DELAWARE HEALTH AND SOCIAL SERVICES

Division of Public Health

Laboratory

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2010

TUBERCULOSIS-POLYMERASE CHAIN REACTION (TB-PCR) TESTING AT DELAWARE'S PUBLIC HEALTH LABORATORY

AMANDA CHAN, M.S., DIANE HINDMAN, MT (ASCP), SM AND GREGORY HOVAN, B.S

Recent reports from the Centers for Disease Control and Prevention (CDC) show approximately 13,000 cases of *Mycobacterium tuberculosis* (TB) were reported in the U.S. in 2008, with 41% of the cases being U.S. born. Current microbiological testing can take up to eight weeks before results can be communicated to patients. There is an overwhelming concern for drug resistant strains of TB (Multidrug Resistant (MDR) and Extensively Drug Resistant (XDR)), and therefore it is imperative that a rapid response program be in place at public health labs to combat this ever growing concern. (1)

Early laboratory confirmation of TB can lead to earlier treatment initiation, improved patient outcomes, increased opportunities to interrupt transmission, and more effective public health interventions. CDC recommends that Nucleic Acid Amplification Testing (NAAT) be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered but has not yet been established. NAAT resting is also recommended when the test result would alter case management or TB control activities, such as contact investigations.

Culture remains the gold standard for laboratory confirmation of TB and is required for isolating bacteria for drug-susceptibility testing and genotyping. In accordance with current recommendations, sufficient numbers and portions of specimens should always be reserved for culturing. Nonetheless, NAA testing is recommended as standard practice for patients suspected to have TB, and all clinicians and public health TB programs should have access to NAA testing for TB to shorten the time needed to diagnose TB from 1-2 weeks to 1-2 days. More rapid laboratory results offer additional advantages for patient care and TB control efforts by avoiding unnecessary contact investigations and respiratory isolation for patients whose AFB smear-positive specimens do not contain *M. tuberculosis*. Rapid laboratory confirmation of TB also can help reduce inappropriate use of fluoroquinolones as empiric monotherapy of pneumonias, a practice which is suspected to lead to development of fluoroquinolone-resistant *M. tuberculosis* and delays in initiating appropriate anti-TB therapy. (2)

Recently, the molecular microbiology/ virology section at DPHL acquired and validated an innovative method for the rapid detection of TB. DNA from *Mycobacterium tuberculosis* complex (MTBC) can be detected in clinical specimens to aid in the diagnosis of tuberculo-

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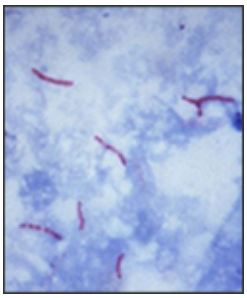
TB PCR Testing at DPHL. Con't

sis using a Real-Time polymerase chain reaction (PCR) assay. Real-Time PCR has a quick turn around time (approximately two hours) and precise results. The assay can detect all members of the MTBC, including: *M. africanum*, *M. bovis*, *M. bovis BCG*, *M. canettii*, *M. caprae*, *M. microti* and *M. tuberculosis*.

The assay targets a specific insertion element sequence, IS6110, which is a stretch of DNA that is exclusive to all members of the MTBC. IS6110 show up in multiple sites at different locations which allows for easy genotyping of different species. *M. tuberculosis* complex genomes may contain 1 to >25 copies, thus the target used in the real-time assay is recognized as a multi copy target. (3)

Respiratory samples, including expectorated sputum, induced sputum, bronchial aspirates, tracheal aspirates, and broncheoalveolar lavage are all tested using this new method. Information is limited regarding NAA test performance for tissue, non-respiratory specimens or specimens from patients under treatment. Further research is needed before specific recommendations can be made on the use of NAA testing in the diagnosis of extra pulmonary TB.

DPHL is now performing the new TB-PCR on respiratory specimens from new patients who are currently not receiving any treatment. Respiratory specimens are initially processed by the microbiology section and separated in order to process an aliquot for culture, and then a heat killed aliquot is submittd to the molecular microbiology/virology section for RT-PCR. Since testing began in February, seventy samples have been tested. Results are uploaded into our online LIMS system where clinicians can quickly review the reports. Even though the test has only been available for a few months, it has already had a positive effect on clinic practice in Delaware by identifying new positive TB patients quicker, as well as ruling out TB in several patients and getting them on more appropriate medications thus avoiding unnecessary contact investigations.



This photomicrograph from the CDC Public Library reveals Mycobacterium tuberculosis bacteria using acid-fast Ziehl-Neelsen stain; Magnified 1000X

References

1. Whittier, Susan. Molecular Diagnostics for TB: To Infinity and Beyond! Columbia University Medical Center. APHL Teleconference Series, December 8, 2009.

2. CDC: Updated Guidelines for the Use of Nucleic Acid Amplification Tests in the Diagnosis of Tuberculosis, **January 16, 2009 / 58(**01**); 7-10.** http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5801a3.htm

3. Coros, Abbie, DeConno, Erin, Derbyshire, Kevin M. IS6110, a Mycobacterium tuberculosis Complex-Specific Insertion Sequence, Is Also Present in the Genome of Mycobacterium smegmatis, Suggestive of Lateral Gene Transfer among Mycobacterial Species. *Journal of Bacteriology, Vol. 190, No. 9.* May2008. p. 3408-3410.

BIOTERRORISM UPDATE

MARION FOWLER, MICROBIOLOGIST II, MT (ASCP)

The Agents of Bioterrorism: Annual Sentinel Lab Wet Workshop was held on April 29 and April 30, 2010 by the Delaware Public Health Laboratory and the National Laboratory Training Network. All of Delaware's sentinel laboratories were invited to this special training. Seventeen microbiologists, who represented all of Delaware's sentinel laboratories, were trained. The morning agenda included an overview of laboratory safety, bioterrorism agent identification, select agent information and the role of the sentinel laboratory plus an overview of the American Society of Microbiology guidelines for the clinical laboratory. A pre-test was administered to each participant in the morning session and a post-test administered after the afternoon session to assess the knowledge gained from the entire workshop.

The afternoon session consisted of a hands-on laboratory exercise using non-select agents and other mimic organisms to provide actual viewing of bioterrorism isolates at 24, 48 and 72 hours on a variety of media. The organisms represented were *Bacillus anthracis, Burkholderia mallei/pseudomallei, Brucella canis, Francisella tularensis* and *Yersinia pestis*. For each bioterrorism organism, colonial morphology , rate of growth, Gram stain and other sentinel laboratory tests for rule-out or referral to DPHL was available at each individual station. After the stations were viewed, five unknown isolates were presented to test the microbiologist's new skills.

The College of American Pathologists-Laboratory Preparedness Exercise (CAP LPX) survey was shipped earlier in April to all of Delaware's sentinel laboratories. Sentinel laboratories were required to call DPHL when the microbiologist was unable to rule-out a BT agent from the three shipped samples. When each hospital contacted DPHL, they were given instructions to package and ship a pretend isolate. The CAP LPX survey not only tests the technical skills of the hospital but the communication between the sentinel laboratories and DPHL.

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BT Update, con't

The next Laboratory Preparedness Advisory Committee (LPAC) meeting is scheduled for June 16, 2010, 9:00am – 12:30pm and will focus on biological laboratory preparedness. The tentative agenda will include a wide variety of topics: vancomycin-resistant Staphylococcus aureus, Tuberculosis polymerase chain reaction, Bureau of Epidemiology restructure, Delaware Electronic Reporting Surveillance System and Delaware Health Information Network, update of Food Emergency Response Network projects, CDC-DPHL Limit of detection study, the CAP LPX survey and Bioterrorism Wet Workshop, and packaging and shipping. Please contact DPHL, Debra Rutledge, Laboratory Manager II, (302)-223-1520 with any suggestions for other topics.

LABORATORY PREPAREDNESS ADVISORY COMMITTEE (LPAC) UPDATE

TARA LYDICK, B.S., CHEMICAL PREPAREDNESS COORDINATOR

Building on the success of the November 2009 Laboratory Technical Working Group meeting, the first Environmental Laboratory Preparedness Advisory Committee, or E-LPAC, met on April 13, 2010 at the Smyrna Readiness Center hosted by the members of the 31st Civil Support Team (CST) of the Delaware National Guard. This session provided the opportunity for members of the laboratory system to meet and discuss opportunities for better integration of laboratory services and provide updates on the current and recent events in the various organizations represented.

The group discussed issues around the response to novel H1N1 influenza and its impact in Delaware, as well as concerns for this calendar year. As the University of Delaware served as the epicenter for Delaware during this outbreak, their perspective, problems, and solutions made for a very interesting discussion. Delaware Emergency Management Agency (DEMA) provided an overview of the May 3, 2010 workshop for the annual Department of Homeland Security Assessment which defined the strategic plan for statewide initiatives by identifying new goals and objectives to fill gaps. The strategic plan is overseen by state and local groups with an interest in implementing it using local resources.

The Department of Natural Resources and Environmental Control (DNREC) reported that their Ecological Assessment Branch laboratory lost funding for fresh water and algal toxicity testing. The branch performs monitoring and surveillance of recreational waters and shellfish bacterial levels to evaluate the need for bed closures and beach monitoring. Bacterial source tracking using DNA methods for surface water quality and nutrient levels continues. In areas of concern increased nutrient levels are being found, but the source is currently unknown. Increased nutrient levels can affect ecosystems and rapidly render populations non-sustainable.

The LPAC discussed further integration of training and physical asset management programs. Joe Miller (Interim Director, Environmental Health & Safety) reported that the University of Delaware utilizes web-based training for their safety training and will investigate if the state laboratories can utilize this system as well. He also reported that they are developing a web-based chemical management tracking system and will forward further information for the group's consideration. The LPAC will continue to investigate web-based training for laboratory personnel reducing the time needed to perform training on-site by each organization.

As part of the laboratory integration effort, three vendors were selected to provide laboratory system wide instrument and equipment maintenance and support. The vendor serves as the first contact point for laboratories needing service and for scheduling preventative maintenance visits. Each state laboratory may select the vendor that best meets their needs. DPHL has selected SUGroup

Lieutenant Colonel (LTC) Brad Knight and Sergeant First Class (SFC) Dale Annis presented CST 101, an overview of the mission and role of the 31st Civil Support Team. LTC Knight and SFC Annis described their day-to-day responsibilities, activation and response requirements, capabilities, and current personnel. A tour of the Smyrna Readiness Center and equipment followed which included the Analytical Laboratory System (ALS), an advanced mobile laboratory operated by SFC Annis. This unit is quite sophisticated and rapidly deployable. With the extensive cooperation of the 31st CST, SFC Annis is actively integrating the ALS and its capabilities into DPHL and the laboratory system response. SFC Annis has been instrumental in bringing M1M (an automated analyzer designed for use with the BioVeris BioVerify test kits for the detection of toxins including botulinum neurotoxins, anthrax, ricin, and staphylococcal enterotoxins A and B) training, to DPHL and has worked on chemical terrorism samples at DPHL in cooperation with DPHL staff. This integration strengthens the potential response to an incident of significance within Delaware and the surrounding area.

As the LPAC continues to identify subject matter experts across the system, we are encouraging individuals to send their name, laboratory organization and location, a webpage or link to their organization or testing, and a brief description of what type of testing and equipment they use to serve as a starting point

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LPAC, con't

for LabList. We envision LabList as an interactive area that allows laboratory system professionals to link with others. The list could help identify someone who has a similar instrument, completes testing in an area of interest, may have insight into a technique or vendor -- the possibilities are endless. Members of the 31st Civil Support Team reported that they utilize the Community of Professionals, an Air Force web-based portal of subject matter experts similar to a secure web-board. As a starting point, DPHL has posted a webpage to serve as a test area for developing LabList. It is located: http://dhss.delaware.gov/dhss/dph/lab/lpac.html. We ask that members of the technical working group send any information they would like displayed on the prototype to Liz Moore (Liz.Moore@state.de.us). We also welcome suggestions on format, content, and feedback on use. We will continue to update and modify the site as information becomes available. Our next meeting will be scheduled either in September or October. Interested personnel should contact Liz Moore; we welcome suggestions for agenda items you feel are relevant to the environmental laboratory, chemical testing, and the state laboratory system response to unknown and known events.

DETECTION OF NOROVIRUS IN FOOD MATRICES

AMANDA CHAN, MS, GREGORY HOVAN, BS, AND JANE GETCHELL, DRPH

Abstract

Norovirus is now recognized as the leading cause of nonbacterial acute gastroenteritis in adults, causing numerous outbreaks worldwide (1). The detection and identification of Norovirus outbreaks is critical. This study compared physical versus chemical cell lysis methods of Norovirus Genegroup II in varying food matrices (2% milk, canned green beans, ground beef, hotdogs, liquid eggs and mixed salad). The methods employed were a Bronson® Cell Disruptor (physical) and Invitrogen TRIzol® reagent (chemical). Positive Norovirus samples were serially diluted and spiked into each food matrix. Each food was processed by both methods, followed by viral RNA extraction using the Qiagen RNeasy Mini Kit. Real-time RT-PCR was used to evaluate the isolation and extraction efficiency. While both methods allowed detection of Norovirus in all the food matrices tested to a dilution of at least 1:103, the cell disruptor method enabled detection of Norovirus at a higher serial dilution in four out of the six foods. Although the cell disruptor method proved to be more sensitive, there were contamination and safety issues which occurred. Therefore, from an overall prospective, the TRIzol method was preferable to the cell disruption protocol.

Introduction

- Norovirus is subdivided into five genegroups (GI-GV), where groups GI, GII, and GIV are found to infect humans. Norovirus GII is most commonly detected in human illness (2).
- The virus is easily transmitted by food, either by sewage contamination or by food handlers during harvesting, processing, and/or preparation (2).
- The Centers for Disease Control and Prevention (CDC) estimates that there are approximately twenty-three million cases of acute gastroenteritis each year, and states that at least 50% of foodborne outbreaks of gastroenteritis can be attributed to Norovirus.
- Norovirus is resistant to environmental degradation, not altered by freezing, and resilient to most chemical treatment processes (2).
- Known positive Norovirus GII samples were serially diluted to 10-8 Norovirus dilutions were added to the food matrices

Methods

Known positive Norovirus GII samples were serially diluted to 10-8

↓

Norovirus dilutions were added to the Food Matrices

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Cell Disruptor Extraction --- TRIzol Extraction

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RNeasy Mini Extraction Kit

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Reverse Transcriptase PCR

↓

Real-time PCR

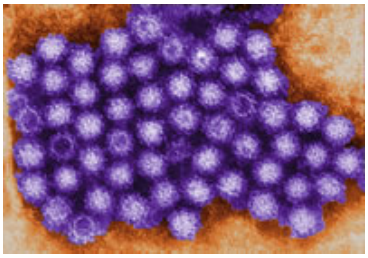

Photo Credit: Charles D. Humphries, CDC image library. Tranamission electron micrograph re: norovirus

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Detection of Norovirus, con't

Results:

Dilution	2% Milk	Green Beans	Ground Beef	Hot Dogs	Liquid Eggs	Mixed Salad	Non-matrix
None	19.614	220.0799	26.5105	16.7335	22.3235	20.3806	23.8376
1:10	24.5185	19.1529	28.4416	19.652	21.5653	19.2772	19.7222
1:100	29.3363	22.0082	32.267	22.58	23.2729	21.6431	22.3936
1:1000	33.4466	16.9127	35.1105	26.1334	27.1137	25.2076	26.2992
1:104	36.9388	30.333	Undet	29.5726	29.6707	28.9726	30.1347
1:105	Undet	34.7076	Undet	33.2474	34.711	32.5324	32.7522
1:106	Undet	37.8048	Undet	37.3261	37.0675	35.3218	Undet
1:107	Undet	Undet	Undet	Undet	Undet	38.4508	37.2751
1:108	Undet	Undet	Undet	Undet	Undet	Undet	37.1816

Table 1. Ct values from samples extracted by the cell disruptor.

Dilution	2% Milk	Green Beans	Ground Beef	Hot Dogs	Liquid Eggs	Mixed Salad	Non-matrix
None	20.3651	22.1936	20.6679	21.0451	17.3719	18.965	25.1387
1:10	23.9113	31.5325	23.8576	24.1557	23.914	24.2541	24.0889
1:100	26.3051	33.4638	26.9463	25.7143	24.6755	27.6002	27.1954
1:1000	28.092	33.4325	30.5527	26.4295	27.6623	31.1252	30.8437
1:104	32.6287	40.3385	33.1136	31.2791	30.7005	36.2691	31.4733
1:105	38.3801	Undet.	37.5183	30.1139	34.003	Undet.	32.9545
1:106	Undet.	Undet.	39.1954	Undet.	Undet.	Undet.	34.8236
1:107	Undet.	Undet.	Undet.	Undet.	Undet.	Undet.	37.4938
1:108	Undet.	Undet.	Undet.	Undet.	Undet.	Undet.	Undet.

Table 2. Ct values from samples extracted by TRIzol

	2% Milk	Green Beans	Ground Beef	Hot Dogs	Liquid Eggs	Mixed Salad*	Non-matrix
Cell Disruptor	10^{-4}	10^{-6}	10^{-3}	10^{-6}	10^{-6}	10^{-7}	10^{-8}**
TRIzol	10^{-5}	10^{-4}***	10^{-6}	10^{-5}	10^{-5}	10^{-4}	10^{-7}

Table 3. Highest dilution detected for the different cell lysis methods.

*Negative controls showed contamination **10 -6 was not detected ***Above the 40 cycle threshold limit

Summary

- Norovirus was detected in all of the food matrices for both extraction types to a dilution of 10-3.
- The physical extraction method enabled detection of Norovirus at a higher dilution in four out of the six food matrices.
- There were continual contamination problems with the cell disruptor method, especially with the mixed salad.
- The cell disruptor could not fit under a Biosafety Cabinet and created aerosols, presenting a substantial safety concern.
- There was more efficient PCR detection for the 1:10 dilution with the cell disruptor extraction, indicative of possible inhibition.
- Overall, the TRIzol extraction was safer and more user-friendly and although less sensitive, the preferred extraction method.

References

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Acknowledgments

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NATIONAL MEDICAL LABORATORY PROFESSIONALS WEEK

CHRISTINA PLEASANTON, M.S.,
DEPUTY DIRECTOR, LAB WEEK COMMITTEE CHAIR

Laboratory Professionals
Get Results

The Delaware Public Health laboratory (DPHL) celebrated National Medical Laboratory Professionals Week April 19 -23. Preparations for the celebration began many weeks earlier with fund raising events. An impressive $196 was raised by Yaohong Zhang who prepared and cooked 210 spring rolls purchased by staff. The week kicked off with staff events including breakfast provided by staff, pizza day, and a pot-luck luncheon, with trivia, games and door prizes throughout the week. Staff events ended with the judging of the Build-A-Bug Contest and an ice cream social. The highlight for the week was the lab's highly successful open house on April 21, during which staff from DPH programs, DPH partners and the general public had the opportunity to learn about the outstanding and vital work that laboratory professionals perform every day of the year. Informational displays were provided by many of our public health partners including the Tuberculosis Elimination Program, the Newborn Screening Program, the Office of Food Protection, the Office of Drinking Water, the Immunization Program and the Office of Lead Poisoning Prevention Program. Outside displays and tours included the Kent County Society for the Prevention of Cruelty to Animals, Odessa Fire Company's Hazmat -4 Field Decontamination Unit, Department of Natural Resources and Environmental Control Emergency Response, Department of Public Health Emergency Response and the National Guard Civil Support Team. Laboratory tours were held throughout the day and were attended by public health officials, state legislators, staff of other state agencies, and staff and students from local colleges and schools. DPHL hosted a very popular hands-on display for students who suited up in protective laboratory coats, masks, gloves and breathing apparatus. The students especially enjoyed pipetting colored liquids and punching dried "blood" spots. Fifty-one guests toured the lab with many more enjoying the partner displays both inside and outside. Dr. Karyl Rattay, DPH director and this year's keynote speaker, spoke about the many partners in the State of Delaware's laboratory system and the vital role played by all the member agencies. The entire week was enjoyable for DPHL staff and served to boost morale and team spirit.

Dr. Karyl Rattay, Director of Delaware's Division of Public Health, welcomes guests to the laboratory open house.

Students observe a specimen under the microscope at the DPH lab.

Amanda Chan, DPHL microbiologist, staffs the Laboratory's display.

Delaware's Special Operations Emergency Response van.

Jeannette Rodman, TB program nurse consultant, smiles for the camera.

Donna Colatrella, DPHL microbiologist, explains procedures to guests at the open house.

John Degour (l) and Ed Hallock from the Office of Drinking Water greet guests.

Kelly Janowski discusses the Blood Lead Program with guests.

EMPLOYEE NEWS

The DPH Laboratory welcomed Brinda J. Modi to the molecular virology section in March of this year. Brinda has a BS degree in microbiology and MLT certification. She was previously employed as a senior fermentation research laboratory technician for four years at a chemical company in Glasgow, Delaware. Brinda is married and she and her husband have an eighteen month old daughter. Brinda will be working as the microbiologist overseeing our Food Emergency Response Network (FERN) grant projects. Welcome Brinda!

Emily Outten returns to the lab after spending the past two years as a Health Program Coordinator in the Division of Public Health's Health Systems Protection section. She will be assuming the role of lab manager I for the virology and molecular sections. Prior to leaving for HSP, Emily was a microbiologist at DPHL for over 6 years. She possesses a Bachelor's Degree in cellular and molecular biology. She spends most of her spare time with her family - her daughter Katherine is 5 and her son Tyler is 18 months old. She also enjoys running in and around Delaware. Emily is very much looking forward to getting back to the lab!

We extend fond farewells to Carrie Paquette-Straub and Amanda Chan, both of whom accepted positions in the private sector.

DELAWARE'S DIVISION OF PUBLIC HEALTH LABORATORY

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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

- Download Test Requisition Form.
- Questions re: specimen collection? - find answers on our Specimen Collection page.
- Review past issues of the LabOrator.

www.dhss.delaware.gov/hdss/dph/lab/labs.html

Karyl Rattay, MD, MS, FAAP, FACPM, Director, Delaware's Division of Public Health

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