

# Laboratory Lines

News from Florida Hospital Clinical Laboratories and Florida Pathology Lab (FPL)

## ER/PR TESTING

We recently completed an evaluation of several immunohistochemical methods for measuring estrogen and progesterone receptors and have shown that in our hands, the Dako pharmDx reagents are the most reliable of those we tested. These kits have a high FDA approval rating of 510(k) and are quite expensive, but we want to offer the best tests available for your patients. You will notice a change in our reporting of ER/PR results. The staining is assessed by a standardized scoring system, the Allred score (1), which is a semiquantitative method that combines the sum of two scores, one for the proportion of cells that stain (PS) and the second for the average intensity of staining (IS), into a total score (TS). The final result is based on the total score, which can range from 0 to 8, with a cut-off for positive of 3 or higher. An example is shown below. Notice that the lowest positive total score of 3 could represent as little as 1% of the cells showing weak staining, which has been our threshold for a positive test in the past and is in line with NIH recommendations (2). This scoring system is strongly recommended by Dako (3) to permit standardized reporting, and although there is considerable debate in the literature regarding cut off levels for reporting positive (4), we concur that this system will help in standardization and give you a more accurate indicator of the level of hormone receptor present in a given tumor. A significant component of the interlaboratory variation in ER/PR testing is a result of differing methodologies and reporting criteria and we hope use of the Dako Pharmdx assay will improve precision of the test. We have no conflict of interest in use of this test or affiliation with Dako and will employ the test based solely on our own research and data available in peer-reviewed literature.

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Director Immunohistochemistry Laboratory  
Florida Hospital

References:

1. Allred, DC, et al, Mod Pathol 1998;11(2):155-168.
2. NIH consensus statement, Vol 17, No 4, Nov 2000.
3. Dako ER/PR pharmDx Interpretation Manual.
4. Fisher, ER, et al, Cancer 2005;103:164-73.

## TISSUE TYPING

Medical research continues to identify disease association with certain HLA genes. This information comes from research done while completing the human genome study. For example, HLA-B27 has a strong association with ankylosing spondylitis. New associations have been documented such as HLA-B\*5701 being a strong predictor of a patient developing a hypersensitivity to the drug abacavir sulfate. The HLA system consists of genes found on the short arm of chromosome 6. These genes are located on 22 HLA loci. Genes, or alleles, located on seven of these loci can be determined by the Tissue Typing Lab. These are HLA-A, HLA-B, HLA-C, HLA-DRB, HLA-DQA, HLA-DQB, and HLA-DPB. There are 617 HLA-A alleles, 960 HLA-B alleles, 335 HLA-C alleles, 626 HLA-DRB alleles, 34 HLA-DQA alleles, 87 HLA-DQB alleles, and 127 HLA-DPB alleles.

Because of the complexity of the HLA system, it is **NOT** possible to have a single test for each HLA allele that can be associated with a disease, drug absorption/sensitivity, etc. Currently, we offer complete typing for HLA-A,B,C (HLA Typing Miscellaneous) and typing for HLA-DRB (DR Typing Miscellaneous). Since most individuals will not require a complete HLA typing for these associations, we have created HLA typing tests specific for HLA loci: HLA-A, HLA-B, HLA-C, HLA-DQA, HLA-DQB, and HLA-DPB. When ordering any of these tests, you will be asked for the specific allele. The amount of blood to be collected is one ACD tube and one EDTA tube. These tests will be performed Monday through Friday, with the results usually available the next working day (24-48 hours). Tests were available for ordering on March 18, 2008.

Since there are a number of HLA typing tests now available, there will be some confusion over when to order which typing test. The simplest rule is to ask, "Is the HLA

typing needed for a transplant, platelet transfusion, or other?" If the HLA typing is for transplantation, the test should be either HLA Typing for Solid Organ Transplant or HLA Typing for BMT. HLA typing for transfusing HLA-matched platelets is HLA Typing Platelet Transfusion. Which locus to order depends upon what antigen/allele the physician is interested in? For example, the alleles for a locus begin with the letter or letters indicating the locus. If the allele sought is A29, then order HLA-A typing. If the allele sought is DQA1 or DQA1\*01, order HLA-DQA typing. If you have questions, please call Tissue Typing Lab at 407-303-1681. Max Marschner, MT, SBB, CHS, MBA Manager, Tissue Typing Lab

### NEWS FROM THE CHEMISTRY LABORATORIES

All Florida Hospital Laboratory campuses have completed the conversion to the Beckman Coulter instrumentation systems for general Chemistry and Immunochemistry testing. This change has brought **new tests in-house at the campuses** where testing was previously sent to Orlando. Among the new tests added at the campuses are: Lactic Acid, LD, PSA, T4, TSH, FreeT4, Fe, TIBC, Folate, VITB12, Ferritin, Cortisol, PTH Intact, Prealbumin, Tobramycin, and BHBT. **Beta-hydroxybutyrate (BHBT) replaces the old Acetone test** and is an actual quantitative test detecting the presence and degree of ketosis.

In the near future Beckman Coulter will be improving their **HDL assay to conform with the National Cholesterol Education Program (NCEP)** requirements for accuracy and precision of their method. We will place a comment on the new and improved results once the assay change is implemented.

Please be aware that the medication liposomal **Amphotericin B (AmBisome)** or any preparation that uses a liposomal envelope to facilitate drug delivery may cause a false elevation of phosphorus by the Beckman Coulter method. The pharmacy has assured us that this is a medication that is rarely prescribed. It is usually prescribed by bone marrow transplant physicians.

**The Rapid HIV screening test or SUDS test is restricted for use by Labor and Delivery and for needlestick requests** by Employee Clinic/Centra Care only. All other testing for the antibody to HIV1/2 will be performed using the usual enzyme immunoassay automated procedure. On March 12, 2008 rapid HIV results began being reported as non-reactive or reactive instead of negative or positive.

This is in keeping with the way we report all other Hepatitis and HIV assays. For any questions please call Dr. Luis Guarda at 407-303-5600 ext.1537 or Susan White, Chemistry Manager at 407-303-5600 ext. 4909.

### REFERENCE LABORATORY SELECTION PROCESS

Please be aware that all reference laboratories utilized by Florida Hospital Laboratories **MUST** comply with all State of Florida regulations for laboratory testing. Florida Administrative Code 59A-7028 requires, "A laboratory must refer specimens for testing only to a laboratory possessing a valid license under Chapter 483, Part I, F.S., authorizing the performance of testing in the specialty or subspecialty in which the referred test is categorized". Therefore, all reference laboratories must have a current State of Florida Clinical Laboratory License in order to perform any testing for our laboratories. In addition before Florida Hospital Laboratories can begin referring specimens to outside laboratories these laboratories must be approved by Florida Hospital's Medical Executive Committee and be added to the Laboratory's Approved Reference Laboratory list. For any questions please call Linda Valdes, Laboratory Administrative Coordinator- Regulations/Safety at 407-303-5600, ext. 5960.

### C. DIFFICILE ANTIGEN

The lab is now able to report the presence of C. difficile in stool generally within eight hours of collection through the detection of glutamate dehydrogenase (GDH). Positive antigen samples are immediately followed with the more clinically significant toxin testing which may include a cytotoxic assay if the antigen is positive but toxin screen is negative. Asymptomatic carriers, most commonly children under 2, will also be antigen positive, toxin negative. If a patient is treated for CDT, **we recommend not retesting the patient within two weeks.** Upon starting therapy, toxin tests should convert to negative within 2-3 days but the antigen may remain positive much longer. For patient protocol questions due to C. difficile results please speak with an infectious disease specialist. For any questions please call Julie Hess, Serology Department Manager at 407-303-5600 ext. 4965