Saint Luke's Regional Laboratories
Clinical Laboratory Letter

October 2014

Molecular Detection of Gastrointestinal Pathogens

Last month's Lab Letter included details of a new molecular gastrointestinal pathogen panel that will be available later this month. This assay detects 22 targets from a single stool specimen including bacteria, viruses, parasites, and toxins.

Shiga toxin production, currently detected by immunoassay, is associated with post-diarrheal hemolytic uremic syndrome (HUS). Although E. coli O157:H7 is the most common cause of HUS in the U.S., several other bacteria may produce shiga toxin, including other enterohemorrhagic E. coli (EHEC). In addition, Campylobacter, Shigella, Salmonella, and Yersinia can produce shiga toxin and subsequent HUS.

Validation of the molecular GI panel by Saint Luke's Microbiology included previously tested specimens that were positive for shiga toxin by immunoassay. In addition to detecting shiga toxin, the molecular panel consistently identified an associated bacterial pathogen producing the toxin, which was not always possible with conventional stool culture testing. Bacterial pathogens detected by the molecular panel along with shiga toxin included Campylobacter, E. coli O157, and Salmonella.

The molecular assay should be ordered as 'Gastrointestinal Pathogen Panel' and requires submission of a fresh diarrheal stool sample from inpatients. Outpatient samples should be submitted in Cary-Blair transport media. The panel will not be available on inpatients that have been hospitalized for more than three days, for whom Clostridium difficile toxin PCR is still the most appropriate initial test. Time to result from initiation of testing in the laboratory is approximately one hour.

Due to the enhanced sensitivity & specificity provided by this technology, conventional diarrheal stool testing with suboptimal performance characteristics (including ova/parasite stains, bacterial stool culture, and viral stool culture) will be phased out early in 2015. Single-target PCR testing for Clostridium difficile toxin, as well as Giardia/Cryptosporidium and Rotavirus antigen testing will remain available in addition to the panel.

Legionella Cultures Discontinued

Legionella pneumophila and related species are uncommon causes of pneumonia in both immunocompromised and normal individuals. Legionella is ubiquitous in natural water habitats, and may colonize man-made water features which can then serve as a source of human infections. Tests for legionella infection include respiratory culture, direct fluorescent antibody (DFA), PCR for respiratory specimens, serum antibody, and urine antigen detection.

The gold standard for diagnosis of legionellosis is culture. However, *Legionella* requires charcoal-containing media for growth, cultures must be held for 14 days, and the reported sensitivity of culture is low at 25-80%. Likewise, *Legionella* DFA has a low sensitivity because large numbers of organisms are required for visualization. Because Saint Luke's Microbiology has not isolated Legionella from culture in several years, effective immediately, these cultures will no longer be performed in-house. Legionella PCR testing of respiratory specimens is available through a reference laboratory, and is the best alternative when this diagnosis is highly suspected.

Serologic and urine antigen tests are also available, when respiratory specimens are difficult to collect. Serologic testing should include both IgM and IgG antibodies obtained both acutely and during convalescence.Antibody response may not be detectable until one to three months after onset of illness.

Urine antigen test detects a specific soluble antigen present in the urine of patients with *Legionella* infections and is the test of choice for diagnosis of early infection. It detects only *Legionella pneumophila* serogroup 1, which causes the majority

of cases of legionellosis. Sensitivity is 70% with specificity near 100%. Antigen excretion may begin as early as 3 days after onset of symptoms and persist for up to 1 year afterward. The test remains positive for several weeks following antibiotic therapy.

Specimen requirement for serologic testing is one SST tube of blood. Specimen requirement for the urine antigen test is 1.0 mL of urine from a random collection. No urine preservatives should be used, and the specimen should be refrigerated after collection.

Serum Free Light Chains

Traditional methods for detection and quantitation of monoclonal proteins include urine and serum protein electrophoresis and immunofixation. For the past 20 years, urine electrophoresis and immunofixation have been the tests of choice for detecting monoclonal free light chains in urine. Patients with light chain disease often demonstrate hypogammaglobulinemia in the serum with detectable monoclonal kappa or lambda light chains in the urine. Unfortunately, these methods are not very sensitive for detection of free light chains. An immunoassay for quantitation of serum free light chains is much more sensitive. This assay measures only free light chains and not the light chains attached to intact immunoglobulin molecules.

Serum FLC assay offers several clinical advantages. It can replace urine electrophoresis and immunofixation in the initial evaluation of patients suspected of having a monoclonal gammopathy. Studies from Mayo Medical Laboratories have demonstrated that 99.5% of cases of multiple myeloma can be detected by a combination of serum FLC analysis with serum protein electrophoresis and immunofixation (Katzman JA, et al. Clin Chem 2009;55:1517-22). The International Myeloma Working Group has concluded that this combination of tests is sufficient to test for monoclonal gammopathies. They no longer recommend 24 hour urine protein electrophoresis or immunofixation for diagnosis, except for amyloidosis (Dispenzieri A, etal. Leukemia 2009;23:215-24). International guidelines also recommend that serum FLCs be performed at the time of diagnosis to provide prognostic information. National Comprehensive Cancer Network (NCCN) also recommends including serum FLC in the diagnostic workup of newly diagnosed patients with plasma cell dyscrasias (Anderson KC. J Nat'l Compr Canc Netw 2011;9:1146-83).

The vast majority of patients who have monoclonal gammopathies are eventually classified as monoclonal gammopathy of unknown significance (MGUS). Overall, individuals with MGUS have about a 1% per year chance of progressing to multiple myeloma or another B cell lympho-proliferative disorder. Quantitation of serum FLCs helps stratify the risk of progression. Lower risk is associated with an M-protein less than 1.5 g/dl, IgG isotype and a normal serum FLC kappa/lambda ratio.

Light chain myeloma accounts for approximately 20% of all cases of multiple myeloma. Screening with serum protein electrophoresis alone misses 40% of cases. Addition of serum FLC measurement detects >99% of cases.

Nonsecretory myeloma accounts for 1 to 5% of multiple myeloma cases and is characterized by the absence of monoclonal proteins in serum and urine by electrophoresis and immunofixation. Serum FLC detects monoclonal FLC in more than 50% of these cases.

Approximately 20% of primary systemic amyloidosis cases have no detectable monoclonal protein by serum or urine electrophoresis and immunofixation. FLC assays detect monoclonal free light chains in 75 to 90% of cases. The International Working Group recommends ordering serum FLC in addition to serum and urine electrophoresis.

Reference ranges are 3.30-19.40 mg/L for kappa free light chain, 5.71 to 26.30 mg/L for lambda light chain, and 0.26-1.65 for kappa to lambda ratio. Lower limit of detection is 1.50 mg/L for kappa and 3.00 mg/L for lambda light chains.

The kappa/lambda ratio is especially important in diagnosing monoclonal gammopathies. An abnormal ratio suggests a clonal expansion of plasma cells. A normal or borderline kappa/lambda ratio in the presence of elevated levels of kappa and lambda light chain levels suggests renal impairment, polyclonal expansion of plasma cells or rarely a biclonal gammopathy with different light chain types. Specimen requirement is a red top tube of blood.

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