



LABletter

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PATHOLOGY & LABORATORY MEDICINE NEWSLETTER

NEWS YOU CAN USE

New Tests Implemented

We recently brought in-house serum free light chain for patients with myeloma and KRAS mutation testing for patients with colon cancer. See more details about these new tests in this issue.

Group B Strep Testing by PCR

The Microbiology section will soon switch from culture to real-time PCR for screening for group B streptococcal infection in pregnant women. This assay was developed and validated in our laboratory and will provide enhanced sensitivity and turn around time (one day). However, since we will no longer be performing culture routinely, it is essential that physicians request antibiotic susceptibility testing when indicated. We will remind you to notify us if this is needed when we report positive results. A notification will be automatically generated in LINKS for patients with penicillin allergy to notify the laboratory to perform susceptibility testing.

PSA Reference Range Changed

In 2007, we changed our cut-off for prostate-specific antigen (PSA) from <4 to <2.5 ng/ml. This coincided with our move to the World Health Organization 96/670 assay standardization (which is lower than the traditional calibration) and to comply with the 2004 recommendation of the National Comprehensive Cancer Network. We have had many requests to return our cut-off to

SERUM FREE LIGHT CHAIN for Monoclonal Immunoglobulin Detection and Management

Jim Faix MD - Director, Clinical Chemistry & Immunology

The traditional way to detect serum monoclonal immunoglobulin has been serum protein electrophoresis followed by visual inspection (looking for an abnormal band). In the past few years, a powerful new tool for detection of monoclonal immunoglobulin has emerged: serum free light chain.

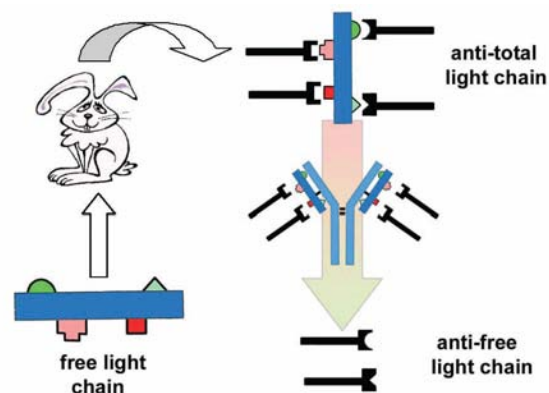
Serum free light chain reagents are not specific for monoclonal free light chain but they reveal the abnormal ratio between kappa and lambda light chains produced when monoclonal free light chain is present. Originally used to detect myelomas in which light chain was the only monoclonal protein produced, it is now clear that most, if not all, patients with myeloma or MGUS have at least small amounts of monoclonal free light chain. We have recently brought this test in-house.

Serum free light chain does not replace protein electrophoresis. Especially in MGUS, the light chain ratio may not be abnormal in patients with a visible abnormal band. But you may wish to consider adding an order for serum free light chain ratio (FLCR) to the traditional screening "serum protein and immunofixation electrophoresis" (SPIE). If an abnormal band or an abnormal free light chain ratio is found, we reflex to a full immunofixation for identification. (Full immunofixation alone is not orderable.)

When following patients with known myeloma or MGUS, we recommend

ordering "serum protein electrophoresis" (SPE) only; the abnormal band will be scanned using densitometry and the concentration compared with the previous specimen. In some selected patients, following the serum free light chain ratio may also be indicated.

Finally, although urine Bence Jones protein has been an important part of the diagnosis of myeloma ever since Dr. Henry Bence Jones examined that unusual urine specimen in 1845, the test for urine protein and immunofixation electrophoresis (UPIE) is no longer needed. It is unlikely that any patient with detectable Bence Jones protein in the urine would not have an abnormal serum free light chain ratio.



Antisera specific for free light chain (kappa and lambda) react with epitopes which are hidden when the light chains are attached to heavy chains. They are made by immunizing an animal with free light chain and then adsorbing the resulting antisera against intact immunoglobulin; only antibodies specific for these hidden epitopes remain.

NEWS YOU CAN USE (CONT.)

the traditional one of 4 ng/ml. Remember that no single PSA cut-off can accurately identify all patients with prostatic cancer. Many factors, including age, race, and family history, need to be taken into account when interpreting screening PSA results.

Successful POCT Survey

The Point-of-Care Testing program at LPCH was recently surveyed by the Joint Commission. Inspectors praised both the nursing staff who perform the tests and the laboratory staff who provide coordination and support.

Quality Improvement Projects

The Stanford Clinical Laboratory recently held its first annual symposium for quality improvement. Sixteen presentations including ensuring proper patient identification; improving inventory management; standardizing data acquisition; and comparing methodologies were reviewed. The winners were:

- A joint project, organized by the Preanalytical and Chemistry areas to improve turnaround time for testing from the Emergency Department, presented by Dr. Raffick Bowen.
- A core lab team project Autoverification in Stanford Clinical Chemistry Lab, presented by Dr. Raffick Bowen

Several other presentations received honorable mentions. The winning presentations will be presented at the Stanford Annual QI Conference.

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KRAS MUTATION TESTING IN COLON CANCER

Andrew H. Beck MD – Molecular Genetic Pathology Fellow

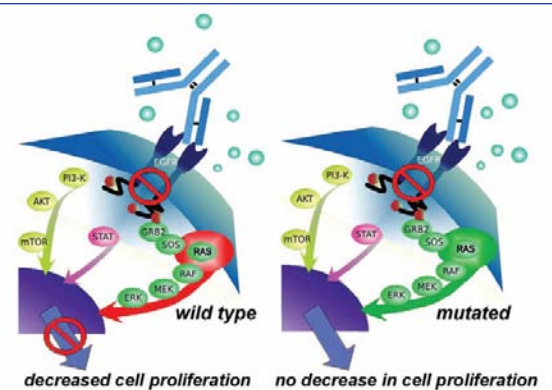
The Molecular Pathology laboratory recently implemented testing for mutations in the KRAS gene. The results of this genetic test determine whether patients with advanced colorectal cancer should receive treatment with chemotherapeutic agents that target the epidermal growth factor receptor (EGFR). EGFR activation plays an important role in helping tumor cells avoid programmed cell death, proliferate, and, in some cases, invade and metastasize. Monoclonal antibodies against EGFR, which block binding by growth factors, have become an important component of personalized care for patients with advanced colorectal cancer. But not all patients respond.

In many patients, the colorectal cancer cells have activating mutations in the gene that codes for KRAS, an important mediator of the signaling cascade induced when EGFR is activated. KRAS initiates a pathway which eventually leads to cell proliferation, and it is clear that tumors with KRAS mutations are largely unaffected by inhibiting EGFR binding. Recent guidelines from a number of organizations, including the National Comprehensive Cancer Network, recommend that anti-EGFR therapy, alone or in combination with traditional chemotherapy, be offered only to patients with tumors that do not have KRAS mutations.

Our test uses PCR amplification of exon 2 of the KRAS gene, followed by mutation detection using a shifted termination assay (STA) technology, which allows enrichment of the mutant signals over the wild-type signal. Products are separated using capillary electrophoresis. The assay detects and differentiates all 12 missense mutations occurring in codons 12 and 13 of the KRAS gene, representing virtually all

of the activating KRAS mutations seen in colorectal cancer. In comparison, the KRAS assay provided by our major reference laboratory is only able to identify the 7 most common KRAS mutations.

Our approach also represents a significant improvement over the traditional technique of gene sequencing, with the ability to detect 5% mutant KRAS in a background of wild-type DNA. This increased sensitivity allows us to avoid performing tumor microdissection, which is required by other less sensitive assays. Physicians can order the test by selecting KRAS on the molecular pathology requisition sheet or through the Epic computer system following biopsy or surgery.



In cell with wild-type KRAS (left panel), antibody to EGFR inhibits binding by growth factors (top) and slows downstream pathways, including those controlled by KRAS (red arrow, bottom right). However, when KRAS is mutated (right panel), these pathways are constitutively active (green arrow, bottom right). Proliferation continues despite the inhibition of EGFR binding and the patient is much less likely to respond to treatment.

The ultimate goal of “personalized genomic medicine” is to utilize genetic testing of patient samples to determine the best therapies for an individual patient. KRAS mutation testing for the treatment of advanced colorectal cancer represents an important advance toward this goal. We hope that in the future we will be able to develop additional genetic tests to improve outcomes for cancer patients.