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Rapid Molecular Detection of Tuberculosis

Traditional testing for pulmonary tuberculosis (MTB) includes AFB smear & culture.

Unfortunately, AFB culture can take up to 6 weeks for results, due to the slow-growing nature of the organisms. Although more timely with regard to results, AFB smears lack sensitivity, and may be negative in up to 50% of culture-confirmed MTB infections. Molecular testing, that improves time to detection of MTB and facilitates treatment and determination of isolation requirements, has been available for several years.

The CDC recommends that molecular testing be performed on at least one respiratory specimen from patients with suspected pulmonary TB for whom the result would alter management or infection control activities (MMWR 2013;62:821-827).

Last fall, a new rapid qualitative PCR became available that allows direct testing of sputum samples for MTB and simultaneously detects the rifampin-resistance *rpoB* gene. The incidence of rifampin resistance is low in the US (1.8% of confirmed TB cases) and most often coexists with INH resistance. A multi-institution evaluation of this assay determined that when a single sputum sample was tested, MTB was identified in 98.2% of smear-positive tuberculosis patients and 72.5% of smear-negative tuberculosis patients. Testing of three sputum samples on smear-negative tuberculosis patients increased sensitivity to a total of 90.2%. Specificity was 99.2% (Boehme et al. *NEJM*, Sept 2010).

This newest MTB PCR assay with molecular detection of rifampin resistance is now performed in Saint Luke's Microbiology. Currently, the assay is FDA-cleared for sputum samples only. Results will be reported as 'MTB detected' or 'MTB not detected' and 'rifampin resistance detected' or 'rifampin resistance not detected'. Molecular testing does not replace the need for traditional testing, hence all sputum specimens received for MTB PCR

must have AFB smear and culture ordered as well. MTB PCR testing will be run daily.

Tissue Plasminogen Activator Coagulopathy Reversal

Recombinant tissue plasminogen activator (rtPA) has been approved to treat ischemic stroke in the first three hours following the onset of symptoms. If given promptly, 1 in 3 patients who receive rtPA have major improvement in their stroke symptoms.

Recombinant tPA produces local thrombolysis by converting plasminogen into plasmin, which then degrades fibrin into fibrin split products. More than 50% of rtPA is cleared 5 minutes after cessation of the infusion and approximately 80% is cleared after 10 minutes. Despite this rapid clearance, rtPA prolongs the prothrombin and activated partial thromboplastin times and decreases fibrinogen levels for as long as 24 hours or more from the time of infusion.

An important complication after treatment of acute stroke with rtPA is symptomatic intracerebral hemorrhage (sICH). Studies suggest that between 3.5% and 6% of stroke patients treated with rtPA develop sICH, and the hemorrhagic complication leads to death in about 50% of patients. Older patients, patients with very severe strokes, and patients who used aspirin before their strokes are all at increased risk for sICH (Yaghi S et al. Symptomatic intracerebral hemorrhage in acute ischemic stroke after thrombolysis with intravenous recombinant tissue plasminogen activator. *JAMA Neurol* 2014; DOI: 10.1001/jamaneurol.2014.1210).

Because coagulopathy related to rtPA lasts up to 24 hours, both early and sustained reversal is necessary to avoid hematoma expansion and neurologic deterioration. The American Heart Association/American Stroke Association treatment guidelines for symptomatic ICH management recommend replacement of coagulation factors and platelets, acknowledging that there is limited evidence to support the strategy. Specifically, the

guidelines call for the use of 10 bags of cryoprecipitate to reverse coagulopathy and one bag of single donor platelets or 6 to 8 bags of random donor platelets.

Each bag of cryoprecipitate contains 200 to 250 mg of fibrinogen and will increase the plasma fibrinogen level of a 70-kg adult by 6 to 8 mg/dL. Generally, 10 bags of cryoprecipitate are given if the fibrinogen level is between 50 and 100 mg/dL and 20 bags are given if it is less than 50 mg/dL. A fibrinogen level should be measured at 30 to 60 minutes after completion of the transfusion to determine if additional doses are needed. The therapeutic goal is to keep the plasma fibrinogen level above 100 mg/dL. Circulating half life of fibrinogen is 3 to 5 days.

The potential benefits of aggressive approaches to managing sICH must be weighed against the potential for worsening thrombosis.

Beta 2 Transferrin for Diagnosis Of CSF Leakage

CSF leakage may be caused by head trauma, cancer, congenital malformation or surgery. The diagnosis of rhinorrhea or otorrhea is difficult to confirm by conventional laboratory tests or radiographic studies. Prompt diagnosis and localization of the source of leakage decreases the risk of meningitis and facilitates treatment decisions.

Serum contains beta-1 transferrin and cerebrospinal fluid contains beta-1 and beta-2 transferrins. The central nervous system contains neuraminidase that partially desialylates beta-1 transferrin to form beta-2 transferrin. Beta-2 transferrin can be used as a biomarker of CSF leakage. Aqueous humor also contains beta-2 transferrin.

Beta-1 and beta-2 transferrin are distinguished by electrophoresis on a high resolution 1% agarose gel, followed by immunofixation with anti-transferrin antibody that is labeled with horse radish peroxidase. The presence of both beta-1 and beta-2 transferrin bands indicates the presence cerebrospinal fluid. This method is sensitive enough to detect the presence of spinal fluid when it comprises only 2.5% of a body fluid. (Normansell DE, et al. Detection of beta-2 transferrin in otorrhea and rhinorrhea in a routine clinical laboratory setting. *Clin Diagn Lab Immunol* 1994;1:68-70).

Preferred specimen is 0.5 mL of otic or nasal fluid in a plastic vial. Alternatively, a saturated cotton swab can be submitted if direct collection is not feasible. It should be transported in a tightly stoppered tube. Reference range is a negative result, meaning that no beta-2 transferrin was detected.

Ethyl Glucuronide and Ethyl Sulfate to Monitor Alcohol Abstinence

Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are biomarkers of alcohol consumption and are used to monitor alcohol abstinence. Each metabolite represents less than 0.1% of the total metabolites of ethanol. They are detectable in urine within minutes of alcohol consumption and remain detectable for approximately three days.

Use of hand sanitizers or the ingestion of mouthwash or cough syrup containing alcohol can result in detectable levels of EtG and EtS. Hand sanitizers usually produce EtG levels less than 100 ng/mL. Mouthwash usually produces EtG levels between 100 and 300 ng/mL.

EtG can be either produced or degraded by bacteria. Bacterial beta-glucuronidase can degrade 50% of EtG within 24 hours after collection. EtS is neither produced nor degraded by bacteria. The presence of EtS increases the likelihood that the source of EtG was ethanol ingestion and not in vitro production.

Screening for EtG is performed by immunoassay. If the screening test is positive, confirmation of EtG and EtS is performed by liquid chromatography and tandem mass spectrometry. The screening threshold for EtG is 500 ng/mL. The confirmation cutoff for is 500 ng/mL for EtG and 250 ng/mL for EtS.

Specimen requirement is 20 mL of urine collected without preservatives. Specimen can be stored up to 7 days refrigerated.

Errata

The July issue contained two errors. Specimen requirement for Protime/INR is a blue top tube of blood, not a lavender top tube. Reference range for total protein is 6.0-8.2 g/dL, not 6.0-8.0.