



# DELAWARE LABORATOR

Summer

2010



## ***FILTER CONCENTRATION AND DETECTION OF BT AGENTS***

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### *Overview*

What if a terrorist gained access to water treatment plants and successfully contaminated Delaware's water system with a potentially deadly toxin or organism? There is significant concern that introduction of an agent could occur post-treatment in distribution mains, storage tanks, booster pumping stations and other water treatment areas, resulting in a potentially dangerous public health emergency.

In such a breach, the identity of the potential agent is unknown and there may be a time delay from discovery of the breach to collection of a sample for analysis. Finding the best collection point can be a guessing game necessitating collection of large sample volumes. When contaminants are added to a water system they will have an uneven distribution because they are initially concentrated in pockets, but disperse throughout the water system over time.

Low concentrations of biological terrorism (BT) agents in the water system may be undetectable by current methods, but still pose a public health risk.

How do we find such small amounts of potentially dangerous contaminants in such large volumes of water? The Laboratory Response Network (LRN) method, "Filter

Concentration and Detection of Bioterrorism Threat Agents in Potable Water Samples", simultaneously concentrates viruses, bacteria, and toxins in large volumes of potable drinking water in preparation for detection of BT agents. In previous testing performed by the Centers for Disease Control & Prevention (CDC), the method detected the following:

#### Bacterial agents

- *Bacillus anthracis* (vegetative cells and spores)
- *Yersinia pestis*
- *Francisella tularensis*
- *Burkholderia thailandensis* and *Burkholderia mallei*
- *Brucella canis*

#### Viral agents

- Vaccinia virus (DNA Virus)
- Venezuelan Equine Encephalitis virus (VEE, RNA Virus)

#### Biotoxins

- Ricin

### *How the Test Works*

As the agent has been dispersed throughout the water system, the collector begins by

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### **Special Points of Interest:**

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obtaining a large volume of potable water (40 – 100 L). The sample is processed using several pre-treatment steps to increase recovery of agents before the process of hollow fiber ultra-filtration. Ultra-filtration is performed with the use of a dialysis filter and peristaltic pump. This process filters the sample to a concentrated volume of 300 – 500 mL of retentate. A small fraction (10%) of the retentate is frozen and stored for future analysis.



This remaining volume is then split in half for additional processing. The first volume is filtered through a gridded 0.2 um filter. The filter is cut into two halves; one of the halves is then cut into quarters. One quarter of the filter is retained and stored at -20°C for future testing. The second quarter of the filter utilizes a bead-beating process and DNA extraction for further real-time Polymerase Chain Reaction (PCR) analysis. The remaining half of the filter is placed on a culture plate. Viable organisms recovered from the plate are identified using the appropriate LRN procedures for the target agent(s) in question.

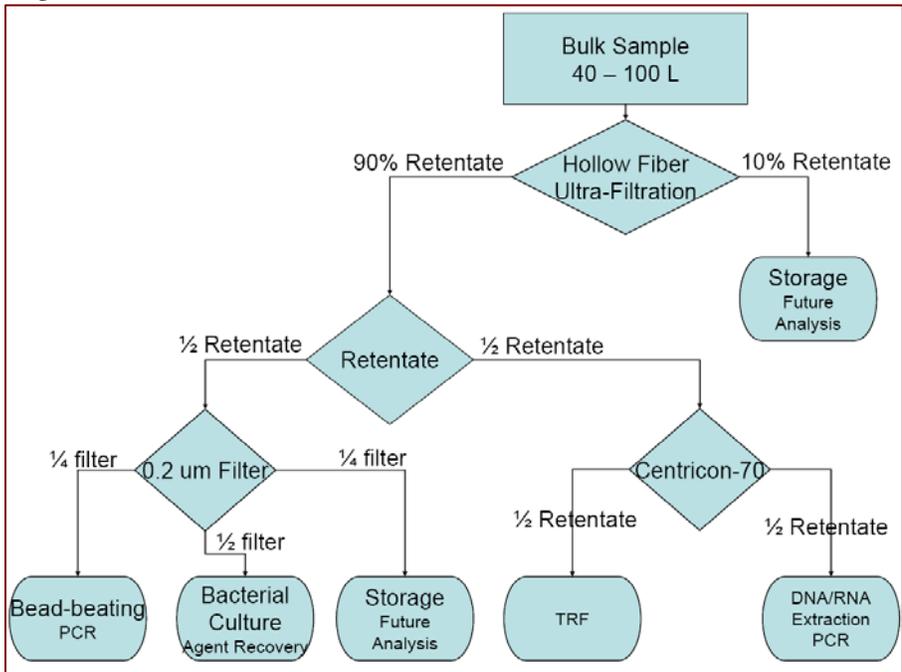
The other half of the retentate obtained from the ultra-filtration process is further concentrated using a Centricon-70 centrifuge concentrator filter. The concentrate can be used directly for Time Resolved Fluorescence (TRF) analysis for Ricin toxin or for real-time PCR using RNA or DNA extraction of target viruses or organisms, such as orthopoxvirus or *Ricinus communis*.

Limitations and Current Progress

As this method concentrates over 40 L of water to a few milliliters, there are some limitations which must be considered. Most organisms and toxins are killed or inactivated by water treatment, so recovery of live organisms or active toxins is unlikely. Water sterilization methods and materials vary from region to region which may result in variation in the volume, concentration and type of organisms found in the system.

Analysis is performed on potable drinking water only; turbid and source water contains varying amounts of particulate matter and dissolved compounds that may clog the filters which may inhibit analytical procedures.

This method is not designed for routine testing. It is designed to be used only in those instances where a credible threat or an identified BT-associated threat exists. As a result, there are currently no validation criteria or routine proficiency testing for this method. To demonstrate competency in this method, Delaware’s Public Health Laboratory (DPHL) is utilizing clean local water for a baseline assessment



and developing our Standards of Operating Procedures to include procedures to transfer concentrates to DPHL bioterrorism staff performing analysis using TRF, PCR, and culture methods. Once CDC evaluates DPHL’s assessment results, DPHL will utilize samples spiked in-house with BT-like agents to determine method limits of detection and detail in-house and external laboratory transfer procedures. DPHL is prepared to keep Delaware’s drinking water safe.

## GEN-PROBE MOLECULAR TEST FOR EARLY DETECTION OF HIV

**DEBRA RUTLEDGE, MBA, MT (ASCP)  
LAB MANAGER II, INFECTIOUS DISEASE LABORATORIES**

Human immunodeficiency virus (HIV) infections continue to be a major cause of disease and death in the United States. The Centers for Disease Control & Prevention (CDC) estimates that 56,300 new HIV infections occurred in the United States in 2006 (1) and, at the end of that year, an estimated 1,106,400 persons in the United States were living with HIV. HIV testing has been available since 1985 for the detection and diagnosis of HIV infection. Over this time-frame, various methodologies have been approved for testing samples for HIV infection including point of care testing, which allows for rapid results in the clinical setting using finger stick and oral fluids as sample types. Point of care testing is optimal for those clients that may not return for follow up, and allows for early access to treatment. Early knowledge of HIV infection is now recognized as a critical component in controlling the spread of HIV infection and improving patient prognosis. (2)

For the past twenty years, the CDC testing algorithm recommends that HIV antibody testing uses an enzyme immu-

noassay (EIA) and western blot combination. Through funding from CDC HIV prevention grants handled through the DPH HIV program, the laboratory has followed this recommendation. In recent years, the majority of initial testing moved to clinical settings using rapid test kits. Positive rapid tests are referred to our lab for western blot confirmation. Our laboratory wanted to find out if we were missing early infections by converting to rapid testing and also to find an alternative to western blot to confirm positive tests. We were able to negotiate an agreement to utilize Gen-Probe equipment that was no longer being used for chlamydia and gonorrhea testing. In June, our lab began work on an evaluation project using the Gen-Probe Aptima HIV-1 RNA Qualitative assay. We are testing pooled samples that have tested negative for HIV antibodies in the clinical setting using rapid tests. We hope to be able to detect the presence of HIV RNA prior to the detection of antibodies and thus be able to get the client into treatment programs earlier in the disease process. Our plans are to test samples for the next 10-12

months to determine the feasibility of converting to this testing completely based on the number of specimens that we may not have identified using rapid testing alone. Several studies have shown that nucleic acid amplification methods can detect infection within the first week of infection. The analytical sensitivity of this method is 30 copies/ml (3). Be on the lookout for the laboratory findings of our evaluation study in future editions of this newsletter. If you are a clinic manager and are interested in participating in this study, please contact me at (302) 223-1520 or email [debra.rutledge@state.de.us](mailto:debra.rutledge@state.de.us).

### Sources

Glynn M, Rhodes P. [Estimated HIV prevalence in the United States at the end of 2003](#). National HIV Prevention Conference; June 2005; Atlanta. Abstract T1-B1101

Valdiserri RO, Holtgrave DR, West GR. Promoting early HIV diagnosis and entry into care. *AIDS* 1999; 13:2317-30.

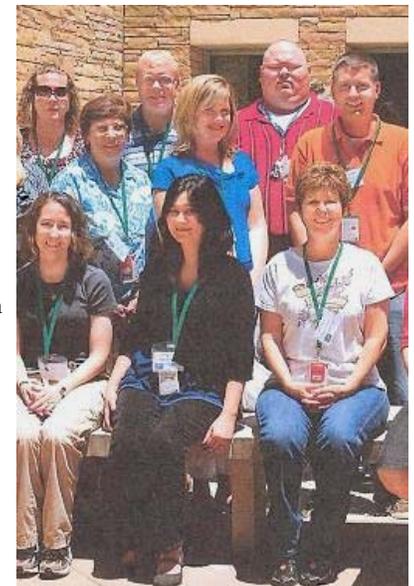
Gen-Probe Aptima HIV 1 RNA Qualitative Assay, Package insert

## DPHL HOSTS VISITOR FROM GUAM

Emily Outten, Virology Lab Manager, attended the "Influenza Detection and Subtyping: FDA-Cleared rRT PCR Flu Panel Assays (Seasonal and 2009 H1N1)", training at the Center for Disease Control & Prevention (CDC) the last week in June. She is now working on getting DPHL standards of procedures in line with the latest FDA approved methods.

Jill Villanueva, a Medical Technologist at the Guam Public Health Laboratory, also attended the training at CDC and after the course APHL arranged for her to visit DPHL to observe our influenza testing processes and see our laboratory set-up. The Guam lab is not currently running any molecular methods for Influenza, only rapid testing. The goal is for Guam to eventually perform the rRT PCR Flu Panel Assays and provide influenza surveillance data to the World Health Organization and CDC.

*Jill Villanueva (in black) and other attendees to the CDC sponsored "Influenza Detection and Subtyping" training in June 2010*



## **BIOTERRORISM UPDATE**

**DEBRA RUTLEDGE, MBA, MT(ASCP), BIOTERRORISM LAB COORDINATOR**

On June 16, 2010 the biological group of the Laboratory Preparedness Advisory committee met at the DPH lab for our biannual meeting. The agenda included a wide variety of topics: Brucella case, Vancomycin Resistant Staphylococcus aureus (VRSA) case, Tuberculosis (TB) real time Polymerase Chain Reaction (PCR) testing (see related paragraphs below), Bureau of Epidemiology re-structure, DERSS and DHIN, update of FERN projects, the College of American Pathologists-Laboratory Preparedness Exercise survey, Bioterrorism Wet Workshop, Chemical terrorism update and packaging and shipping. There was good representation from our partners and sentinel laboratories at this meeting. Facilities unable to attend may access distributed information on the laboratory web page.

<http://www.dhss.delaware.gov/dph/lab/LPAC.html>

### **Brucella Case**

On June 9, 2010, the DPH laboratory confirmed brucellosis by culture in a 58 year old, female, New Castle County resident. The patient had no history of recent travel, or contact with pets, farm animals or wildlife, and has been unemployed since 2008. The patient experienced similar symptoms following consumption of unpasteurized milk and cheese products one year ago and was treated at that time with a short course of antibiotics. While person-to-person transmission is rare, humans can be infected by eating or drinking raw milk and cheese made with unpasteurized dairy products. They can also be exposed to bacteria through skin wounds when hunting or working in slaughterhouses, meat packing plants, and poultry plants.

### About Brucellosis

- Brucellosis is a bacterial disease transmitted from animals to humans. Approximately 100 to 200 human cases are reported annually in the United States. If aerosolized, brucellosis has the potential for use as an agent of bioterrorism.
- Transmission occurs through direct contact with infected mammals (commonly through abrasions of the skin). In the United States, transmission occurs more frequently by ingesting unpasteurized milk or dairy products. Transmission may occur through inhalation of aerosols, and while handling cultures in laboratory settings. There is no evidence of person-to-person transmission. Brucellosis is most often seen in farmers, ranchers, veterinarians, and others who work directly with animals. Employees in certain types of laboratories, slaughterhouses and meat inspectors may also be infected. Sporadic cases and outbreaks occur among consumers of unpasteurized milk and milk products, especially soft cheeses.
- The incubation period is variable, ranging from 5-60 days but may be several months.
- Symptoms may last for several days, months, or even a year or more if not adequately treated. The onset of the disease can be acute or insidious and include fever, night sweats, headache, weakness, sweating, arthralgia, myalgias, weight loss and anorexia. Physical findings include enlargement of the lymph nodes, spleen or liver, and occasionally arthritis. Complications of brucellosis include meningitis, endocarditis, and osteomyelitis.
- Laboratory criteria for diagnosis: iso-

lation of Brucella species from a clinical specimen; or fourfold or greater rise in Brucella agglutination titer between acute and convalescent phase serum specimens obtained >2 weeks apart and studied at the same laboratory; or demonstration by immunofluorescence of Brucella species in a clinical specimen.

- Prolonged antibiotic treatment for at least 6 weeks is necessary to achieve a cure. Combination therapy (2 antibiotics) is recommended. Monotherapy is associated with a high rate of relapse. Doxycycline and rifampicin are the treatments of choice.

### For More Information

- CDC website at: [www.cdc.gov/ncidod/dbmd/diseaseinfo/brucellosis\\_g.htm](http://www.cdc.gov/ncidod/dbmd/diseaseinfo/brucellosis_g.htm)
- DPH, Bureau of Epidemiology: (302) 744-1033 or 1-888-295-5156.

### **Real-time PCR for *Mycobacterium tuberculosis complex***

New molecular methodology for TB, which was implemented by DPHL in February 2010, enabled the lab to detect 2 highly suspect TB cases within 24 hours of specimen receipt. In both cases, patients were exhibiting classic symptoms including coughing up blood. DPH lab received the first specimen from Kent General Hospital on July 20, 2010 in the late morning and used Real time PCR to confirm the case within approximately 4-5 hours of specimen receipt. The other was received on July 28 from Beebe Hospital and had Real-time PCR and smear positive results within 24 hours of receipt. This rapid confirmation and reporting allows for better patient case management. Traditional methods require culture of the bacterium, which takes about 14 days.

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More information on the TB PCR method can be found on the Spring 2010 edition of our newsletter. <http://www.dhss.delaware.gov/dph/lab/files/laborator.pdf>

**VRSA**

The Delaware Public Health Laboratory had the dubious distinction of confirming the eleventh VRSA (vancomycin resistant staph aureus) isolate in the US. The patient, from Nanticoke Hospital in Seaford, was transferred to a PA hospital for treatment. Testing of contacts from the hospital, dialysis unit and nursing home was negative for VRSA by DPH lab using CDC guidelines.

**CDC Reminds Clinical Laboratories and Healthcare Infection Preventionists of their Role in the Search and Containment of Vancomycin-Resistant *Staphylococcus aureus* (VRSA), May 2010**

The Centers for Disease Control and Prevention (CDC) has recently confirmed the 11<sup>th</sup> case of vancomycin resistant *Staphylococcus aureus* (VRSA) infection since 2002 in the United States. This serves as a reminder about the important role of clinical laboratories in the diagnosis of VRSA cases to ensure prompt recognition, isolation,

and management by infection control personnel. This is an important opportunity for all laboratories to revisit their step-by-step problem-solving procedure or algorithm for detecting VRSA that is specific for their laboratory. A sample algorithm is available at [http://www.cdc.gov/ncidod/dhqp/ar\\_visavrsva\\_algo.html](http://www.cdc.gov/ncidod/dhqp/ar_visavrsva_algo.html) and highlights the recommended testing methodologies for detecting VRSA and actions based on testing results.

Furthermore, because of exchange of genetic material from vancomycin-resistant enterococci (VRE) to methicillin-resistant *Staphylococcus aureus* (MRSA) in the emergence of VRSA, CDC is asking clinical laboratories, when patients are identified with suspected or confirmed VRSA, to ensure that all VRE, MRSA, and VRSA isolates from these patients are saved. Following confirmation of VRSA, CDC recommends that all three isolate types (i.e., VRE, MRSA, and VRSA) be shared with public health partners, including CDC.

Immediately, while performing confirmatory susceptibility tests, notify the patient's primary caregiver, patient-care personnel, and infection-control personnel regarding the presumptive identification of VRSA so that appropriate infec-

tion control precautions can be initiated promptly. It is also important to notify local and state public health departments.

Coordination with public health authorities is critical. CDC has issued specific infection control recommendations intended to reduce the transmission of VRSA. However, these may need to be customized to the healthcare settings (e.g., dialysis, home healthcare). Infection control precautions should remain in place until a defined endpoint has been determined in consultation with public health authorities.

VRSA infection continues to be a rare occurrence. A few existing factors seem to predispose case patients to VRSA infection, including:

- Prior MRSA and enterococcal infections or colonization
- Underlying conditions (such as chronic skin ulcers and diabetes)
- Previous treatment with vancomycin

Appropriate antimicrobial prescribing by healthcare providers, adherence to recommended infection control guidelines, and, ultimately, the control of both MRSA and VRE are necessary to prevent further emergence of VRSA strains.

**Historical U.S. VRSA Case Count and Geographical Information:**

State	Year	Age	Source	Diagnosis	Underlying Conditions
NY	2004	63	Urine from a nephrostomy tube	No infection	Multiple sclerosis, Diabetes, kidney stones
MI	2005	78	Toe wound	Gangrene	Diabetes, vascular disease
MI	2005	58	Surgical site wound after panniculectomy	Surgical site infection	Obesity
MI	2005	48	Plantar ulcer	Osteomyelitis	MVA, chronic ulcers
MI	2006	43	Triceps wound	Necrotizing fasciitis	Diabetes, dialysis, chronic ulcers
MI	2007	48	Toe wound	Osteomyelitis	Diabetes, obesity, chronic ulcers
MI	2007	54	Surgical site wound after foot amputation	Osteomyelitis	Diabetes, hepatic encephalopathy
MI	2009	53	Plantar foot wound	Plantar soft tissue infection	Diabetes, obesity, lupus, rheumatoid arthritis
DE	2010	64	Wound drainage	Prosthetic joint infection	Diabetes, end-stage renal disease, dialysis

## EMPLOYEE NEWS



Linda Popels, Ph.D. has moved to the clinical microbiology section where she is the laboratory manager. Previous to this, Linda worked in the environmental chemistry section at DPHL as a volatile organic compounds chemist. She has a Bachelor of Science Degree in biology (chemistry minor) and a Ph.D. in oceanography. Linda is married and has a 3 year old daughter. She is excited about her new position at the lab and learning her new responsibilities quickly. She is sorely missed by the environmental chemistry section.

Congratulations Linda!

## INTERN REPORT

My name is Kialeen Hay and I am currently going into my senior year at the University of Delaware. I will graduate in May 2011 with a Bachelors of Science degree in Medical Technology with a minor in Biological Sciences. In May 2010, I accepted the internship at Delaware's Public Health laboratory (DPHL). As an intern, I have the opportunity to shadow and perform various laboratory tests and techniques. I also have the opportunity to help with a validation and verification of the seasonal influenza test method, as well as 2009 Influenza A (H1N1)pdm Real Time PCR on the MagNA Pure Compact. I am grateful for the amount of knowledge and experience that I have gained.

I currently work at Union Hospital in Elkton, MD and at DPHL. DPHL opened my eyes to a completely different spectrum of biological testing. Some routine work that I perform at DPHL is similar to the routine work done everyday within a hospital lab, but the part that I enjoy the most is being able to see the work that is not routine hospital work. This includes polymerase chain reactions (PCR), pulse field gel electrophoresis, pyrosequencing, western blot, and various extraction methods.

As part of my internship I worked on a project to help with method comparison and validation of the MagNA Pure Compact versus the Qiagen Viral RNA manual extraction method. The project allowed me to learn how to use the Magna Pure Compact, get more experience with PCR and learn to troubleshoot. I am excited that while I gained knowledge, I was able to contribute to the lab. I truly do appreciate all the time and energy all the employees put into mentoring me.



## DELAWARE'S DIVISION OF PUBLIC HEALTH LABORATORY



Delaware Public Health Laboratory  
30 Sunnyside Road  
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**Built: 1990**

**Business Hours: 8 a.m. – 4:30 p.m.**

**Purpose:** The Division of Public Health Laboratory currently offers consultation and laboratory services to state agencies, Delaware Health and Social Services and Division of Public Health programs including:

- HIV surveillance and prevention
- Immunization
- Lead
- Epidemiology
- Newborn Screening
- STD prevention
- TB Elimination
- Drinking water
- Preparedness

**VISIT OUR WEBSITE:**

[www/dhss.delaware.gov/dhss/dph/lab/labs.html](http://www/dhss.delaware.gov/dhss/dph/lab/labs.html)

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If you have questions regarding these articles or would like to receive a hard copy of this newsletter, contact the Delaware Public Health Laboratory at 302.223.1520. To receive this newsletter by email, contact Liz Moore, editor, at [liz.moore@state.de.us](mailto:liz.moore@state.de.us).

"To Protect and Enhance the Health  
of the People of Delaware"

