Welcome Letter from our Deputy Director of Administrative and Support Services

by Jennifer L. Newman, MPH

It is my pleasure to introduce the re-launch of the Maryland Public Health Laboratory Newsletter, formerly known as the Critical Link. Our Newsletter is a natural and consistent extension of our renewed commitment to communications, aiming to reach a broader audience through multiple platforms, such as Facebook and other internet media. New features of our newsletter will include broader spectrum stories that communicate the substantial public health impact the Department of Health and Mental Hygiene’s Laboratories Administration has on you. We are also eager to inform you about our future activities.

(Continued on page 2)
Welcome Letter from our Deputy Director

Baltimore has been the home of the Maryland State Public Health Laboratory since its inception in 1898, 115 years ago. As I write this, the construction team recently completed the concrete superstructure for the six laboratory floors and two mechanical penthouse floors. The exciting construction progress can be followed by visiting the Laboratories Administration's website (http://dhmh.maryland.gov/laboratories/SitePages/Home.aspx). We will also be providing relevant updates in our newsletter. The building is still tentatively set for completion in May 2014. The 235,000 square foot facility is a $170 million project, located in the Science and Technology Park at Hopkins (north of the Johns Hopkins Medical Institutions campus). The project is the fruition of a partnership between the Maryland Economic Development Corporation, Forest City – New East Baltimore Partnership, and the Maryland Department of Health and Mental Hygiene. The new state-of-the-art public health laboratory will provide Maryland with the capacity to meet current and emerging public health demands, including response to bio-terrorism, chemical terrorism, or radiological events. The new laboratory will provide a 29 percent increase in usable space over our current facility. It will also feature cutting-edge technologies and the flexibility to incorporate future technologies to meet the changing needs and laboratory science of the future. We have occupied our existing facility since 1974, so the planning is a very significant undertaking but one in which we are thrilled to be participating.

East Baltimore is a deliberate and burgeoning location for the new Maryland Public Health Laboratory. The construction project is employing members of East Baltimore and the surrounding Baltimore City community, through all phases of development. Once we are operating in our new location, we are eager to expand our commitment to public health through educational public health lessons in the classroom setting, and positive visibility in the community. The Laboratories Administration will be encouraging careers in science through formalized channels, such as the Maryland Business Roundtable for Education. We are equally eager to attract and retain young scientists in a cutting-edge public health science laboratory.

Moving forward, the Maryland Public Health Laboratory Newsletter will be issued on a quarterly basis. We encourage our readership to subscribe via e-mail at mdphl.newsletter@maryland.gov, and also provide feedback at the same address. We hope that you enjoy our revamped issue. We are excited to bring it to you.


Unusually High Number of Pertussis Cases in Maryland in 2012

145 laboratory-confirmed cases compared to 34 cases for the same time in 2011

Bordetella pertussis, the agent of pertussis or whooping cough, is fastidious, aerobic gram negative coccobacilli. It requires special media for isolation and was described by Rodet and Gengon in 1906. The organism is a strict human pathogen, and no animal or insect source or vector is known to exist.

Pertussis cases in Maryland are unusually high. So far this year, Maryland has 316 confirmed and probable pertussis cases (compared to 103 for the same time period in 2011) of which 145 are laboratory-confirmed compared to 34 cases for the same time in 2011. (Data gratefully provided by Kurt Seetoo and Tara Thallmayer from the State of Maryland DHMH Center of Immunization).

B. pertussis produces multiple antigenic and biologically active products including pertussis toxin, filamentous hemagglutinin, fimbriae, tracheal cytotoxin, and adenylate cyclase, among other factors. Collectively, these virulent determinants are responsible for the clinical feature of pertussis disease, and an immune response by the host following infection which does not appear to be permanent.

The disease pertussis is an acute, primarily toxic-mediated, respiratory infection. Once the bacteria attaches itself to the cilia of the respiratory epithelial cells, it produces toxins that paralyze the cilia and cause inflammation of the respiratory tract, which ultimately interferes with the clearing of the pulmonary secretions.

Other species of bordetellae, including B. parapertussis and, less commonly, B. bronchiseptica or B. holmesii, are associated with cough illness. The clinical presentation of B. parapertussis can be similar to that of classic pertussis. B. parapertussis, however, does not secrete the pertussis toxin and generally causes less severe infection. Co-infections of B. pertussis and respiratory syncytial virus are observed frequently in infants.

The disease is transmitted from person-to-person through large respiratory droplets generally by coughing or sneezing, with an incubation period ranging between 4-21 days (commonly 7-10 days).

Patients with pertussis go through three phases (stages) of illnesses. The first stage, the catarrhal stage, is characterized by the insidious onset of coryza (runny nose), sneezing, low grade fever, and occasional cough, with this cough gradually becoming more severe. This phase usually lasts one to two weeks. The second, the paroxysmal stage, is characterized by numerous and rapid coughs due to difficulty expelling thick mucus from the tracheobronchial tube. It is during the catarrhal and early paroxysmal phases of the disease that patients are most infectious and can remain infectious for more than six weeks. The third, or

(Continued on page 4)

Summary of Diagnostic Tests for Pertussis

Compiled by CDC’s Pertussis and Diphtheria Laboratory

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Optimal Timing</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>12–60%</td>
<td>100%</td>
<td>&lt;2 weeks post-cough onset</td>
<td>Very specific (100%)</td>
<td>Low sensitivity; 7-16 day delay between specimen collection and diagnosis</td>
</tr>
<tr>
<td>PCR</td>
<td>79–99%</td>
<td>86 – 100%</td>
<td>&lt; 4 weeks post-cough onset</td>
<td>Rapid test; more sensitive than cultures; organisms do not need to be viable positive post-antibiotics</td>
<td>Not FDA approved tests or standardization; potential for false positives; DNA cross-contamination can be problematic</td>
</tr>
<tr>
<td>Paired1 Sera</td>
<td>99 – 92%</td>
<td>72 – 100%</td>
<td>At symptom onset and 4-4 weeks later</td>
<td>Effective indication of mounting antibody titers</td>
<td>Late diagnosis; no FDA approved tests or standardization</td>
</tr>
<tr>
<td>Single1 Sera</td>
<td>36 – 76%</td>
<td>99%</td>
<td>At least 2 weeks post-cough onset, ideally 4-8 weeks post-cough</td>
<td>Useful for late diagnosis or post-antibiotics</td>
<td>Not FDA approved test or standardization; possibly confounded by recent vaccination; diagnostic cut-off not validated</td>
</tr>
</tbody>
</table>

1 Not part of CDC/CSTE case definition (Exception: MA single point ELISA assay)
2 Sensitivity and specificity values obtained from Wendelboe and Van Rie, 2006
3 Data currently being validated at CDC (except paired sera)

Table 1. from the APHL pamphlet “What’s All The WHOOP About?” http://www.aphl.org/aphlprograms/infectious/Documents/Pertussis_Brochure-Final3.pdf
convalescent, stage of the disease is characterized by gradual recovery, and the cough becomes less paroxysmal and disappears in two to three weeks.\(^2\)

Patients with pertussis are highly infectious and individuals are considered infectious until they have completed five days of appropriate antibiotic treatment.

Prior to the 1940s, pertussis was a major cause of morbidity and mortality among children in the United States. The introduction of a whole cell pertussis vaccine (containing killed cells of \(B.\) \(pertussis\)) in the 1940s resulted in a dramatic decrease in the number of infections and deaths among vaccinated individuals. Side effects to the use of whole cell vaccine (seizures, pain at the injection site, fever) lead to the development and replacement of this vaccine with acellular pertussis vaccine. The acellular pertussis vaccine contains five different components of the bacterial virulent factors and includes pertussis toxin (PEX), pertactin (Prn), filamentous hemagglutinin (FHA), and fimbriae 2 (Fim2) and fimbriae 3 (Fim3) antigens. Initially, the acellular vaccine was licensed in the early 1990s as part of the fourth and fifth booster series. In 1997, all five doses of the childhood pertussis vaccine series were using the acellular vaccine structure. In 2005, a single dose adolescent and adult booster was recommended.

Waning immunity over time, genetic changes among \(B.\) \(pertussis\), and the fact that the components used in the acellular vaccine came from isolates circulating in the 1950s which are different from the currently circulating strains, may provide some explanation to the resurgence of pertussis worldwide and in the United States.\(^3\)

The laboratory diagnosis of pertussis can be established by culture, molecular, or serological methods (see Table 1).

Culture is considered the gold standard as it is the only 100% specific method for identification, because very rarely have patients been found to harbor \(B.\) \(pertussis\) without any symptoms.\(^4\) Since culture has excellent specificity, it is particularly useful for confirming pertussis diagnosis when an outbreak is suspected. Many other respiratory pathogens have similar clinical symptoms to pertussis and co-infections do occur. Culture is best done from nasopharyngeal (NP) specimens collected during the first two weeks of cough when viable bacteria are still present in the nasopharynx (see Fig. 1).

After the first two weeks, sensitivity is decreased and the risk of false-negatives increases rapidly. A properly obtained NP swab or aspirate is essential for optimal results. Throat swabs are less suitable since \(B.\) \(pertussis\) exhibits tropism for ciliated respiratory epithelium, which is not found in the pharynx. A study was conducted to compare throat and NP swab specimens for culture diagnosis and to evaluate relative sensitivity. Of 38 pertussis cases studied, 36 (95%) had positive NP culture, while only 16 of 36 (44%) had positive throat cultures. In that study, there were no cases of nasopharyngeal-negative, throat-positive cultures.\(^1\)

Samples should be taken before antibiotic treatment is started. Culture will become negative within five days of antimicrobial therapy. Cultures from untreated individuals will remain positive for up to three weeks.

Dacron swabs are recommended for specimen collection for both culture and Polymerase Chain Reaction (PCR). Best results are obtained by transporting specimens at room temperature the same day taken. If delays are expected (not transported the same day), place inoculated tubes into an incubator at 35-37°C. Cool transport of specimen suppresses overgrowth of other organisms, but decreases the number of Bordatellae by > 75%. Transport time should be as short as possible.\(^5\)

\(B.\) \(pertussis\) and \(B.\) \(parapertussis\) are slow growing microorganisms and it may take three to seven days and two to three days of incubation, respectively, before observing colonies on plated media. The turn-around time for culture results is at least seven to ten days from specimen receipt.

Due to the fastidious nature of \(B.\) \(pertussis\) and \(B.\) \(parapertussis\), commercial systems for the identification of GNRs are not reliable for identifying these species.\(^1\)

Although \(B.\) \(pertussis\) and \(B.\) \(parapertussis\) are resistant to most oral cephalosporins, they are in-vitro susceptible to penicillins, macrolides, quinolones, SXT, chloramphenicol and tetracyclines. Antimicrobial susceptibility testing is not standardized at this time.
(Continued from page 4)

Unusually high number of Pertussis cases in Maryland in 2012

The second method used in the diagnosis of pertussis is the PCR. PCR is a rapid test and has an excellent sensitivity for the detection of both B. pertussis and B. parapertussis organisms. PCR should be tested from NP specimens taken up to three weeks following cough onset, but may provide accurate results for up to four weeks of cough in infants or unvaccinated persons. After the fourth week of cough, the amount of bacterial DNA rapidly diminishes, which increases the risk of obtaining falsely-negative results.

The DHMH Molecular Biology Division working in conjunction with the Division of Public Health Microbiology developed, validated and implemented a Bordetella pertussis PCR assay in early 2006. This assay is based on the detection of two gene targets. A mobile insertion sequence (IS481) that is present in multiple copies in both B. pertussis and B. holmesii is used as the screening assay with an established sensitivity of one infectious organism per PCR reaction. Specimens that demonstrate the presence of the IS481 target sequences are then reflexed to a less sensitive but highly specific assay based on a B. pertussis chromosomal marker (BPERT 485), which when reactive confirms the presence of B. pertussis DNA in the specimen. PCR has the advantage of detecting both viable and non-viable organisms. Viable isolates are only recovered in culture from approximately 25-30% of the B. pertussis PCR reactive specimens that are detected by the DHMH Laboratories. The turn-around time for the majority of specimens being reported is within 48 hours of receipt.

A PCR-positive, but culture-negative result, is common at later stages of the disease in vaccinated patients, in patients who are under antibiotic treatment, or who have recently had close contact with patients with culture-proven pertussis.

The third method used in the laboratory diagnosis of pertussis is serological methods. Serologic testing could be useful for adults and adolescents who present late in the course of their illness, when both culture and PCR are likely to be negative. At this time, however, there are no FDA-approved serological diagnostic tests. The currently available serologic tests measure antibodies that could result from either infection or vaccination. Post-vaccination antibody levels do not interfere with diagnosis because the antibody levels decline below 94 IU/mL by six months post-vaccination. A positive serologic response simply means that the person has been exposed to pertussis by either recent or remote infection, or by recent or remote vaccination. As such, the use of serologic assays cannot differentiate infection from vaccine response, nor can they be relied upon for case confirmation of pertussis infection.

Both paired and single sera can be used in the diagnosis of pertussis disease. A single serum point taken at optimal time (two to six weeks post symptoms) is sufficient to measure antibody levels.

The Maryland State Public Health Laboratories Administration offers a single-specimen serology test for pertussis as recommended by the CDC (Centers for Disease Control and Prevention). The test is designed to confirm outbreak cases, as adults and adolescents may not seek medical attention promptly, and isolation of B. pertussis by culture or PCR is not likely. The anti-pertussis IgG antibody ELISA is used to detect elevated IgG antibody titers that tend to rise two weeks after the onset of symptoms and persist after the infection is resolved. This assay should not be used to assess immunity to pertussis or for clinical diagnosis. Patients should be over the age of 11, present with a cough ≥ two weeks, and not have been vaccinated against pertussis in the past six months. The results should not be interpreted in children younger than 11 years old due to potential interference caused by antibody formation after childhood vaccinations (see Table 1).

If you have any questions on the laboratory diagnosis of pertussis, please call the Division of Public Health Microbiology at 410-767-6125 or 410-767-6135.

This article written by Dr. Jafar Razeq with assistance from Dr. Leena Trivedi and Naomi Barker.

REFERENCES

1 ASM Manuel, 10th Edition.
4 EID: 15 (8), 1206-1213, 2009.
5 JCM 1987:25(6), 1109-1110.
6 JCM; 1997; 35(10): 2435-43.
Iodine Water Purification

Treating the water with iodine does not only kill bacteria and other disease-causing organisms

Imagine, you are vacationing in the Dominican Republic, and you are enjoying the sights. It is time for bed and you go to sleep feeling fine. Suddenly, in the middle of the night, you are awakened by severe stomach cramps followed by unrelenting diarrhea. You think, what has caused my sickness?

The spread of disease is easily accomplished by contaminated water.\(^1\) Sadly, over one billion people in the world do not have access to safe drinking water. Of those, millions die from water-related diseases.\(^2\) Emergency situations, such as tornadoes, floods, and hurricanes, can cause situations where the potable water supply is jeopardized and becomes contaminated with surface water or other contaminating substances,\(^2,\) such as disease-causing microorganisms, gasoline, pesticides, and more.\(^3\)

The scenario imagined at the beginning of this article is a situation that many of us may have experienced. Almost 30% to 70% of travelers suffer from some form of “Travelers’ Diarrhea.” Common causes include Escherichia coli, Salmonella, and other bacteria, or viruses, all of which can be transmitted by a contaminated water supply.\(^4\) By simply using a contaminated water supply to brush your teeth, a severe illness may ensue. This may include diarrhea and vomiting, which can lead to dehydration.

One method of water sanitation is to treat the water with iodine.\(^5\) Iodine is an effective germicide and is successful in eliminating Giardia lamblia.\(^6\) Iodine not only kills bacteria but also kills other disease-causing organisms.\(^5\) However, iodine is not an immediate sanitizer. Iodine needs to be in contact with the water for at least 20 minutes at a residual of 0.5 to 1.0mg/l.

Iodine disinfection was developed by Harvard University and the United States Army in the 1940’s.\(^4\) Iodine is a solid black crystal that dissolves in water. There is, however, a direct correlation between temperature and the ability for iodine to dissolve. The higher the temperature, the more the ability of iodine to dissolve. Iodine treatment is effective in purifying water over a large pH range and will not lose its purification ability up to a pH of 10.\(^7\) After the treatment phase, iodine can be removed from the water by using a carbon filter.\(^8\)

Figure 1 shows how iodine is used in a bypass saturator system. No electricity is needed for this type of system. The iodine is held in the solution tank attached to the water supply and a small amount of water is diverted through it. The water is then injected back into the water line. The valves shown in the diagram are in place to adjust the amount of iodine that is put into the water system. The water is then collected into the contact tank until it is used, residentially or commercially. Since the solubility of iodine fluctuates directly with temperature change, it is imperative to monitor and adjust the system as necessary.\(^5\)

For example, there are many hand-pumps on the biking or hiking trails along the C&O Canal from Washington, DC to Cumberland, Maryland that use iodine to sterilize the water supply. Western Maryland Regional Laboratory tests the water samples in the area seasonally, April 15 through November 15, to make sure the iodine sterilization is effective. When a sample fails bacteriological testing, the Department of Natural Resources will remove the handle to the pump to prevent the public from having access to the water. The water treatment will be adjusted and retested until the water quality is safe for the public.

Continual use of iodine is not advised, although no literature supports detrimental outcomes. Occasional use of iodine sanitation for camping, vacations, emergencies, etc. are appropriate examples.\(^5,\) Iodine tablets to sterilize drinking water have been developed and can be bought at most camping supply stores. These would be great to use when the unexpected occurs and there has been no preparation or plan made for the lack of sanitary water. Tincture of iodine could also be used in the absence of iodine tablets. Nevertheless, any usage of an iodine product needs to follow recommended directions of use.\(^5\)

As many of us travel, there could be a need for iodine

(Continued on page 7)
Iodine Water Purification

Our hobbies, vacations, and emergency circumstances sometimes put us in an environment where iodine is used to sterilize water or iodine tablets are needed to make the water safe for our consumption.

References
1 Dix, Paul. Registered Maryland Milk Sanitarian, Maryland Department of Health and Mental Hygiene, Personal Interview, Iodine Water Systems Used in Dairy, 1100EST September 11, 2012 at Western Maryland Regional Laboratory.

Community Outreach of the Laboratories Administration

Encouraging students to pursue a career in laboratory-based science to ensure a future work force

One of the Laboratories Administration’s core functions is to recruit and train the next generation of public health laboratory scientists. To do this requires a network of college and graduate students majoring in a public health laboratory science. The Laboratories Administration has for many years provided lab tours and sent our staff scientists to participate at local high school career fairs. These initiatives support the development and maintenance of this network and encourage high school students to consider majoring in laboratory science upon graduation. Kicking off the Department of Health and Mental Hygiene’s (DHMH) Community Outreach Initiative took this network support a step further. Under the auspices and guidance of the Superintendent of Baltimore City School’s Office, we proposed the “Public Health Educational Workshop on Foodborne Disease Outbreak Investigations.”

This workshop is intended to serve two purposes. The first is to interest students in science and introduce them to the value and satisfaction associated with careers in public health and laboratory science. The second purpose is to support public relations and community interest in the Maryland State Public Health Laboratory’s mission and how it has a positive effect on local residents.

In the fall of 2010, the Laboratories Administration and the Infectious Disease and Environmental Health Administration hosted twenty eighth-grade students from the Bluford Drew Jemison S.T.E.M. Academy on North Caroline Street in East Baltimore. They were invited for the first half-day “Public Health Educational Workshop on Foodborne Disease Outbreak Investigations” where the students were actively engaged through educational hands-on multisensory activities and a video. They were introduced to public health concepts such as physical characteristics of bacterial pathogens, microscopy techniques, outbreak investigation methods, and the importance of food safety. The students learned surveillance and case study techniques used by epidemiologists, environmental health professionals, and sanitarians, and had an opportunity to see the critical work these professionals perform. The Laboratories Administration hopes this initiative will serve to spark the students’ interest in math and biological sciences.

As part of the continual community outreach, the Laboratories Administration hosted a Career Forum aimed at high school summer program students, for the BioTechnical Institute (BTI) of Maryland. BTI is partnering with East Baltimore Development, Inc. to identify bioscience and laboratory-based career opportunities in the Baltimore area. The Laboratories Administration

(Continued on page 8)
Community Outreach of the Laboratories Administration

staff feel career day forums are an effective way to make students aware of the vital work public health scientists perform every day and to highlight the diverse careers offered in this vocation. The goal is to show there is a science career for everyone in laboratory science.

On July 25, 2012, twelve high school seniors of the Baltimore area high schools and five BTI administrators visited the Maryland Public Health Laboratory for a Career Forum. The day began with an introduction from the Laboratories Administration’s Director, Robert Myers, Ph.D. During the discussion, Dr. Myers engaged the students and talked with them about his interests, his educational background, what influenced his career choices, and his career path. He started as a Public Health Scientist in the Rabies Lab and rose to Laboratory Administration Director. The students had opportunities to ask Dr. Myers their own questions relating to science, medicine, public health, and bioterrorism.

After the discussion with Dr. Myers, BTI students met Chemical Terrorism Lead Scientist Shannon Black, Core Sequencing Laboratory Developmental Scientist Julie Haendiges, Accessioning Laboratory Technician Brandon Holman, Enteric Laboratory Lead Scientist Celere Leonard, Mass Spectrometry Laboratory Scientist II Jamie Myers, and Rabies Laboratory Supervisor Kenneth Okogi, for an open discussion session. Students were given example discussion questions to help create a conversation with the scientists. They talked individually with these scientists, and in a small group setting. They were encouraged to ask about types of careers, and tips or pointers about high school, college, internships, and career advice.

It was a great experience for both BTI students and the Laboratories Administration scientific staff. Students had the opportunity to interact with a wide variety of public health scientists, and the scientific staff had the chance to discuss their own personal career experiences at the Laboratories Administration, share educational and real life situations, and connect with students to learn what it is like approaching career choices today.

Many of the Laboratories Administration’s partners and past and current employees are residents of Baltimore City. Many others have deep roots in the City that go back over a hundred years. The State public health laboratory and Baltimore City have always depended on and supported one another and will continue to do so in the future.

This article written by Rachel Vaden Michael.