

The LAB Report

Published quarterly for clients of Marquette General Health System Laboratory services

Spring 2002

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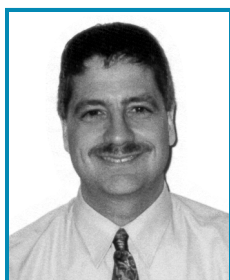
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FLOW CYTOMETRY at MGHS

By Martin M. Renaldi, MT (ASCP)

The MGHS main campus laboratory is pleased to announce the arrival of the latest in cutting-edge technology – analytical flow cytometry. In October of 2001 our laboratory installed Beckman Coulter's Epics XL-MCL flow cytometer. The capabilities of this instrument are immense and our expectations are to use it to its fullest capacity on an incremental basis.



Martin Renaldi

Although flow cytometry has been around for a number of years, it has been only recently that the technology has been gaining popularity in the clinical laboratory. This is due in part to the fact that not many technologists have been exposed enough to the process to feel comfortable in performing it. There is a significant learning curve associated with the technology; however, with time and experience, workshops, and some late night reading, the science of flow cytometry will prove to be extremely interesting and beneficial to your laboratory environment.

Probably the most common application of clinical flow cytometry involves cell surface marker assessment through the use of antigen specific fluorescent reagents. When appropriately selected and applied, the markers, called Cluster Designations or CD's, 'tag' the surface of the cell. The cells with known CD tags are

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BIOLOGICAL WARFARE and ANTHRAX

By Gigi Renaldi

B.S. M.T., M.S. Microbiology

ANTHRAX. Due to unfortunate recent events, anthrax is now a household word. In the past, anthrax has been referred to as a *possible threat* if it was ever made into a biological weapon. Troops were vaccinated against anthrax during the Gulf War and now the threat of attack by a biological weapon has become reality. Our post offices, government offices, and the general public are on constant alert of being exposed to anthrax.

The Centers for Disease Control (CDC) have a complete protocol for agents of biological warfare that can be found at their website, www.bt.cdc.gov. As laboratorians, we are part of the "first response" by health care workers when a covert attack by biological agents has occurred. Health care workers are the first to see the symptoms, culture the organism, or diagnose the agent.

Some of the biological agents of concern are small pox (*variola major*), anthrax (*bacillus anthracis*), plague (*yersinia pestis*), tularemia (*francisella tularensis*), botulism (botulinum toxin), and viral hemorrhagic fevers

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(filoviruses and arenaviruses). Why these agents specifically? They are stable as an aerosol form and can infect via the aerosol route. Civilians are also highly susceptible to these agents, which means a high morbidity and mortality rate. Some of them are spread by person-to-person contact and they are also difficult to diagnose and treat. In addition, these particular agents have been studied for biological warfare and some even previously developed for that purpose.

Anthrax is a disease caused by the bacteria *Bacillus anthracis*, a Gram-positive sporeforming bacillus. Anthrax is a zoonotic disease that is transmissible to humans through the handling or consumption of contaminated animal products. In areas where anthrax exists, herbivorous wildlife mammals and domesticated livestock are at highest risk for the disease. Animals can become infected with anthrax by grazing on contaminated land, eating contaminated feed, or drinking from contaminated water holes. *Bacillus anthracis* spores can remain viable in soil for many years. Although outbreaks are very infrequent in North America, anthrax has been reported in deer in Louisiana, Texas, up into the Midwest and among wood buffalo in the Northwest Territory of Canada.

Humans may contract anthrax by handling products or consuming undercooked meat from infected animals. Infection may also result from inhalation of *B. anthracis* spores from contaminated animal products such as wool or the intentional release of spores during a bioterrorist attack. Human-to-human transmission has not been reported. Three forms of anthrax occur in humans: cutaneous, gastro-intestinal, and inhalation.

Cutaneous infections occur when the bacterium or spore enters a cut or abrasion on the skin, such as when handling contaminated wool, hides, leather or hair products (especially goat hair) from infected animals.

A raised itchy bump or papule which resembles an insect bite appears in the beginning and then develops into a fluid-filled vesicle, which ruptures to form a painless

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The Clinical Laboratory Sciences Museum

(And a little lab history just for kicks!)

By Michele Toyras MT, ASCP

The Department of Clinical Laboratory Science at Northern Michigan University has some exciting news! The CLS Department has moved into a new state-of-the-art laboratory and offices in the new Seaborg Science Complex. They have created a museum display case outside of the new laboratory. This is a wonderful display that shows how far the profession has come over the years.

The CLS Department is looking for help from hospitals, alumni, and friends to contribute items for display in their museum. So get down on your hands and knees and clean out that old cupboard! You never know what you may find. Things to consider:

- Old equipment
- Old supplies and apparatus
- Old pictures of labs
- Pictures of previous graduating medical technology classes or personnel

Your institution's name will be displayed with your contributions and any pictures. For more information, please contact Lucille Contois at 906-227-1660 or

lcontois@nmu.edu.

While trying to dig up something I could donate to the museum, I thought I would also dig up some history on laboratory medicine. It is hard to specify an exact date for the beginnings of the clinical laboratory. The history of disease is as old as the history of mankind.

When searching for the first indications of laboratory medicine, writings from 1500 B.C. indicate that there was knowledge of intestinal parasites (tapeworms were probably somewhat of an enigma). Hippocrates, who lived between 460-370 B.C., left behind writings that showed he knew of diseases such as malaria and tuberculosis.

During the middle ages, the best indication of the presence of diabetes was sweet tasting urine that seemed to attract ants. It makes me wonder who the first person was to drink urine and proclaim,

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Michele Toyras

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Flow cytometry

then passed through a laser beam that 'excites' the fluorescent dyes to higher wavelengths. This 'Stokes shift' is the leading issue in identifying the lineage of the questionable cell population.

The laser beam of the flow cytometer also has the ability to measure standard cellular characteristics such as size, granularity, and content by simply passing through the laser aperture without any pre-treatment. This capability is of course the basic concept of most standard laboratory hematology analyzers.

The flow cytometer has other significant capabilities as well, such as DNA tagging (the detection of the presence of

cellular DNA) and tumor cell ploidy analysis for the assessment of cancer cell populations.

Currently our laboratory is in the process of implementing T and B cell surface marker testing (TBSMs) as well as a method to detect residual WBCs (rWBCs) in all of our leukocyte-reduced blood products. Once these tests are on board our attention will be directed to the evaluation of leukemia/lymphoma immunophenotyping and tumor cell DNA ploidy analysis.

For more information into this exciting new technology and the progress of our endeavors, please call the MGHS main laboratory at 906-225-3050 and ask for me, Martin Renaldi. We look forward to serving you.

STATISTICALLY SPEAKING

Bordetella Pertussis

By Michele Toyras MT, ASCP

Pertussis, or whooping cough, is endemic throughout the world. It is generally a disease of infants (50% of cases occur in children less than one year old). Pertussis is serious in children under the age of two.

It is said that the disease is troublesome but rarely serious in older children and adults, except in the aged. However, I know of one lady who would call it more than troublesome. Yes, indeed, one of our very own was stricken down with this nasty illness

back in September, and is still coughing to this day. So what are the chances of catching this extremely contagious illness?

- In the U.S., 5,000-7,000 cases are reported each year.
- The incidence of pertussis has increased steadily since the 1980's. Since 1990, peaks have occurred every 3 to 4 years.
- The highest incidence since 1967 was reported in 1996, when 7,796 cases of pertussis were reported. In 1967 there were approximately 2.9 cases per 100,00 population.
- According to the CDC, the incidence has remained relatively stable among children younger than 5 years, most of whom are protected by vaccination.
- In contrast to 1990-1993, during 1994-1996, the average incidence among persons 5-9 years, 10-19 years, and 20 years or older increased 40%, 106%, and 93%, respectively.
- Pertussis is highly contagious, with up to 90% of susceptible household contacts developing clinical disease following exposure to an index case.

When analyzing national data and statistics, it is important to understand that

valid statistics cannot be compiled unless there is an improvement in diagnosis and reporting. One possible explanation for what appears to be an increase in incidence may simply be increased reporting.

So as lab professionals, what can we do to ensure accurate data? We can ensure that specimens are collected and trans-

Guidelines for optimal collection and transport must be followed, otherwise there is little hope of recovering the organism in culture.

ported properly by educating physicians and other health care professionals. We can also ensure that all isolates are reported to the Health Department.

Bordetella is a fastidious organism that requires specific media for transport and culture. Guidelines for optimal collection and transport must be followed, otherwise there is little hope of recovering the organism in culture.

The traditional method for obtaining a culture specimen was for the patient to cough directly onto the culture plate or for the physician to obtain a nasopharyngeal specimen that was transported in Regan-Low media. But because culture methods offer little sensitivity, MGHS has stopped performing Bordetella cultures and now sends all specimens to Mayo for the rapid PCR Bordetella assay. PCR offers a much higher sensitivity and specificity and provides a faster turn-around time in comparison to the culture method.

For collection, use a flexible wire swab to collect a specimen from the posterior nasopharynx. Place the swab in an Amies with charcoal sheath transport. Specimen can be kept ambient or refrigerated.

Feel free to contact Don St. Amour at 906-225-3050 if you need further information about our protocol. Also, the CDC (www.cdc.gov) and WHO (www.who.org) have information regarding symptoms, treatment, vaccination, and epidemiology.

Meet a Member of Our Staff

By Michele Toyras MT, ASCP

Meet Dave

Brown, our Laboratory Employee of the Quarter. Dave has been with us since 1989, working in our core lab on the midnight shift. He



Dave Brown

has an associate degree in Technology and a bachelor of science degree in Biology, both from Northern Michigan University. Before he came to us, he worked at Southwest Veterinary Diagnostics in Arizona, Consultants Laboratory Inc. in Wisconsin, and at Baraga County Memorial Hospital. Dave likes to go camping, fishing, and attend sporting events. When asked what his favorite sport was, he replied, "Go Pack!" We are very proud to have Dave as a member of our team.

Fly Our Flags High!

By Gigi Renaldi

B.S. M.T., M.S. Microbiology

Everyone is getting into the spirit of patriotism due to the attack on our beloved country and flags are being displayed more than ever! Did you know that there is proper etiquette for flying the flag of the United States of America?

Here are just a few flag-flying tidbits that may interest you. Did you know that when a flag is displayed 24 hours a day, it needs to be properly illuminated? If you need to retire an old flag, you should contact your local VFW Chapter to help you properly dispose of your flag, usually by ceremonial burning. The flag should never be used as wearing apparel, bedding or drapery. The flag should never be displayed with the union down, except as a signal of dire distress in instances of extreme danger to life or property.

Properly fly your flags high and be proud of our country. Visit www.ushistory.org/betsy/flagetiq.html for many, many more flag-flying tips!

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CLS Museum

“Wow! That’s mighty sweet tasting! Hurry and get the ants!” I can honestly say I would not have chosen this profession back in those days. It wasn’t until 1848 when the first quantitative urine test was performed by Hermann von Fehling.

Until the early 1920’s, most of the “lab work” was still being performed by physicians. Around this time, the role of the pathologist and the clinical lab began to be more prominent as hospital size and utilization grew. The American Board of Pathology was formed in 1936 and defined the requirements of the clinical pathology specialty.

The medical technology that developed during World War II had a significant effect on the advancement of laboratory medicine. Advances such as diagnostic radioisotopes, exfoliative cytology, molecular biology and fluorescent studies were introduced.

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ANTHRAX

ulcer (called an eschar), with a characteristic black necrotic area in the center.

To collect a specimen, soak two dry sterile swabs in vesicular fluid from an unopened vesicle. In the eschar stage, rotate two swabs beneath the edge of the eschar without removing the eschar. Toxins released by *B. anthracis* cause pronounced edema, and lymph glands in the area may also swell.

With appropriate antibiotic therapy, the lesions become sterile within 24 hours. Approximately 20 percent of untreated cases of cutaneous anthrax result in death either because the infection becomes systemic or because of respiratory distress caused by edema in the upper thoracic regions.

The *gastrointestinal* form of anthrax may follow the consumption of contaminated meat from infected animals and is characterized by an acute inflammation of the intestinal tract. Initial signs of nausea, loss of appetite, vomiting, and fever are followed by abdominal pain, vomiting of blood, and severe diarrhea. The mortality rate is difficult to determine for gastrointestinal anthrax but is estimated to be 25-60 percent.

If possible, a stool specimen should be

Almost all clinical studies were performed manually in the 1950’s. Shockingly, there was little or no precautions taken for the handling of patient specimens. Blood and urine were treated as if they were water! Laboratory equipment consisted mostly of test tubes, beakers, Bunsen burners, and pipettes. Specimens were read on a spectrophotometer or colorimeter and results plotted on log sheets. Does anyone remember doing that in Clinical Chemistry class? Now I’m starting to feel old.

The 1960’s saw the beginning of government interest and participation in health care. Government involvement allowed for new clinical laboratory funding to arise. This allowed for money to be spent on new developments in instrumentation, giving rise to more advanced laboratories. When computers made their way into the clinical laboratory in 1969, it made a dramatic change in laboratory

collected and in later stages of the disease, blood cultures will grow *B. anthracis* especially if the specimens are collected before antibiotics are administered.

An anthrax vaccine for humans is licensed for use in the U.S. It is reported to be 93 percent effective in protecting against cutaneous anthrax and may also be protective against aerosol anthrax.

Inhalation anthrax results from inhaling *B. anthracis* spores and most likely follows an intentional aerosol release of *B. anthracis*. After an incubation period of one to six days (depending on the number of inhaled spores), disease onset is gradual and nonspecific.

Fever, malaise, and fatigue may be present



instrumentation. Automation and computers have greatly increased efficiency and eliminated many labor intensive and time consuming processes.

The imposition of DRG’s in 1983 ensured that more money would go to medicine, the hospital industry, and clinical laboratories. This made possible future studies, new instrumentation, and an increase in health care.

Automated instruments in the 1970’s and 1980’s allowed for faster turn-around time, discrete sampling and random access. This, in turn, provided more flexibility, accuracy, and versatility.

Where will we be in 10 years? 20 years? With all of the advances in areas such as molecular biology and flow cytometry, it will be exciting to see what lies ahead. Will phrases like “by PCR” be archaic? Whatever the future may bring, let’s all just be thankful we will never again mouth-pipette!

initially, sometimes in association with a nonproductive cough and mild chest discomfort. These initial symptoms are often followed by a short period of improvement (ranging from several hours to days), followed by the abrupt development of severe respiratory distress with labored breathing, perspiration, high-pitched whistling respiration, and bluish skin color.

Shock and death usually occur within 24-36 hours after onset of respiratory distress, and in later stages, mortality approaches 100 percent despite aggressive treatment. A sputum specimen should be collected for culture and Gram stain, along with blood cultures. Again, blood cultures are more reliable in the later stages of disease (2-8 days post exposure).

How do we protect ourselves against anthrax? An anthrax vaccine for humans is licensed for use in the United States. It is reported to be 93 percent effective in protecting against cutaneous anthrax and animal studies suggest that the vaccine may also be protective against aerosol anthrax. The anthrax vaccine is distributed by BioPort Corporation in Lansing, Michigan. Since anthrax is in the news frequently, we’ll be the first to know when the vaccine becomes available to us.