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PCR Triumphs in *Clostridium difficile* Toxin Detection

Clostridium difficile is a Gram-positive, spore-forming, anaerobic bacillus that is associated with pseudomembranous colitis. The disease formerly known as *C. difficile*-associated disease (CDAD) is now called *C. difficile* infection (CDI). CDI ranges in severity from mild diarrhea to fulminant colitis. Risk factors for CDI include antibiotic use within three months prior to symptom onset and exposure to a health-care setting. Colonization with *C. difficile* is common in hospitalized patients (20-40%), while only 3% of healthy adults are colonized. CDC data indicates that the incidence and severity of CDI has been increasing since the year 2000. The recently described hypervirulent *C. difficile* strain has now been reported in 38 states, & presents a new challenge for infection control. Community-acquired infections, many without identifiable risk factors, are increasing as well.

Alterations of normal gut flora, resulting in overgrowth of *C. difficile*, are believed to initiate CDI. Production of exotoxins A & B by the organism subsequently results in colonic mucosal damage, and detection of these toxins is the basis for diagnostic laboratory tests. The majority of U.S. laboratories utilize enzyme immunoassay (EIA) for this purpose, due to rapid turn-around time & ease of use. However, the sensitivity & specificity of EIA is widely variable, as low as 50% and 70%, respectively. More accurate toxin detection is achieved by cell culture cytotoxicity assays, however this technique requires 48 to 72 hours to obtain results. Conversely, there are recent favorable reports in the literature regarding the utility of real-time PCR for CDAD diagnosis. (Ann Intern Med. 2009;151;176-179, JCM 2009; 47; 3211-3217)

A multi-disciplinary team at Saint Luke's Hospital including laboratory, ID & GI physicians, has evaluated *C. difficile* toxin testing by EIA vs. PCR. The PCR test, performed by the Molecular

Diagnostics section of Saint Luke's Regional Laboratories, targets the *tcdA* & *tcdB* genes that encode for toxin A & B. Parallel testing of 204 residual stool samples submitted for *C. difficile* toxin EIA was performed by PCR, with discordant samples submitted to a reference laboratory for further testing by PCR with different gene targets. There were 121 negative samples and 83 positive samples by EIA. Of these, 124 samples tested negative and 76 samples were positive by both PCR assays. The SLRL PCR was positive in 4 samples, which were negative by both EIA & the reference laboratory PCR. The overall sensitivity and specificity of the SLRL PCR test, compared to the reference PCR method, were 100% and 97% respectively.

The evaluation also included data from 18 patients with multiple inconsistent results by EIA. Based on the majority of results, 13 of these cases were judged to be true negative & 5 true positive. PCR evaluation of these specimens yielded consistent results in all cases. In 3 true positive cases, PCR detected toxin up to one day earlier than EIA.

Beginning in mid November, Saint Luke's Regional Laboratories will replace *C. difficile* toxin A/B testing by EIA with *C. difficile* toxin A/B gene detection by real-time PCR. Due to improved sensitivity & specificity of the PCR assay, only one stool specimen per patient should be submitted for testing. Specimens should be submitted fresh (refrigerated unless transported to the laboratory immediately) or in Cary-Blair transport media. Testing will be performed daily, including weekends.

HbA1c for Diagnosis of Diabetes

Hyperglycemia has been the sole diagnostic criterion for diabetes since the development of blood glucose assays more than 100 years ago. Despite being the gold standard, measurement of blood glucose is less accurate and precise than most physicians realize. Blood glucose

measurements are also subject to several limitations including:

- 8-12 hour fasting specimen requirement
- Diurnal variation requiring morning collection to capture peak levels
- Large biological variation of 5-8%
- Nonstandardized instrument methods with >12% bias compared to the reference method
- Glycolysis after collection, even in sodium fluoride tubes, causing falsely decreased glucose levels

Traditionally, measurement of HbA1c levels has been largely restricted to monitoring diabetic patients. Very recently, HbA1c was endorsed for diagnosis of diabetes by an International Expert Committee (Diabetes Care 2009, 32 (7):1327-1334). A diagnostic cutpoint of 6.5% was recommended, based on the risk for developing microvascular complications such as retinopathy. Patients who have an HbA1c of 6.0 to 6.4% are considered at high risk for developing diabetes and cardiovascular disease in the future. These individuals should be identified and counseled about lifestyle modifications such as exercise and weight loss. An elevated HbA1c should be confirmed with a repeat measurement on a different day, except in those individuals who are symptomatic and also have plasma glucose over 200 mg/dL. HbA1c testing is indicated in children in whom diabetes is suspected but the classic symptoms and a casual glucose >200 mg/dL are not found. Analysis should be performed on central laboratory instruments and not with point of care devices, which have not been shown to be sufficiently accurate or precise for diagnosis.

The advantages to using HbA1c are:

- Better index of overall glycemic exposure & risk of complications
- Low intraindividual variability (<2%)
- No requirement for fasting or timed specimen
- Standardized methods with precision <2%
- Less affected by acute illness or stress
- Good stability after blood collection
- Single test can be used for both diagnosis and monitoring

Some arguments against using HbA1c include the limited number of studies that have been

performed thus far to derive the diagnostic cutoff and the increased expense of HbA1c compared to glucose.

A couple of issues appear somewhat confusing. First, the upper limit of normal for HbA1c is 6.0% and the diagnostic cutoff for diabetes is 6.5%, leaving a gray zone of values that are not normal but not high enough to qualify as overt diabetes. However, the risk for diabetes based on glycemia is a continuum and there is no single threshold at which risk clearly begins. The other apparent contradiction is that the diagnostic threshold for diabetes is defined as 6.5% while the recommended treatment target, which is based on glucose testing, remains at 7.0%. Additional clinical research will be needed to resolve these important issues.

Cocaine has Become More Dangerous

Cocaine is often deliberately diluted with other substances at some stage of production, packaging or distribution to increase the apparent quantity (salt, sucrose, lactose, starch, or ascorbic acid), produce a complementary effect (procaine, benzocaine or tetracaine) or attenuate side effects (diltiazem or hydroxyzine). Recently, the Special Testing and Research Laboratory of the U.S. Drug Enforcement Administration has detected levamisole in more than 50% of Columbian cocaine shipments that have been intercepted. It is a veterinary deworming agent that can be purchased in bulk on the Internet. Levamisole may provide a complementary effect to cocaine by acting as a ganglion nicotinic acetylcholine receptor agonist.

The major adverse effect of levamisole is severe agranulocytosis, possibly by stimulating formation of IgG and IgM anti-neutrophil antibodies and anti-neutrophil cytoplasmic antibodies (ANCA). Physicians should consider the possibility of levamisole adulterated cocaine in patients who present with otherwise unexplained fever and agranulocytosis.

Levamisole is not detected by routine drugs of abuse screening and is difficult to detect by gas chromatography/mass spectrometry because the elimination half-life is only 5.6 hours and only 2-5% of the drug is excreted unchanged in urine.