

WHAT'S NEW?

New Director of Clinical Virology

Benjamin Pinsky, MD PhD, Assistant Professor of Pathology, is the new Director of the Clinical Virology section of the Stanford Clinical Laboratory. Dr. Pinsky received his PhD and MD degrees from the University of Washington, and came to Stanford in 2007 for his training in Clinical Pathology and Molecular Genetic Pathology. Dr. Pinsky's research includes the development of molecular diagnostic approaches for identifying and characterizing pathogens. He devised the tests that were used during last year's H1N1 influenza pandemic. Dr. Pinsky can be reached at bpinsky@stanford.edu or at pager#: 13118.

College of American Pathologists Inspection a Success

The Stanford Clinical Laboratory's recent inspection by the College of American Pathologists was highly successful. A total of 17 laboratory sections had no deficiencies and a minority of laboratory sections had a small number of citations. Dr. Daniel Arber, Medical Director, Dr. Sharon Geaghan, Co-Medical Director of Pediatrics, and Ms. Celli Frost, Administrative Director, Anatomic Pathology & Clinical Laboratories congratulated the laboratory staff for their efforts.

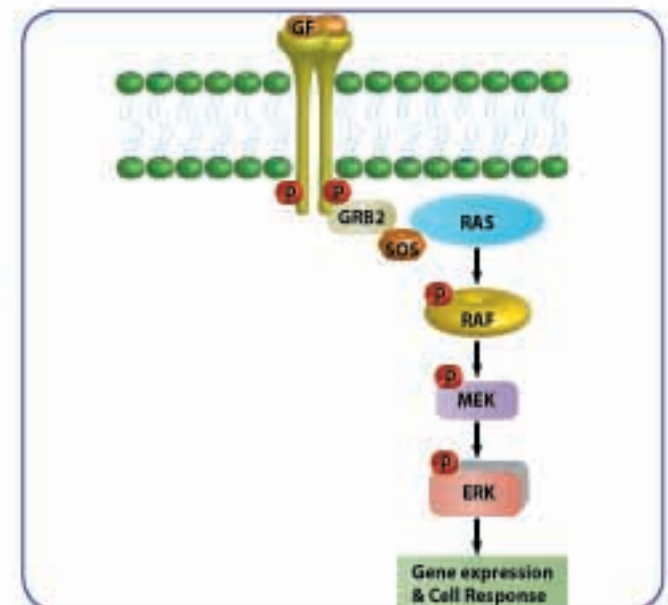
BRAF – ROLE AND IMPLICATIONS IN CANCER BIOLOGY

NILESH DHARAJIYA, MD
FELLOW, MOLECULAR GENETIC PATHOLOGY

BRAF, a member of Serine-threonine kinase family of proteins, plays an important role in the signal transduction and cellular response activated by growth hormone or other extracellular stimuli. BRAF mutations were initially identified with high frequency in patients with malignant melanoma. Later on, BRAF mutations have also been detected in colorectal carcinoma, papillary thyroid carcinoma, ovarian carcinoma and a small proportion of lung adenocarcinoma.

BRAF mutations cluster in the glycine-rich loop and activation segment of the kinase domain. These mutations destabilize the inactive conformation of BRAF, thus promoting activation of the kinase. The most frequent mutation in BRAF is a valine to glutamine change at codon 600 (V600E) resulting in a 500-fold increase in kinase activity. Current literature suggests that BRAF mutations are observed in patients with colorectal carcinoma arising secondary to somatic mutations in the DNA mismatch repair system, but not in patients with hereditary non-polyposis colon cancer or germline mutations in DNA mismatch repair enzymes.

Tyrosine kinase inhibitor therapy has sparked interest in mutational analysis of BRAF along with EGFR and KRAS genes. Patients with metastatic colon cancer carrying BRAF mutations show poor response to anti-EGFR



EGFR binding by growth factors (top) initiates downstream pathways that result in cell growth and proliferation.

tyrosine kinase inhibitor therapy. In contrast, lung adenocarcinoma patients with mutations in the tyrosine kinase domain (deletions in exon 19 and point mutation L858R in exon 21) of EGFR show improved response to tyrosine kinase inhibitors, whereas KRAS codon 12 and 13 mutations impart poor response.

The Stanford Molecular Pathology Laboratory offers testing for mutations in BRAF, KRAS and EGFR. The assays include the V600E mutation in BRAF, codon 12 and 13 mutations of KRAS and exon 19 in-frame deletions as well as the L858R mutation in exon 21 of EGFR. The specimen types include fresh or frozen tumor tissue or formalin-fixed paraffin-embedded tissue blocks.

NEW TEST ANNOUNCEMENTS

Recently implemented new tests include testing for mutation in BRAF and the move of *C. difficile* testing from the Microbiology section to the core laboratory at Stanford Hospital. See articles in this issue for more information.

Additional Respiratory Virus Testing Available

In the wake of last year's success implementing influenza A nucleic acid testing, this year we developed nucleic acid testing for two additional respiratory viruses, [Respiratory Syncytial Virus \(RSV\)](#) and human [Metapneumovirus \(MPV\)](#).

These viruses, members of the family Paramyxoviridae, subfamily Pneumovirinae, cause acute upper respiratory tract infections in patients of all ages, and were the second (RSV) and third (MPV) most frequently identified respiratory viral pathogens in our patient population during the 2009 season. Only influenza A 2009 (H1N1) was found more often. Perhaps most importantly, these viruses commonly cause severe lower respiratory tract disease in infants and children, including bronchiolitis, pneumonia, and acute respiratory failure resulting in significant morbidity and mortality.

These sensitive PCR tests have been validated on both upper and lower respiratory tract specimens and can be ordered individually (test codes: **LABRSVPCR** or **LABMPVPCR**) or as a panel (test code: **LABRMPCR**) that detects both viruses.

LABletter

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REPEATING *C. DIFF* TESTING MAY NOT MAKE A DIFF

NIAZ BANAEI MD – DIRECTOR, MICROBIOLOGY SECTION

Clostridium difficile is an anaerobic gram positive rod that causes diarrhea. Risk factors include hospitalization, ongoing antibiotic treatment, and severe underlying disease. Therefore, *C. difficile*-associated diarrhea is a significant concern at both LPCH and Stanford Hospital.

The most sensitive method for detecting *C. difficile* infection is stool culture; however, this method takes several days to perform. In order to provide a faster turn-around time, most hospital laboratories have relied on immunoassay for the toxin produced by the bacteria. Recently, many have moved to real-time polymerase chain reaction (PCR) assay targeting the toxin B gene, which is much more sensitive than immunoassay of the toxin itself.

Stanford's clinical laboratory moved to PCR last year, using an assay developed in the Microbiology section located at the Hillview site. In order to provide even faster results, we are moving this test to the core laboratory at Stanford Hospital, using a commercially available system. The new assay will produce results in less than 2 hours, helping to quickly determine the need to place patients in contact isolation. As with the prior in-house assay, only symptomatic patients who are producing at least 3 loose stools per day should be tested. Since approximately 50% of newborns are colonized with *C. difficile*, patients should also be at least 1 year old.

Physicians are accustomed to ordering follow-up testing of patients with negative results, because of the significant number of false negative results seen using immunoassay. However, PCR testing is more sensitive; therefore ordering only one PCR test is sufficient. We verified this high degree of performance in a recently published study (see figure). We showed that repeating PCR testing more than once within a week of the initial negative result provided no new clinical information in 99% of patients. Because up to 20% of

hospitalized patients may be colonized with *C. difficile*, repeating negative PCR testing may even result in false-positive results.

The usefulness of repeat PCR testing in patients with positive results is not clear. In our study, two-thirds of the patients with positive results had at least one negative result on repeat testing within two weeks, but there is not sufficient evidence to support relying on such results as evidence of "cure". Instead patients should be followed clinically.

For these reasons, the laboratory will no longer accept samples from patients who have already had PCR testing within the last seven days unless special permission is obtained from the Pathology resident on-call.

Testing begins December 6, 2010 in the Hematology section of the Stanford Core Laboratory (test code: **CDTPCR**). Any questions, please contact Dr. Tracy George, Director Hematology Laboratory (tigeorge@stanford.edu) or Dr. Brent Tan, Associate Director Hematology Laboratory (btan@stanford.edu)

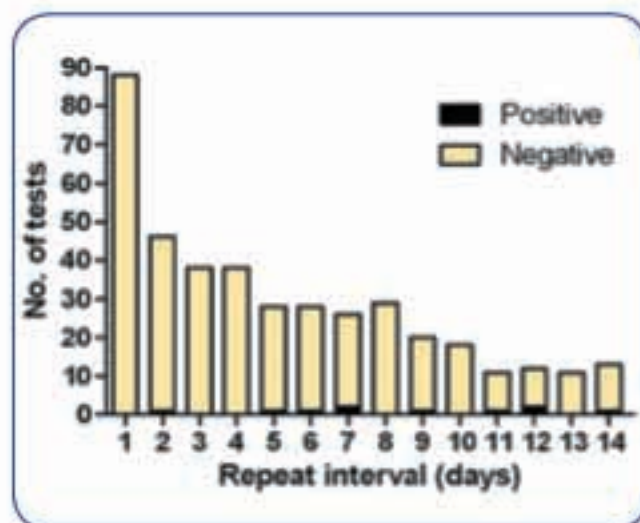


Figure legend

Repeating *C. difficile* PCR testing following an initial negative result was unhelpful in the overwhelming majority of patients, especially within the first week after the initial testing (from *Journal of Clinical Microbiology* 2010; 48:3738-3741).