



# LABletter

NOV/DEC 2008

PATHOLOGY & LABORATORY MEDICINE NEWSLETTER

## NEWS YOU CAN USE

### New Tests Implemented

New types of testing for Mycobacteria were recently implemented in the Microbiology section, including a rapid, PCR-based method for identification and new approaches to antibiotic susceptibility testing. Celiac Disease antibody testing was recently implemented in the Special Chemistry section. (See separate articles in this issue.)

### Laboratory Accreditation Renewed

The Stanford Department of Pathology's clinical laboratories passed their bi-annual inspection by the College of American Pathologists in October. A team of 15 inspectors from the University of Texas Southwestern Medical Center extensively reviewed our Anatomic Pathology and Clinical Laboratory services at Stanford Hospital, the Clinical Laboratory at Hillview, and the satellite laboratories at 730 Welch Road and the Bass Center. We are accredited for another two years.

### Dr. Dan Arber Named Medical Director

Dan Arber MD became Associate Chair of Pathology for Clinical Services and the Medical Director of the SHC Clinical Laboratory and Anatomic Pathology Services in November. Dr. Arber has oversight responsibility for all services in clinical pathology and anatomic

## RECENT ADVANCES IN MYCOBACTERIAL DIAGNOSTICS: Rapid Identification & Susceptibility Testing

Niaz Banaei MD - Associate Medical Director of Clinical Microbiology & Virology

Infections by pathogenic mycobacteria result in a variety of illnesses, ranging from lung disease to osteomyelitis. Globally, *Mycobacterium tuberculosis* is the most important species. However, in the United States, non-tuberculous mycobacteria account for the majority of mycobacterial infections. For instance, *M. tuberculosis* complex organisms made up only 14% of the pathogenic isolates recovered from patient specimens in our laboratory in 2007.

Timely detection and identification of positive acid-fast bacillus (AFB) cultures is of great importance for both clinical decision making and for public health measures. Rapid identification allows patients who are infected with *M. tuberculosis* to receive appropriate respiratory isolation, and helps to avoid unnecessary administration of potentially toxic antibiotics to patients infected with other organisms. Unfortunately, commercially available methods are either limited in their diagnostic scope or require expertise which is not feasible for the routine clinical microbiology laboratory.

We recently implemented a simple multiplex, real-time polymerase chain reaction (PCR) assay that can identify most of the pathogenic mycobacteria we see in our laboratory (see Figure 1). Our protocol differentiates groups of mycobacteria in each of two separate reactions and was also validated for the direct detection of *M. tuberculosis* complex in positive respiratory specimens. We have also

developed similar PCR assays to definitively identify members of the *M. tuberculosis* complex and the *M. chelonae-M. abscessus* group. The small percentage of cultures not defined by PCR will be identified using RNA gene sequencing and high-performance liquid chromatography.

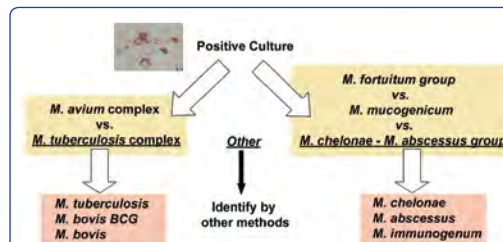


Figure 1: PCR identification of Acid-Fast Bacilli

The initial two PCR reactions characterize the organism as a member of one of two groups of commonly seen species, or likely to be a rare isolate. The subsequent PCR reactions further identify the *M. tuberculosis* complex and the *M. chelonae-M. abscessus* group.

Antibiotic susceptibility results are essential for providing individually tailored regimens for certain mycobacteria. We recently validated an automated system to detect susceptibility of *M. tuberculosis* complex isolates to first-line drugs. We also have adapted the E-test © system for susceptibility testing of some nontuberculous isolates. We will not routinely perform antibiotic susceptibility testing on other non-tuberculous mycobacteria unless the patient was previously treated or is not responding to present therapy.

## NEWS YOU CAN USE (CONT.)

pathology at Stanford, including those provided to patients and physicians at SHC and LPCH, as well as to clients of our outreach testing program. Dr. Arber will continue to serve as Director of Clinical Hematology and Hematopathology. Sharon Geaghan MD will continue in her present roles as Co-Medical Director of the SHC Clinical Laboratory for Pediatrics (a role that reports to the Medical Director) and Chief of Pathology at LPCH.

Drs. Arber and Geaghan ([darber@stanfordmed.org](mailto:darber@stanfordmed.org), [sgeaghan@stanfordmed.org](mailto:sgeaghan@stanfordmed.org)) want to ensure that we provide the highest quality of services in laboratory medicine and anatomic pathology. They welcome suggestions about priorities for enhancing further the laboratory medicine and pathology services offered to patients and physicians at SHC and LPCH.

#### Rapid Respiratory Virus Testing Again Available

Rapid testing for RSV and both influenza A and B is again available as a stat request from November 2008 until April 2009. The results of rapid testing are useful for initial screening but negative results should be confirmed by direct fluorescent antibody (DFA) testing. Please use the new collection kit (with two swabs). If you only have the old kit (with only one swab), call us at 650-724-8632.

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# SCREENING FOR CELIAC DISEASE WITH A NEW COMBINATION OF TESTS

Jim Faix MD – Medical Director of Clinical Chemistry & Immunology

Celiac disease was once considered to be of low prevalence but is now known to affect almost 1% of individuals of European descent. It has become a significant condition to rule out when patients complain of chronic diarrhea.

Also known as gluten-sensitive enteropathy, celiac disease has both a genetic component (susceptibility) and a triggering external agent (gluten). Gluten is the protein component of the endosperm of grain kernels, and the flour contains small soluble proteins called gliadins. These contain a segment which is relatively resistant to protease digestion and which, when expressed on the surface of antigen presenting cells, incites an intense inflammatory response. Overtime, the absorptive villi of the small intestine become blunted and malabsorption ensues (see Figure 2).

Certain major histocompatibility complex (MHC) proteins are especially good at presenting the offensive peptide, but there is more to the genetic component because many individuals with these MHC proteins do not experience celiac disease. And, although the native primary amino acid sequence of the gliadin peptide is important for binding to the antigen presenting cell's MHC, binding is enhanced when certain amino acid residues are deamidated by the enzyme tissue transglutaminase (see Figure 3). For unknown reasons, this enzyme (TTG) becomes a target for autoantibody production in celiac disease. Originally identified by indirect immunofluorescence as an antibody against reticulin or the endomysium of smooth muscle, anti-TTG antibody is now measured by ELISA. Although celiac patients develop both IgA and IgG anti-TTG antibodies, the IgA isotype is an especially sensitive and specific marker.

Although patients with celiac disease develop antibodies against gliadins (as well as against other food antigens), so do many patients with other inflammatory disorders of the small intestine. So, anti-gliadin antibodies have never been very specific markers. For some time, it has been known that anti-gliadin reactivity in celiac patients was

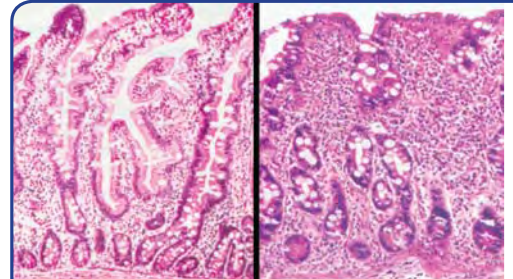


Figure 2: Villous Atrophy in Celiac Disease

Normal small intestinal mucosa (left) shows frond-like villi. In celiac disease (right), infiltrating lymphocytes destroy these, causing malabsorption.

enhanced using deamidated gliadin peptides (DGP), especially for IgG antibodies. These may be as specific as anti-TTG antibodies, and may respond more rapidly to the institution of a gluten-free diet, although additional studies are needed.

Beginning this month, Stanford Clinical Laboratory will offer **IgA anti-TTG (order code: ATTG)** and **IgG anti-DGP (order code: GDGP)**. Both will be available separately, but both will also be included in a new **Celiac Disease Antibody Screen (order code: CDAS)** which also includes total IgA (to rule-out the presence of IgA deficiency). All positive ELISA results will be confirmed by indirect immunofluorescence (the traditional anti-endomysial antibody assay).

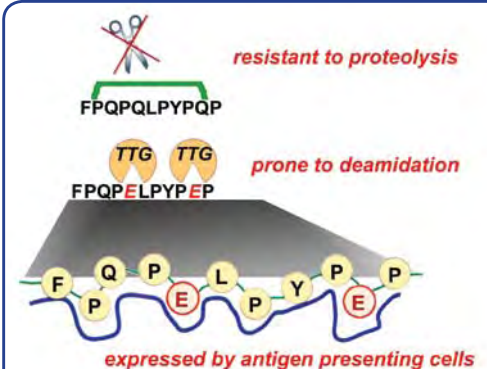


Figure 3: Antibody Targets in Celiac Disease

The triggering peptide derived from gliadin is resistant to digestion by proteases. Deamidation of some glutamine (Q) residues to glutamic acid (E) by tissue transglutaminase (TTG) helps it to stimulate T cells when processed by antigen presenting cells. Patients develop antibodies to both the enzyme which catalyzes the deamidation as well as to the deamidated peptides themselves.