

December 2015**MALDI-TOF Madness in Microbiology**

For decades, bacterial and fungal cultures have been the mainstay of clinical microbiology laboratories. Traditional identification of cultivated organisms includes observation of growth characteristics, various stains, and manual or semi-automated biochemical tests. Newer tools include molecular identification of a rather small group of select pathogens. Recently, a revolutionary technology has become available that significantly impacts the time needed to identify a wide range of infection-causing bacteria and fungus. This technology (Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry, abbreviated MALDI-TOF) allows reporting of accurate, inexpensive results to the treating physician the same day a bacterial culture turns positive, instead of 2 or more days later.

The principle behind MALDI-TOF is proteomics. For identification of a bacterial colony from a culture plate, the organism is suspended in a matrix, which is then placed into the mass spectrometry instrument. The suspension is vaporized by a laser and discharged into the flight tube. The mass-to-charge ratio is measured, and the organism's profile is compared to a database from which identification to the species level is generated. Time for bacterial identification from mass spectrometry is ≤ 3 minutes, compared to 24 hours or more for traditional testing.

Literature comparing this technology to customary bacteriology indicates that less than 10% of bacteria causing infections are identified by conventional methods by 24 hours after growth is observed. Less than 90% of bacteria can be identified correctly to species level by conventional methods. The accuracy of MALDI-TOF identification is 98.3% and can be performed from a single bacterial colony, the same day culture growth appears.

Saint Luke's Microbiology laboratory began using MALDI-TOF for identification of Gram-negative

bacteria in August 2015, and recently expanded to Gram-positive bacteria and yeast. Physicians will notice improved turnaround times and accuracy for pathogen identification. Additionally, the laboratory is collaborating with the Antimicrobial Stewardship Program to leverage this new technology to the best advantage for patient care.

GFR Calculation Options

Optimal estimation of renal function in the clinical setting is frequently based on the use of various formulas. Some of these formulas such as one developed by Cockcroft and Gault (1976) is based on creatinine clearance and is as follows:

$$CCr = \frac{\{[140 - \text{Age (yrs)}] \times \text{Weight (kg)}\}}{72 \times \text{Scr}} \times (0.85 \text{ if female}),$$
 where Scr is serum creatinine and CCr is creatinine clearance.

In addition to serum creatinine level, body weight and BMI introduces bias in determination of renal function in the above equation. Therefore, KDIGO 2012 Guidelines published for the Evaluation and Management of Chronic Kidney Disease (CKD) recommends using glomerular filtration rate (GFR) employing equations based on serum creatinine levels. Such equations were formulated more than a decade ago and have been in use for an accurate assessment of renal functionality. Originally, a total of 7 equations were formulated from data obtained from a multi center study that performed evaluation of effect of dietary protein restriction and strict blood pressure control on progression of renal disease (Modification of Diet in Renal Disease Study (MDRD) (Levey AS *et. al* in 1999)). Of these, the abbreviated MDRD formula showed fairly accurate prediction of GFR over a wide range of values (15-29 ml/min per 1.73 m²), smallest overall bias, and ease of implementation in the clinical laboratory owing to use of a standardized method for determination of serum creatinine levels. The equation is as follows:

$GFR = 170 \times [Scr]^{-0.999} \times [Age]^{-0.176} \times [0.762, \text{ if patient is female}] \times [1.180, \text{ if patient is African American}]$

More recently, another equation formulated based on serum creatinine levels, namely Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), although considered a more accurate predictor of renal function, has not been validated outside of the original study and is therefore not widely used. A recent study performed direct comparison of all the above mentioned equations in a subgroup of patients who are old, underweight, or overweight and concluded that the highest accuracy for GFR estimation was reached with the abbreviated MDRD and CKD-EPI equations in a clinical setting (Wieneke, MM *et. al.* Clin J Am Soc Nephrol 5:1003-1009, 2010).

Saint Luke's Health System Chemistry laboratories use the abbreviated MDRD equation for reporting estimated GFR on all patients. Of note, SLHS Citrix includes a clinical calculator with both Cockcroft-Gault and MDRD equations for GFR calculation. For accurate comparison with the reported laboratory data, health care providers should make sure that the calculator option is set to use the abbreviated MDRD equation.

ANA Or ANNA

ANNA or anti neuronal nuclear antibodies are frequently requested in error in patients with suspected autoimmune connective tissue disorder (CTD). Neuronal antibodies are mainly seen in patients with CNS disorders including paraneoplastic and non-paraneoplastic. A recent review (Graus F. *et. al.* J Neurol (2010)257:509-517) attempted to subclassify these autoantibodies based on the location of the antigens targeted. Group 1 antibodies target intracellular antigens and are further subclassified into group Ia onconeural antibodies (Hu (ANNA1), Yo (PCA1), Ri (ANNA2), CV2 (CRMP5), amphiphysin, Ma2), cancer specific group Ib antibodies (SOX and ZIC), and group Ic non-paraneoplastic syndrome antibodies (glutamic acid decarboxylase (GAD), adenylate kinase 5 and Homer 3) that identify - stiff-person syndrome (SPS), cerebellar ataxia, and limbic encephalitis (LE). These antibodies are most likely non-

pathogenic but useful for diagnosis. The presence of these antibodies should be interpreted in the context of clinical setting, since many patients with small cell lung cancer and no evidence of paraneoplastic syndrome are positive for antibodies such as Hu, CV2, and amphiphysin. Group II antibodies recognize neuronal surface antigens and include group IIa antibodies associated with characteristic CNS syndromes (such as antibodies to potassium channels, AMPA and GABA_B receptors associated with LE, NMDA receptor antibodies associated with encephalitis, and antibodies against glycine receptors associate with SPS with encephalitis). Group IIb includes antibodies detected in patients with paraneoplastic cerebellar ataxia associated with lung cancer (P/Q type calcium channels antibodies) or Hodgkin disease (metabotropic glutamate receptor type 1 antibodies).

ANA or anti-nuclear antibodies on the other hand are hallmark of many autoimmune connective tissue disorders and include antibodies directed at various cellular compartments such as nuclear envelope, mitotic spindle apparatus, cytoplasmic organelles, and cell membrane. Laboratory detection of these antibodies is useful in diagnosis and monitoring of various autoimmune diseases (AID) such as systemic lupus erythematosus (SLE), Sjogren's syndrome, scleroderma, mixed connective tissue disease, polymyositis, and dermatomyositis. ANA testing has high sensitivity (92%-95%) but low specificity (53%) and is therefore a good screening test for AID. A positive ANA test with high titer of >1:42 is typically followed with more specific tests including anti-dsDNA and anti-smith antibodies to rule/in or rule/out SLE. Other autoimmune tests requested such as anti-Jo-1, anti-Scl-70, anti-CENP (anti-centromere), anti-SS-B/La, ANA, anti-mutated citrullinated vimentin (anti-MCV), EC4d, and BC4d helps distinguish SLE from other CTDs.

Specimen requirement for ANA testing is 1 ml serum (serum gel tube), stored and transported at room temperature. The test is run Monday to Friday. If the test is positive the staining pattern and titer of the antibody is reported. ANNA, also known as neuronal cell antibody testing, is a send-out test and also requires 1 ml serum (plain red top or serum gel tube), stored and transported frozen.