

The LAB Report

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MGHS REGIONAL REFERENCE LAB UPGRADES ITS TECHNOLOGY FOR SCREENING PAP SMEARS

Advancements in ThinPrep Imaging System achieve a higher level of certainty in cervical cancer screening

By *Jim LaJoie,*
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Marquette General Health System's Regional Reference Laboratory has greatly expanded its Pap smear processing capabilities for cervical cytology screens.

The reference laboratory has acquired a state-of-the-art, computer-assisted screening system that facilitates the performance of the ThinPrep Pap Test — a cervical cancer diagnosing screening method that has become

the gold standard for patient care.

The MGHS lab is the only institution in Upper Michigan to provide Image-Directed Cytology (IDC), and is the fourth institution in the nation to have both the T3000 Thin Prep Pap Processor and Imager.

Combining advanced screening technology with human expertise, the Imaging System provides digital enhancements of the ThinPrep Pap test — a modality that is significantly more effective than the conventional Pap smear for the detection of cervical lesions.

"We have acquired this

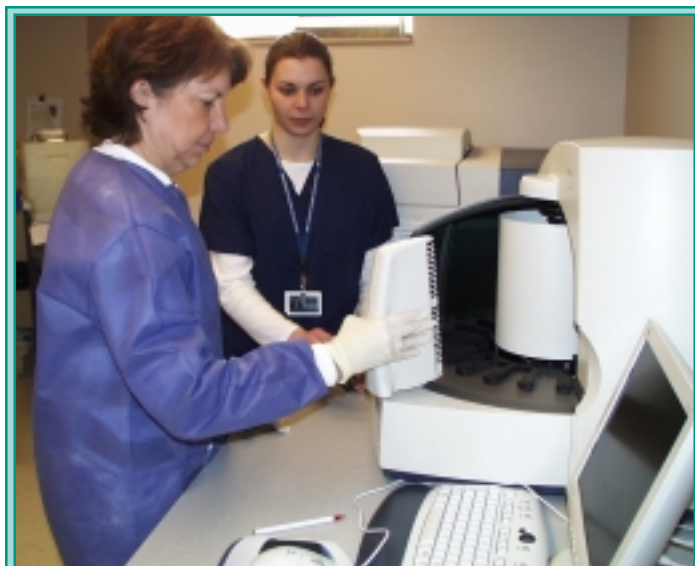
technology because physicians and patients we serve have grown to expect that we will provide them with the latest technological advances available in medicine," said John Rhoades, Laboratory Director at Marquette General.

The dual screening approach, Rhoades stressed, increases sensitivity and improves specificity over manual screening, further reducing the rate of false negatives. Clinical trials found a 39 percent reduction in false negative readings with the Imaging System.

"The Imager electronically marks the most significant fields of view on the pap slide (based on biological markers associated with abnormal cells, i.e., increased nuclear DNA content and cell-crowding)," said Cathy Bammert, Anatomic Pathology Section Head at Marquette General. "This enables our cytotechnologists to focus their time and interpretative skills on relevant cells."

Added Dr. John Weiss, a board-certified anatomic and clinical pathologist on staff at Marquette General: "Our regional reference laboratory is committed to providing our patients with the finest technology to detect early cervical disease. This system allows us to do that."

With IDC, a patient's slides are first processed on the ThinPrep Pap



Pam Carlson, a Cytology prep technician in the MGHS Regional Reference Laboratory, prepares a slide for computer-assisting imaging while cytotechnologist Jill Kimmes looks on. The MGHS reference lab has acquired an innovative screening system that facilitates the performance of the ThinPrep Pap Test. Marquette General is just the fourth institution in the nation to have both the T3000 Thin Prep Pap Processor and Imager.

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AABB SETS NEW REQUIREMENTS TO PREVENT BACTERIAL SEPSIS

The American Association of Blood Banks (AABB) and the College of American Pathologists (CAP) have developed new accreditation requirements as part of an effort to prevent bacterial sepsis associated with platelet transfusions. The AABB Standard 5.1.5.1 listed in the 22nd edition of the Standards for Blood Banks and Transfusion Services, became effective on March 1, 2004. It includes developing methods to limit the inoculation of bacteria into blood components and to detect bacterial growth during storage of platelets at room temperature. The CAP Checklist Questions became effective back in December 2002, and is a Phase I requirement that an inspected facility have a bacterial detection method in place for platelets at the time of their inspection. (Phase II requirement is expected to become effective sometime this fall with the printing of new rules).

Bacterial contamination of blood components continues to be a significant problem. All blood components are susceptible to bacterial contamination, however, platelets are more susceptible due to their storage at room temperature. Platelet – transfusion - associated sepsis is now recognized as the most frequent infectious complication of transfusion therapy, surpassing greatly the incidence of transfusion associated viral transmission.

Bacterial contamination of platelet products is estimated to occur in approximately 1 in 1000 to 1 in 3000 platelet units. The incidence of severe episodes of transfusion associated bacterial sepsis is estimated to occur in about one-sixth of

By Sue Morris, MT (ASCP)

contaminated platelets transfused. It is very likely that many instances of clinically significant occurrences of bacterial contamination of platelet components are neither recognized or reported due to the failure to associate chills, rigors, and/or fever (common signs and symptoms of platelet transfusion) with possible bacterial contamination.

The Center for Disease Control's (CDC) BaCon study (1998-2000) attempted to estimate the incidence of clinically significant bacterial contamination associated with the transfusion of platelets. This study used strict criteria that required the isolate be detected in both the recipient and the blood component and that a clinically defined transfusion reaction be recognized. The BaCon study had confirmed reports of bacterial contamination at rates of 162-288 per year, with fatality rates of 5-18 per year. These results are thought to have underestimated the incidence of contamination, particularly when compared to hospital based surveillance methods. John Hopkins conducted their own study and estimated platelet bacterial contamination with resulting post transfusion sepsis at 420 per million random platelets transfused, and 75 per million single donor platelets transfused with fatality rates of > 100 deaths per year.

The AABB Standard 5.1.5.1 requires a combination of strategies to first limit the initial introduction of bacteria into the blood component

and second to detect any subsequent growth of bacteria during room temperature storage. Bacteria can contaminate a blood component if:

1. The donor has an undetected bacteremia at the time of donation.
2. There is skin contamination at the site of the venipuncture.
3. There has been a defect in the integrity of the storage bags.

Donor screening may detect a donor with bacteremia if the donor's temperature is greater than 99.5°F (37.5°C) at the time of the donation. The prevention of contamination at the site of venipuncture is possible with good aseptic techniques for preparation of the site. Site preparation begins with a minimum 30-second vigorous scrub, with varying directions of a 3 inch diameter of skin with an Iodopropyl-PVP scrubstick. A second scrub is done with a Cliniswab Providone – iodine USP swabstick making concentric circles spiraling outward from the center of the site, and this is allowed to stand for at least 30 seconds. Venipuncture is then performed without palpating or touching the sterile area. (AABB Standard 5.6.2 which is referenced in 5.1.5.1 refers to venipuncture site preparation in the 22nd edition of the Standards).

A recently developed method for prevention of the introduction of bacteria through skin contamination involves the use of a diversion pouch during blood collection. Apheresis blood collection kits now provide an attached diversion pouch which collects the first 32 mls. of donor blood. This prevents the venipuncture skin plug from entering the storage bags and the diverted blood can then be used for the required donor testing. (ABO/Rh, Antibody screen, and Transmissible Disease testing). There are currently no diversion processes available with whole blood collection bags due to problems in manufacturing. There are plans to have them available on the market in the near future. These improved collection techniques along with a nationwide increase in apheresis platelet collections should help limit the initial introduction of bacteria into platelet components, thus meeting the first requirement of Standard 5.1.5.1.

Detection of bacterial contamination in stored platelet components is the next requirement that must be met. The method of detection should be practical, specific, sensitive, rapid, and cost effective. Culture methods for bacterial detection achieves the best combination of sensitivity and specificity. Two culture methods, the BacT/ALERT (Biomérieux Inc., Durham, NC) and Pall Bacterial

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THINPREP ADVANCEMENTS

Processor that produces uniform, easy-to-read slides. The slides are then stained using a special, preformulated stain that allows measurement of cellular DNA content.

Once stained, the slides move to the digitized screening process. There, the Imaging System digitally scans every cell and cluster on the patient slide and electronically marks "areas of interest" for a cytotechnologist to review.

The cytotech uses a robotic scope to review the marked fields of interest. If abnormal cells are identified in any of the fields of view, the cytotech will screen the entire slide before referring it to a pathologist for final review.

According to Bammert, the new technology

allows Marquette General to accept additional Pap smear cases from all over the country.

"We provide rapid turn-around-time and highly competitive prices," said Bammert, adding that most insurance companies reimburse for the ThinPrep Pap Test with Imaging.

A fully accredited, full-service clinical laboratory, the MGHS reference lab features an ASCP-certified staff of cytotechnologists and pathologists to provide the highest quality cytology services. The lab has served clients nationwide for several years.

Women are encouraged to ask their physician to request a Thin Prep with Imaging test at their next yearly gynecological exam.

For more information about the ThinPrep Imaging System available at Marquette General, call Bammert at 225-7097, or 1-800-562-9753, ext. 7097. She can also be contacted via email at cbammert@mgm.org.

HELP! IS IT ACA, APA, APS, OR LA?

By SueAnn Hampton, MT (ASCP)

Antiphospholipid antibodies are a family of immunoglobulins that act in response to phospholipid-binding proteins. They are IgG, IgM, IgA or a mixture, but they differ in their shape, and the way they react to phospholipids. This difference is important in laboratory testing. The family includes: **APA** - antiphospholipid antibodies (antiphosphatidyl serine, and others);

and the two most common, known as: **ACA** - anticardiolipin antibodies, **part of the APA group**, since cardiolipin is a phospholipid, and **LA** - lupus anticoagulants, originally detected in people who had lupus, the name now a misnomer.

APA's primary binding structure is lamellar and they conform in a bilayer configuration. Some **bind cardiolipin, an anionic phospholipid**, hence the name ACA. Usually, though, **they bind proteins** (e.g. beta-2glycoprotein I, prothrombin, and annexin V) not phospholipids (making APA a misnomer, too). **APAs are detected only with an ELISA method of testing** where ELISA plates are coated with negatively charged phospholipids (i.e. cardiolipin, phosphatidyl serine, and phosphatidic acid).

LA's binding structure is hexagonal. Phospholipids change to a hexagonal configuration after membrane trauma or injury. **LA's** recognize this shape and **bind the protein anionic phospholipid complexes** stopping them from serving as surfaces upon which coagulation enzymes can interact to form fibrin. In short, **LA interferes with phospholipid-dependent clot based assays** (APTT, PT, Dilute Russell Viper Venom test, and Kaolin Clotting Time) and prolongs their test times.

APA and LA antibodies can be alloimmune or autoimmune and are part of a well-known autoantibody syndrome called **APS (Antiphospholipid Syndrome)**. The syndrome is frequently associated with venous thrombosis commonly manifested as thrombophlebitis, DVT, and PE, and arterial disease such as migraines, TIAs, memory loss, strokes, peripheral artery disease, angina, and MI. In women, APS is implicated in recurrent fetal loss (15%), fetal growth retardation, fetal thrombosis, prematurity, and infertility. Other diseases antiphospholipid antibodies may be seen in are:

- autoimmune SLE (30-50% of patients) or RA
- alloimmune antibodies that arise from drug exposure: Chlorpromazine (antipsychotic) – most common, Procainamide (cardiac anti-arrhythmic),

Hydralazine (anti-hypertension), Quinidine (anti-fever), Phenytoin (anti-epileptic) certain antibiotics.

- alloimmune antibodies from bacterial or viral infections (childhood infections, adult common colds), syphilis, and HIV positive patients (up to 50%)

- lymph disorders (malignant lymphoma, certain leukemia's)

- and, should this surprise, when there is no underlying disease (1-2 %)

The approach to lab testing is divided into evaluating for LA and ACA as most of the alloimmune antibodies appear as ACA. **ACAs are transient** and have little clinical consequence, although a few have been implicated in thrombosis. They're reported as specific isotopes (IgG, IgM, IgA) but it's still debatable if these isotopes are associated with different patterns of disease. Keep in mind, testing for ACA alone could miss an APA that doesn't react with cardiolipin. A few patients require evaluation for other phospholipid antibodies, such as phosphatidylserine or phosphatidic acid.

Where ACAs are transient, **LA's are typically chronic** and are often associated with thrombosis. It's important to repeat tests at least 6 wks after a positive finding to rule out transient alloimmune antibodies and confirm the more persistent autoimmune ones. **To officially be a LA**, tests must be positive on at least two occasions more than 3 months apart. Here are a few caveats to keep in mind:

- Since the LA and APA relationship is still not fully understood, and there's **no one test** to screen for antiphospholipid antibodies, it's recommended to test for both.

- Approximately 60% of patients possess both APA and LA antibodies.

- ACA is 5 times more common than LA but patients with LA have a higher risk for thrombotic events than those with ACA (risks are 11.1 and 3.2 respectively).

- APS occurs in up to 30% of individuals with chronic antiphospholipid antibodies. Two-thirds suffer from thrombophlebitis, DVT, and PE, while the remainders have arterial thrombotic events. Levels of APA may fall during a thrombotic episode.

- A small portion of individuals with APS may have thrombocytopenia and hypoprothrombinemia and may experience bleeding.

- LA is one of the most common causes of a prolonged APTT second only to heparin.

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PREVENTION OF BACTERIAL SEPSIS

Detection System (BDS – Medsep Corp., Lovina, CA) have been approved for QC testing of leukocyte reduced apheresis platelets and are currently licensed by the FDA. Sampling of the platelet product takes place after a 24 hour holding period, allowing for any bacteria in the product to grow to sufficient levels for detection. The culture is then incubated for a minimum of 24 hours before no growth can be reported out and the release of the product can take place. Studies have shown that these two culture methods detect the majority of contaminated units yet the 24 hour holding period and the 24 hour incubation time required causes a significant delay in the accessibility of the platelet product.

Other methods of bacterial detection use observation techniques to either detect stained bacteria itself or detect metabolic changes in the platelet product caused by bacterial growth. These testing methods reflect the platelet condition immediately prior to transfusion and also use smaller volumes which make them appropriate for use on whole blood derived platelets as well as apheresis platelets. These methods include swirling, staining, and measuring glucose and pH levels by dipstick or automated methods.

The swirling method involves visually checking the platelet product through a light source for a shimmering swirling appearance caused by the discoid morphology of the platelets. As the acidity of the platelet unit rises due to bacterial growth, it inhibits the metabolic function of the platelets, and they lose their discoid shape and round into spheres. This method is very subjective, has low specificity, and is not an approved method for detection except in emergency situations.

Staining methods used to detect bacteria can be relatively inexpensive but require additional staff to perform and read the slide. Gram Stain, Wright Stain, and Acridine Orange Stain can all be used to stain bacteria. The sensitivity of these staining methods are only slightly better than the reagent dipstick method with the Acridine Orange Stain being most sensitive and the Wright Stain the simplest to perform and interpret. This method is best

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PREVENTION OF BACTERIAL SEPSIS

performed at the time of issue for maximum sensitivity, but may cause significant delays depending on staffing and the stain used.

The method used for bacterial detection in platelets here at Marquette General Health System is the reagent dipstick method. The growth of bacteria in a platelet product causes the consumption of glucose and the production of organic acids. Measurement of the glucose and pH levels of a platelet product can therefore be used as a bacterial detection method. Measurement can be done either by dipstick (reagent stick) or automated techniques. Most facilities chose the dipstick-reagent strip method because it is the simplest to do, its sensitivity is only slightly less than the staining methods, and it can be done right at the time of issue. Testing performed at other institutions as well as our own facility, determined that a glucose concentration of < 250 mg/dl or a pH level of < 7.0 was indicative of possible bacteria contamination. Inoculation studies validated the detection of contamination by dipstick method (with blood culture confirmation) of platelet units inoculated with *S. epidermidis*, *K. pneumoniae*, *S. aureus*, and *E. coli*. In addition, non-inoculated platelets that were at the end of their 5 day storage period and tested negative by dipstick method were confirmed negative by culture.

A platelet product that fails bacterial screening cannot be released for transfusion or distribution, must be submitted for culture, and then discarded. We also quarantine any other blood products from that donation, and their final disposition is determined by the Medical Director after review of the culture results.

Technologies that could in the future replace what is currently available for bacterial detection include cytometry, nucleic acid testing, dielectric phoresis, and immunoassays. One promising system, the Verax PGD (Pan Genera Detection) looks a lot like a rapid pregnancy test and is simple to perform. Another focus is on methods such as pathogen inactivation, bacterial decontamination, and growth inhibitors. These efforts to limit the introduction of bacteria and detect its presence in platelet products will not only significantly improve the safety of these products, but may, in the future, allow for the extension of the platelet outdate to seven days which will also help with the supply issue that has plagued blood centers and hospitals nationwide.

BAYER bDNA TESTING

By Gail Koski

In June of 2004 the main campus laboratory at Marquette General Health System instituted testing for the direct quantitation of Human Immunodeficiency Virus Type 1 (HIV-1). The assay can quantitate HIV-1 virus RNA over the range of 75 to 500,000 copies per milliliter allowing the physician the ability to monitor the "viral load" in previously established HIV-1 infected individuals. This assay is not intended for the primary detection of HIV infections but rather provides an assessment of the patient's extent of infection prior to initial therapy and also is instrumental in monitoring the efficacy of the therapy as it progresses.

Testing is performed using the Bayer System 340 bDNA analyzer and the Versant HIV-1 (bDNA) assay kit. Branched DNA

(bDNA) is a nucleic acid technology that was designed specifically to quantify nucleic acids, either DNA or RNA.

In bDNA technology, probes are attached directly to the target nucleic acid sequence (HIV-1) and branched DNA molecules are added to each target nucleic acid molecule to boost the signal that the target sequence delivers. This technology is referred to as signal amplification.

The amount of signal produced is directly proportional to the amount of target nucleic acid present. This allows for direct quantification that is precise, accurate, and reproducible. We are pleased to be able to offer this test at Marquette General Health System.

NEW LH750 HEMATOLOGY ANALYZER

By Michelle Toyras, MT (ASCP)

MGH Laboratory is committed to providing the highest quality services by using cutting edge technology and state-of-the-art instrumentation. In keeping with this commitment, the Coulter LH750 was chosen as our new primary hematology analyzer. It has replaced the Gen-S, but operates under the same general principles as its predecessor.

The Coulter method counts and sizes cells by detecting and measuring changes in electrical resistance when a cell in a conductive fluid goes through a small aperture. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between two electrodes, one on each side of the aperture. This causes an electrical pulse that can be counted and sized. Three separate apertures will perform this function and an average will be taken to provide the RBC, WBC, and platelet counts. WBC differential analysis and classification is accomplished through three measurements. Low-frequency current measures volume, high-frequency current senses cellular content by changes in conductivity, and laser light characterizes cellular surface, shape and reflectivity.

Reticulocytes are counted by being stained, incubated, and then differentiated from mature red cells and other cell populations by light scatter, direct current measurements, and opacity characteristics. Hemoglobin is calculated from blank and sample readings. Other results and indices are either calculated or derived from the histograms.

With the LH750, the Beckman Coulter AccuGate algorithm uses adaptive contouring methods designed for finding optimal separation between overlapping clusters of data. The technique also uses multidimensional data to distinguish the presence of even the faintest subpopulations. The 2-D and 3-D full-color data plots that are derived from this information are outstanding. Technologists can use the scatter plots to determine if a normal distribution is present, or if abnormal forms and interfering cells/particles are identified.

We are very pleased with this new analyzer. The instrument itself, ease of operation, the software, and the accuracy of results are excellent. You can be assured that your hematology work is in good hands at the MGH Laboratory.