Women of AP/CP: Professional Development Program Featured in Podcast

by Suzie Thibodeaux, MD PhD

Drs. Ann Gronowski and Carey-Ann Burnham have spearheaded efforts within Anatomic and Clinical Pathology (AP/CP) to develop a professional development program specifically geared towards women in the field. The program involves once monthly meetings, where a variety of topics are discussed with the intention of considering the female perspective in the process. Examples of topics from a variety of speakers both internal and external to the department include: unconscious bias, negotiation, navigating difficult conversations, the promotion process, professionalism and professional etiquette, salary gender gap, among many others.

The program has subjectively been met with great enthusiasm since its inception in November 2012, and Drs. Gronowski and Burnham have collected information via survey to capture objective data on the program’s success. The data was published in June of 2018 in the Journal of Applied Laboratory Medicine and featured in the journal’s podcast as well.

Article:
http://jalm.aaccjnls.org/content/3/3/498

Podcast:
http://traffic.libsyn.com/aaccjalm/JALM_201811_Gronowski.mp3
Automated Syphilis Testing at BJH
by Neil Anderson, MD

On 1/23/19 the BJH core laboratory will transition manual serologic testing for syphilis to automated testing performed on the BioPlex 2200.

Syphilis testing at BJH has historically been performed using the traditional screening algorithm as follows:

1. **Screen (non-treponemal test)** Rapid Plasma Reagin (RPR) performed 24/7
2. **Positive RPR**
3. **Confirmation (treponemal test)** Fluorescent Treponemal Antibody (FTA) test performed Monday/Wednesday/Friday

As of 1/23/19 syphilis testing will be performed using the traditional screening algorithm as follows:

1. **Screen (non-treponemal test)** Automated Rapid Plasma Reagin (RPR) performed 24/7
2. **Positive RPR**
3. **Confirmation (treponemal test)** Automated Treponemal Antibody test performed 24/7

Transitoning to an automated methodology allows us to provide rapid results from both screening and confirmatory assays on a 24/7 basis. Additional benefits include the fact that automated testing is less prone to laboratory error and result variation based on human interpretation. Since we are adopting automated versions of both the RPR and treponemal tests, we will be able to continue offering testing utilizing the traditional syphilis screening algorithm. Positive RPR results will continue to be both titered and reflexively tested for treponemal antibodies.

Providers that wish to order both the RPR and treponemal antibody tests upfront will continue to be able to do so. This approach might be utilized when a diagnosis of tertiary or ocular syphilis is being considered as screening using RPR alone is less sensitive in these settings.

Another important consideration is that serologic testing may be negative in the setting of primary syphilis (isolated genital chancre). Although RPR testing should still be ordered when primary syphilis is suspected, results may be negative due to the lack of a fully developed immune response. As such, physicians are urged to interpret RPR results in the context of the entire patient presentation and consider empiric treatment if clinically indicated.

Testing will be performed on serum from blood drawn in red-top tubes. Automated RPR testing can be ordered in Epic using the “RPR, serum” orderable and automated treponemal testing can be ordered using the “Treponemal Antibody Blood” orderable.

If you have any questions, please contact Neil Anderson, M.D., Assistant Medical Director of Microbiology (314-362-1307).
On February 11, 2019, the HLA Laboratory implemented a newly validated flow crossmatch procedure, called Halifaster flow crossmatch (Hum Immunol. 2018;79:28-38), that has been widely adopted in many transplant centers in the US and Canada.

The main benefits of the Halifaster method include a lower false positive rate and a more rapid workflow that does not compromise testing quality or sensitivity.

Key updates include:
1) pretreatment of lymphocytes by pronase to reduce nonspecific antibody binding,
2) pooled negative sera from multiple patients used as the negative control to reduce variation,
3) incubation at room temperature rather than 4°C to reduce the binding of “cold”, clinically insignificant antibodies,
4) shortened assay times

An extensive validation study was conducted during the past 11 months in which hundreds of crossmatches were performed. Statistical cutoff values are now lower, the positive cutoff is now >17 Median Channel Shift (MCS) for T cells >23 MCS for B cells. Sensitivity of the flow crossmatch approaches 100% for donor-specific HLA antibodies (DSA) with mean fluorescence intensity (MFI) values >3000, as determined by the single-antigen bead assay. The T cell flow crossmatch is slightly more sensitive than B cell crossmatch for detecting class I DSA, while the B cell flow crossmatch can readily detect class II DSA. The false positive rate in the validation study was 1.4%. Historical, false-positive results by the existing BJH flow crossmatch method were correctly determined to be negative by the new Halifaster method. However, false-positive results may still occur with the Halifaster method. Such false-positive results may be caused by either non-HLA antibodies or autoantibodies and should be interpreted in conjunction with additional laboratory and clinical data.

The reporting format of flow crossmatch in EPIC remains unchanged except that pronase treatment and the revised cutoff values will be indicated in the clinical report. A brief description of the Halifaster method and its implementation date will be added to the disclaimer for your reference.

The HLA laboratory will continue to monitor the performance of the Halifaster flow crossmatch and evaluate feasibility of replacing the complement-dependent cytotoxicity (CDC) crossmatch with the Halifaster method as the default prospective crossmatch method.

If you have further questions regarding the Halifaster crossmatch, please contact the HLA Laboratory Supervisor, Donna Phelan, Associate Medical Director Dr. Bijal Parikh, or Medical Director Dr. Chang Liu.
Volume Matters: Best Practices for Blood Culture Collection

by Melanie Yarbrough, PhD

Detection of pathogens in the setting of bloodstream infection is a critical function of the clinical microbiology laboratory. Although many factors influence the yield of blood cultures, the single most important factor for successful pathogen detection is the volume of blood collected. Potential consequences of collection of an inadequate amount of blood include delayed time to blood culture positivity and falsely negative culture results.

Blood culture detection rate increases with the quantity of blood submitted. When bloodstream infection is suspected, 3 to 4 blood culture sets should be submitted within a 24 hour period. In adult patients, 20 mL of blood should be collected per blood culture set, which should be split equally between the aerobic (BACT/ALERT FA Plus, green top bottles) and anaerobic (BACT/ALERT FN Plus, orange top bottles) bottles. For children, the appropriate volume can be approximated by collecting 1 mL of blood per year of patient age.

An assessment of 1000 blood cultures from adult patients that were submitted to the BJH Microbiology Laboratory in 2017-2018 revealed that less than 5% contained lower than 2 mL of blood. Over 76% contained more than 5 mL of blood, while the remaining 19% contained 2-5 mL of blood. This is a marked improvement from 2016, in which 44% of cultures were submitted with < 2 mL of blood. These data are very encouraging, and we hope to see ongoing improvement.

For questions regarding blood culture collection, please contact Melanie Yarbrough, PhD (myarbro@wustl.edu) or Carey-Ann Burnham, PhD (cburnham@wustl.edu), Medical Directors of Clinical Microbiology or the Microbiology Laboratory at 314-362-3898.
Implementation of the BACT/ALERT VIRTUO Blood Culture System in Microbiology

by Carey-Ann Burnham, PhD

The BJH Microbiology Laboratory has recently implemented a new blood culture system, the bioMerieux BACT/ALERT VIRTUO (Figure 1). Similar to the previous blood culture system, the VIRTUO is a continuously monitored blood culture system and each blood culture set will include a pair of bottles—and aerobic (green top) and an anaerobic (orange top) bottle (Figure 2). Correct labeling of the bottles is essential (Figure 3, next page). In adults, 20 mL of blood should be collected for each blood culture set, and the volume should be distributed equally between the two blood culture bottles.

An advantage of the new system is that the blood culture bottles are plastic and thus can be sent to the laboratory via the pneumatic tube system. Blood culture bottles are loaded onto the VIRTUO system upon arrival in the microbiology laboratory, and are continuously monitored for up to 5 days for evidence of microbial growth. Positive blood cultures are processed in real time, and positive blood culture samples are analyzed using the microbiology laboratory’s Kiestra Total Laboratory Automation System.

Implementation of this new blood culture system was a huge team effort—including the laboratory and our partners in nursing and phlebotomy—at all the sites served by our central laboratory (including BJH, St. Louis Children’s Hospital, Parkland Hospital, Christian Hospital, and Alton Memorial Hospital).

For questions regarding the new system or this transition, please contact Carey-Ann Burnham, Medical Director of Microbiology (cburnham@wustl.edu) or the BJH Microbiology Laboratory at 314-362-3898.

Figure 1. Ribbon cutting ceremony for launch of the new instrument (Jennifer Schmidt, Technical Coordinator, and Elizabeth Robinson, Assistant Manager of Microbiology).

Figure 2. VIRTUO Blood Culture Bottles
Implementation of the BACT/ALERT VIRTUO Blood Culture System in Microbiology, cont.

Correct Label Placement

Figure 3. (a) Correct Label placement.

Incorrect Label Placement

Placing the label incorrectly over the Barcode, the Black Lot Number area, the fill-to-mark or the Barcode Pull Tab and into the Accession/Demographic area will obstruct critical reference points for the VIRTUO Imager.

Incorrectly placing the label in a horizontal position across multiple reference points will cause failure in volume to be recorded and the label will not be read.

Figure 3. (b) Incorrect label placement.
When the Lab Sees Red

by Mitchell Scott, PhD

When you receive a laboratory result with a comment such as “hemolyzed, result may be falsely elevated”, “hemolyzed, unable to report” or “icteric, result may be falsely decreased” do you ask, “How does the lab know this?” or, “How did the lab detect the hemolyzed or icteric nature of the sample?”

Both exogenous and endogenous constituents in a blood sample can interfere with laboratory tests. Exogenous interfering substances such as drugs, dietary supplements and IV fluids will not be discussed further in this article. The most common endogenous constituents that interfere with common laboratory tests are soluble hemoglobin from erythrocyte lysis (hemolysis), high bilirubin (icterus) and high concentrations of lipids (lipemia). These can interfere because many laboratory tests depend upon the detection of light absorption/transmission at particular wavelengths. If those wavelengths correspond to the “red” (hemolyzed) or “yellow” (icteric) spectrum, profound negative or positive colorimetric interferences can occur. Lipemic samples will scatter light and can affect many tests that measures light absorption/transmission to quantify the test analyte. In addition to spectrophotometric interferences, hemolyzed samples will falsely elevate test results for analytes that are present at very high concentrations in erythrocytes relative to blood such as potassium and LDH. Approximately 6% of the samples received in the BJH Core Laboratory have an endogenous interfering substance with the most common interference being hemolysis. From some areas of the hospital, hemolysis rates approach 20%; with the frequency of this problem, it imperative that the laboratory can identify samples that will produce inaccurate results.

The BJH core laboratory receives over 7000 specimen tubes daily and samples are centrifuged in a closed automated system making it impossible to identify hemolyzed, icteric or lipemic samples by manual visual inspection. Rather, the automated analyzers have channels dedicated to determining the presence and extent of hemolysis, icterus and lipemia of every sample before laboratory tests are completed. These are called “index” channels and provide a semi-quantitative value for soluble hemoglobin, bilirubin and lipids. The analysis for indices is performed by bichromatic light absorption where the primary wavelength corresponds to the peak absorption spectrum for hemoglobin (570 nm), bilirubin (480 nm) and lipemia (660 nm). All tests undergo extensive validation when first introduced including studies to determine the extent and direction (false increase or decrease) of an endogenous interference using samples spiked with varying amounts of hemoglobin, bilirubin or intralipid. From these data the amount of an interferrant that causes clinically significant changes in a test result is determined and rules are written in the laboratory information system to prevent results from being verified when the index value reaches a certain threshold. A comment is then appended indicating direction of the interference or that we are unable to report due to extreme interference.

So, the answer to the question “How did the lab know that?” is that: “red”, “yellow”, and “cloudy” samples are identified by an automated system and the extent and direction of the interference is determined by studies during initial method validation.