lymphoid infiltrate by documenting clonality and/or aberrant antigen expression, the combination of IHC with routine histologic evaluation remains the basis for the final WHO classification of most cases of lymphoma and PCD. For these reasons, FC and IHC should be seen as complementary.



Positive clonal IgH gene rearrangement study in B cell lymphoma

Although we will not attempt to provide guidelines for constructing FC and IHC panels to evaluate for lymphoma and PCD, suffice it to say that such panels must include an adequate number of antibodies to identify all the major cell populations in the infiltrate. Two useful references on IHC testing in neoplastic hematopathology include Dabbs D [7] and Sun T [8].



Demonstration of a c-MYC gene rearrangement in Burkitt lymphoma

Optimizing Polymerase Chain Reaction (PCR) Evaluation:

PCR evaluation has replaced Southern blotting as the major molecular method for assessing B or T cell clonality in atypical lymphoid infiltrates. PCR evaluation may be applied successfully to either unfixed (e.g., fresh or frozen) or fixed tissue (generally formalin-fixed tissue). However, if both unfixed and fixed tissues are available for a particular specimen, it is GREATLY PREFERABLE that the PCR be performed on the unfixed tissue, because of the higher quality of the DNA. At PhenoPath, we use a unique DNA extraction methodology that allows us to scrape unstained paraffin-embedded tissue sections for PCR evaluation. This approach allows us to target the area of interest on a particular slide, and reduces the effect of PCR inhibitors.

Optimizing Cytogenetic Evaluation:

A portion of all bone marrow aspirates from patients with known or suspected CLL/SLL or plasma cell myeloma should be sent, in preservative-free heparin, for standard cytogenetic evaluation (karyotyping) to look for prognostically relevant chromosomal abnormalities. While cytogenetic evaluation of lymph node biopsies can often provide useful information, we do NOT currently consider such evaluation to represent a standard-of-care practice in lymph node pathology, as FISH evaluation almost always suffices to look for diagnostic or prognostic chromosomal abnormalities.

Optimizing Fluorescence in Situ Hybridization (FISH) Evaluation:

FISH evaluation, in which fluorescently labeled nucleic acid probes are applied to the nuclei of either whole or sectioned cells, has both diagnostic and prognostic application to lymphoma and PCD. A salient example is the identification of the t(11;18) in B cell-predominant gastric lymphoid infiltrates, which both confirms the diagnosis of extranodal marginal zone lymphoma, and suggests that the lymphoma is unlikely to respond to anti-Helicobacter pylori therapy. FISH evaluation is also an important aid in establishing prognosis in chronic lymphocytic leukemia/small lymphocytic lymphoma and PCD, particularly in cases in which standard cytogenetics has not been performed, or did not yield adequate cell growth for evaluation.

In our experience, careful tissue fixation in NBF results in high diagnostic yield for FISH studies, as the DNA tends to be adequately preserved for this purpose. In contrast, fixation in alternative solutions such as B5 or Prefer™ (the latter is a glyoxyl-based fixative) frequently damages the DNA to such an extent that FISH evaluation may not be possible; prior decalcification also frequently compromises FISH evaluation.

The evaluation/scoring of FISH studies of hematolymphoid-associated chromosomal abnormalities, whether done manually or by automated morphometric image analysis, is a complex procedure requiring extensive assay validation. Therefore, such studies should only be done in specialized laboratories with extensive experience.

Optimizing Microbiological Studies: If infectious etiologies are in the clinical differential diagnosis, then a portion of the biopsy should be allotted, in a sterile manner (generally intra-operatively), for appropriate culture media.

References:

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