PhenoPath is pleased to announce the availability of two new FISH studies. These tests are available on formalin-fixed, paraffin-embedded (FFPE) tissues.

**t(9;22) BCR/ABL FISH**

Chronic myeloid leukemia (CML) is a myeloproliferative disorder representing 15-20% of adult leukemias. CML has been traditionally diagnosed by detection of the Philadelphia chromosome (Ph) which is the hallmark of this disease and is the result of a translocation between the BCR gene on chromosome 22 and the ABL gene on chromosome 9. FISH studies offer a highly specific and sensitive method for detecting this translocation in blood, bone marrow and FFPE tissues. The t(9;22) is also found in ~20% of adult precursor B cell acute lymphoblastic leukemias, and is associated with an unfavorable prognosis. In addition, the few patients with acute myeloid leukemia (AML) harboring a t(9;22) translocation involving BCR/ABL are known to have an unfavorable prognosis, and in a recent ECOG study, interphase FISH has been shown to be highly accurate in helping to stratify AML patients into cytogenetically defined risk categories (Vance GH et al., *Leuk Res* 31:605-9, 2007; Slovak ML et al., *Blood* 96:4075–83, 2000). In normal cell nuclei, this FISH assay yields two orange and two green signals, but in nuclei of cells harboring a reciprocal BCR/ABL translocation, FISH results in a single orange and single green signal and two orange/green fusion signals. In FFPE tissue, nuclei are often transected and thus, only a single fusion signal may be seen (see image).

**RARA Breakapart FISH**

Acute promyelocytic leukemia (APL) is a distinct form of acute myeloid leukemia which accounts for 5 to 10% of cases and is characterized by a proliferation of myeloid precursors blocked at the promyelocyte stage of differentiation. The abnormal promyelocytes may be rare or numerous in the peripheral blood. Patients with APL are typically relatively young among AML patients and have a better prognosis, as long as the frequent complication of disseminated intravascular coagulation (DIC) is adequately managed. Therefore, proper subclassification of this leukemia is critical for optimal patient management (i.e., to alert the treating oncologist that there is a significant risk of DIC). Typically, these patients are treated with all-trans-retinoic acid (ATRA), a relatively well-tolerated therapeutic agent that promotes myeloid differentiation. Myeloid differentiation of these abnormal promyelocytes can be followed by standard chemotherapeutic agents with a reduced risk of DIC.

(continued on page 2)
FISH tests can be used to establish a definitive diagnosis of APL. There is a strong association of the t(15;17)(q22;q12) and its associated PML/RARA fusion gene with this subtype of AML. FISH studies are capable of detecting a t(15;17)(q22;q12) in almost all cases. The rapid nature of this FISH-based testing, along with its high specificity and sensitivity, are significant advantages in the clinical setting. Moreover, the use of RARA breakapart FISH probes permits the identification of variant RARA gene translocations that would be missed using standard t(11;18) dual color, dual fusion FISH probes. The three main variant translocations involving the RARA gene include: t(11;17)(q23;q21) involving the promyelocytic leukemia zinc finger gene (PLZF) on 11q23; t(5;17)(q23;q21) involving the nucleophosmin (NPM) on 5q23; and t(11;17)(q23;q21) involving the nuclear matrix-associated gene (NUMA) on 1q13. Acute promyelocytic leukemias involving the variant t(11;17)(q23;q21) have been reported to be resistant to ATRA whereas those with variant t(5;17)(q23;q21) appear to respond to ATRA. In normal cell nuclei, this breakapart probe set yields two paired orange/green signals, but in the nuclei of cells harboring a translocation involving the RARA gene, one paired (normal allele) and two unpaired signals are observed (see image on page one).
In January 1999, with the introduction of routine HER2 testing of breast carcinomas, PhenoPath Laboratories commenced a quality assurance program to maximize the accuracy of testing by immunohistochemistry (IHC) and by fluorescence in situ hybridization (FISH). For all cases submitted to our laboratory for HER2 testing by FISH, an accompanying IHC test is performed in parallel. In addition, for all cases referred for IHC testing which demonstrate a 2+ (equivocal) score, a HER2 test by FISH is performed.

Quarterly reviews of the concordance data between IHC and FISH are performed by a pathologist. All cases in which there is a discrepancy between IHC and FISH, that is IHC (0, 1+) and FISH amplified or IHC 3+ and FISH non-amplified, are reviewed. The IHC slides and the digital images are reviewed by all the pathologists at our daily Pathology Case Conference. In addition, an audit of a subset of cases submitted for HER2 testing is also performed. The primary data and clinical records from approximately 10% of cases tested by both IHC and FISH and 5% of cases tested only by IHC are reviewed by a pathologist.

The initial results of this concordance data (Yaziji H et al, *JAMA* 291;1972- 1977, 2004) have recently been updated. An analysis of 6604 cases, from 2003 to 2006, showed a high rate of concordance between IHC and FISH. 1904/1919 (99.2%) of those cases 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.

Recently published guidelines from ASCO and CAP mandate that laboratories performing HER2 tests show high concordance rates between IHC and FISH (95%). At PhenoPath, we have achieved this extremely high rate of concordance, at least in part due to the institution of a comprehensive quality assurance program, ongoing for years, which permits continuous visual feedback of IHC results with FISH-determined HER2 gene status.
Dr. Mark R. Wick, of the University of Virginia at Charlottesville, Virginia, will present "Diagnostic Immunohistochemistry in Dermatopathology: An Update" at the Quarterly Pathology/Immunohistochemistry Conference on Thursday, November 29, 2007. The format of the conference is a social hour commencing at 6:30 p.m. followed by Dr. Wick's lecture at 7:30 p.m. A light catered dinner will be served during the social hour.

Dr. Wick is currently a Professor of Pathology in the Divisions of Surgical Pathology and Cytopathology, and Autopsy Pathology, Associate Director of Surgical Pathology, and Director of Pathology Residency Training at the University of Virginia at Charlottesville. He received his M.D. from the University of Wisconsin, and completed his anatomic and clinical pathology residency training at the Mayo Clinic. Dr. Wick is a physician who has continued to practice as a general anatomic pathologist with particular interest in immunohistochemistry, dermatopathology, thoracic pathology and soft tissue pathology.

Dr. Wick's research focuses on protein chemistry and immunohistology of human neoplasms, and clinical outcomes analysis.

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