

The LAByrinth

Indiana State Department of Health Laboratories Newsletter

2013 ISDH Surveillance of Fresh Produce

By Tom Cronau and Hesham Elgaali, Ph.D.



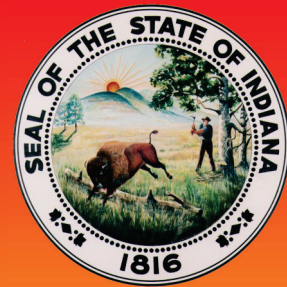
During the past summer, the Indiana State Department of Health (ISDH) Food Protection Program initiated an extensive surveillance of fresh produce grown and/or sold in Indiana. In May 2013, their team of ten Food Safety Inspection Officers and two Food Safety Farm Consultants began collecting and submitting samples of cantaloupe and other produce sold or grown in Indiana. The farm inspectors

focused on cantaloupe and watermelons although other fresh produce may be collected. *Salmonella spp* and *Listeria spp* were the targeted organisms. The other sanitarians collected various types of fresh produce sold in Indiana. This included lettuce, collard greens, spinach, broccoli, strawberries, cilantro, peppers, parsley, basil, zucchini, cucumber and chard. In addition to the targeted pathogens, this additional produce was also screened for general cleanliness by testing for coliforms and performing an Aerobic Plate Count. It was obvious that all of this fresh produce should be thoroughly washed before consuming.

The ISDH Containers Section staff of three Laboratory Technicians has been kept busy by ensuring that all of the field staff had sufficient supplies to collect, package and ship the samples to the ISDH Laboratories. When possible, the insulated shipping boxes are reused. An adequate inventory of the necessary supplies is maintained at the ISDH Laboratories. This is in addition to their routine duties of processing orders for and sending out drinking water collection/submission kits as well a variety of clinical specimen collection/submission kits to customers statewide.

The ISDH Laboratories provide a unique combination of analytical services to support these activities. The Food and Dairy Microbiology Laboratory staff did the initial sample preparation, isolation, detection and identification of the targeted organisms. They used Food and Drug Administration (FDA) approved screening and confirmation protocols that included traditional culture techniques as well as rapid screening instrumentation such as BAX PCR and VIDAS immunoassay to rapidly and accurately provide the initial findings to the investigators. This, in addition to the routine microbiological testing of meat and dairy products collected and submitted as part of the extensive surveillance efforts of the Indiana Board of Animal Health.

The ISDH Media Preparation Lab, staffed by two Laboratory Technicians, provided many liters of universal pre-enrichment broth specific for the



Indiana State Department of Health Laboratories

William VanNess II, M.D.
State Health Commissioner

Judith Lovchik, Ph.D
Assistant Commissioner
Laboratory Services

Our Mission:

The Indiana State Department of Health Laboratories partners with other public health agencies to provide timely and accurate information needed for surveillance and outbreak investigations to protect and improve Hoosier health.

Inside this Issue:

2013 ISDH Surveillance of Fresh Produce	1-2
Carbapenem Resistant Enterobacteria Update	2-5
Communicable Disease Rule Training	6
ISDH Virology is on Fire!	7
June 2013 Hero Award	8
Kudos to Food and Dairy Laboratory	9

for the isolation of *Salmonella* and Buffer Enrichment *Listeria* Broth formulation specific for *Listeria*. They also provided the specialized agar plates required to confirm the initial findings using traditional culture techniques. Their ability to make and provide the required media at short notice was critical to the success of the testing program.

The ISDH Enteric and Reference Bacteriology Laboratories also provided additional assistance by isolating and serotyping the microorganisms. Then Pulse Field Gel Electrophoresis (PFGE) were performed on the isolated organisms. The data from the PFGE pattern was analyzed and entered into the national database, PULSENET. This provided valuable additional information to the epidemiology investigators to potentially link the produce samples to clinical isolates associated with suspected food-borne illness incidents. The importance of this collaboration between the laboratories and the investigators was quite evident in last year's *Salmonella* cantaloupe investigation and outbreak which had extensive involvement from the public health investigators in Kentucky and the FDA. During the investigation of 2012, over 100 samples of cantaloupe, watermelon and environmental samples (water and swabs) were tested. The following strains of *Salmonella* were found and identified in the produce: Anatum, Newport and Typhimurium. Some were linked by PFGE matches to previous clinical isolates.

At this time, the surveillance efforts have included environmental samples (Soil, water, swabs) in an attempt to identify the source of the *Salmonella* and *Listeria spp* where it has been found on the cantaloupe. Hopefully, this year's surveillance and investigative efforts will identify problems in the field before they become problems for consumers. Also, the environmental sampling and testing will provide valuable insight to the farmers to minimize or eliminate the problem in the future.

ISDH CRE Carbapenem Resistance *Enterobacteriaceae* Update

By Jon Radosevic

Emerging resistance of gram negative bacteria and a rise in prevalence of Carbapenem Resistant *Enterobacteriaceae* (CRE) (bacteria of the intestinal tract) have been noted in medical facilities nationwide.

These organisms were first detected in the mid-to-late 90's in North Carolina and have become endemic in the northeast United States. The two most prevalent and deadly CRE are the *Klebsiella pneumoniae* carbapenemase (KPC), and New Delhi metallo-beta-lactamase (NDM-1). Both types of these CRE can be resistant to most all of the antimicrobial agents.

Recently, a grant from the APHL/CDC was awarded to the ISDH Laboratory.

The grant was focused in part to study the recognition, prevalence, and reporting of suspected CRE organisms by clinical laboratories in Indiana and also to enhance the communication/education to medical institutions regarding this issue.

Since January 2013 the ISDH laboratory has received suspected CRE isolates from clinical labs around the state. The ISDH lab began antibiotic susceptibility testing (AST) using an overnight disc diffusion method (12-disc AST) as described by Dr. Paul Schrenkenberger, Loyola University School of Medicine in Chicago. This method was presented during the SCACM meeting in Indianapolis in October 2012.

The 12 disc AST results are recorded and compared with the automated system AST results that are received from the submitting clinical labs. This part of the study is to compare the susceptibility data results using two different methods and to better understand the recognition and reporting of the suspected CRE isolates based on this testing.

The ISDH laboratory also provides further testing of these organisms to confirm the presence of KPC, and/or NDM-1 strains, using the Modified Hodge test, the MBL E-test, and molecular test markers for KPC/NDM-1 detection.

The APHL/CDC grant for this project started in January 2013 and ended the first of July 2013. However the CRE testing of suspected CRE organisms has continued. The data chart shown below outlines information on the predominate organisms received from January through most of August 2013.

85 / total # isolates tested during Jan-Aug 2013	Predominate organisms rec'd Jan-Aug 2013	total # isolates tested	% of total isolates tested	total # positive for the KPC genetic marker	% positive KPC of total isolates tested	total # isolated from urine culture	% of total isolated from urine culture	57 total # isolates from urine culture
85 total isolates tested	<i>Klebsiella pneumoniae</i>	44	52	39	46	33	58	57 total isolates from urine
	<i>Escherichia coli</i>	10	12	4	5	7	12	
	<i>Enterobacter cloacae</i>	24	28	0	0	14	25	
	total	78	92	43	51	54	95	

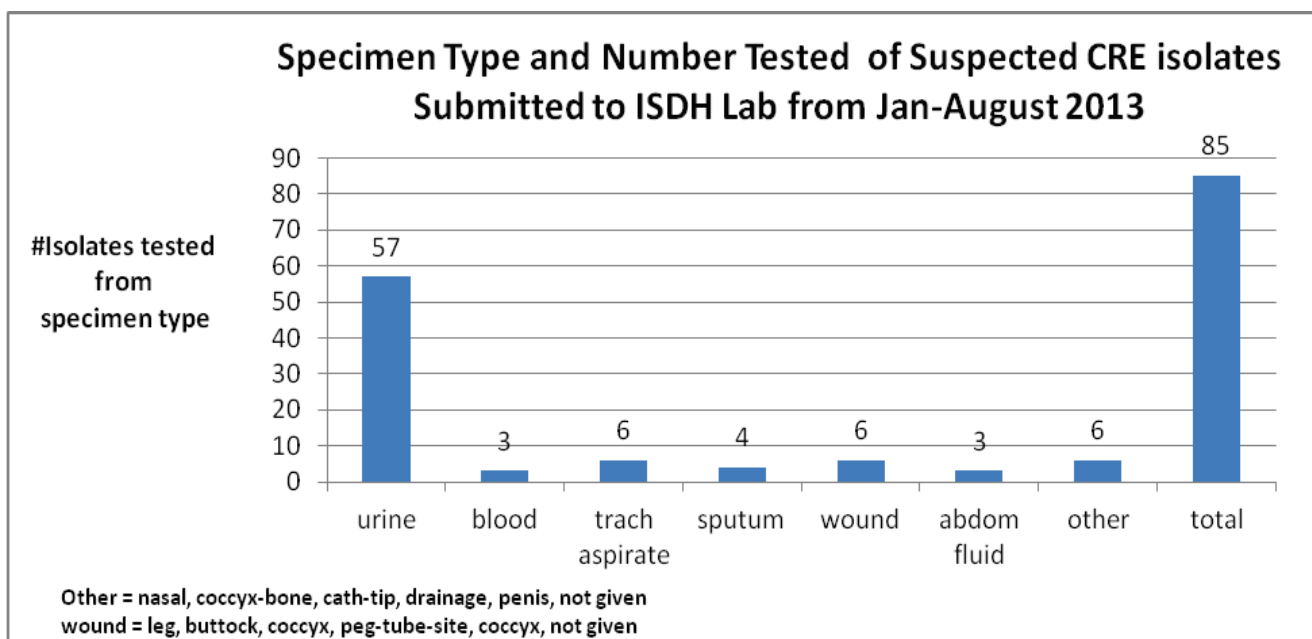
As of the end of August 2013, 85 isolates of suspected CRE have been received and tested. As noted by the data table above, three organisms have been the most prevalent submitted for the AST comparative testing, identification and confirmation of the KPC/NDM-1 strain type.

Out of the 85 suspected CRE isolates received, 44 (52%) were *Klebsiella pneumoniae* and 39 (89%) of these organisms were confirmed as KPC's.

The other two most commonly received organisms were *E. coli* and *Enterobacter cloacae*. These two organisms accounted for another 40% of the organisms received.

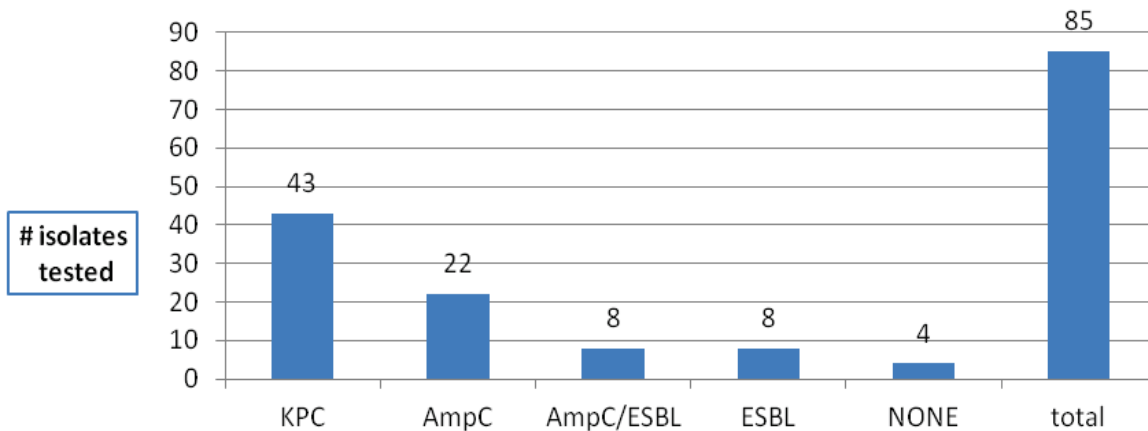
Cumulatively, these three organisms represented 92 percent of the suspected CRE isolates submitted, with 95 percent of that group isolated from a urine culture. Urine was the most common source of isolation for all organisms received (57 out of 85).

The other organisms received and tested (5%) were *Hafnia alvei*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Proteus mirabilis*, *Providencia stuartii*, and *Serratia marcescens*. All tested negative for KPC/NDM-1.



Enterobacteriaceae Update (continued from page 3)

Mechanism of Resistance Determined from 12-Disc AST Testing and Molecular KPC/NDM-1 Testing



NONE = isolate was susceptible to nearly all 12 antibiotics

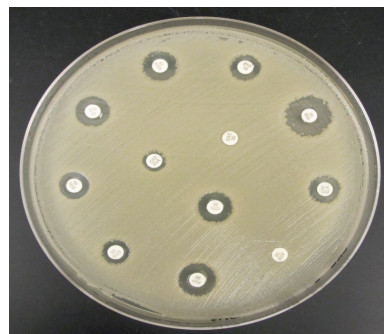
Based on the 12 disc antibiotic susceptibility testing results for these isolates, 95 percent of these were multi-drug resistant to some extent, or displayed some level of resistance to at least two or three different classes of antibiotics.

A large portion of the isolates, 38 (44%) displayed some level of resistance to many of the cephalosporin antibiotics tested as noted by the AmpC and ESBL groups displayed in the graph.

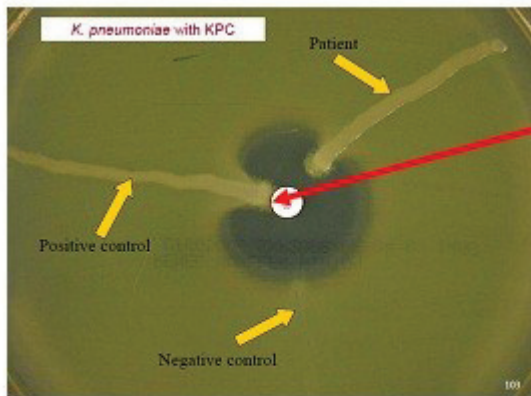
Four isolates received showed no resistance mechanism and were susceptible to nearly all of the antibiotics tested. Please see the table and photograph below for specific antibiotic resistance evidence.

Of all the isolates received at the ISDH laboratory, the majority were submitted by just a few hospital labs. We do have approximately 20 different clinical labs in Indiana participating with this project.

12-Disk Antibiotics	
Cefepime 30ug	Ceftazidime
Cefotaxime 30ug	Cefoxitin 30ug
Cefotaxime 30ug + Clavulanic Acid 10ug	Cefotetan 30ug
	Aztreonam 30ug
Ceftriaxone 30ug	Ertapenem 10ug
Ceftriaxone 30ug + Clavulanic Acid 10ug	Imipenem 10ug
	Meropenem 10ug



Pictured on next page are examples of two phenotypic lab tests showing the (MHT), Modified Hodge test used to confirm the presence of a carbapenemase/KPC producing organism, and the (MBL Etest), metallo-beta lactamase Etest, used to confirm a NDM-1 carbapenemase producing organism.



Modified Hodge Test (MHT) Dr. P Schreckenberger

1. **Positive control MHT**
Shows the indentation of growth or cloverleaf growth effect as a positive test.

2. **Patient labeled sample**
shows no/little indentation or negative result.



MBL Etest for NDM-1 Dr. P Schreckenberger

1. **Positive MBL result**
MIC of Imipenem (IP) bottom of strip is reduced by at least three doubling dilutions in presence of EDTA (IPI, top half of strip).

1. The ISDH lab CRE poster information (APHL/CDC grant data)

<https://myshare.in.gov/ISDH/islabs/Shared%20Documents/Forms/AllItems.aspx?RootFolder=%2FISDH%2Fislabs%2Fshared%20Documents%2FCRE%20Confirmatory%20Testing&FolderCTID=0x012000280FB539E8A199489AE443156EA7F0AE&View={73147AC1-4EEC-4BDB-8A4F-88D3B224C424}>

2. The ISDH Lab and ISDH Epidemiology CRE survey results and discussion.

<http://www.state.in.us/isdh/25499.htm>

Indiana CRE Survey of Carbapenem-resistant Enterobacteriaceae (CRE)

3. CRE Testing Method Information

http://www.scacm.org/PhenotypicDetectionAntibioticResistance_rev5.pdf

Phenotypic Detection of Beta-Lactamase Resistance in Gram-Negative Bacilli Testing and Interpretation Guide (Rev .2-21-12) Paul C.

Schreckenberger, Ph.D., Violeta Rekasius, MT(ASCP)

Contacts for further information and to submit suspected CRE organisms to the ISDH Laboratory:

Shelley Matheson, State Laboratory Training Coordinator, Indiana State Department of Health Laboratories Phone 317-921-5890
smatheson@isdh.in.gov

Jon Radosevic, Supervisor, ISDH Reference Bacteriology, 317-921-5860 jradosev@isdh.in.gov :

Communicable Disease Rule Training

By Jyl Madlem and Shelley Matheson

During 2011 and 2012, the ISDH Laboratories Outreach Team and ISDH Epidemiology Resource Center (ERC) collaborated on a new training program designed to educate laboratories and providers on the Indiana Communicable Disease Rule (CDR) by providing guidance on compliance for reporting communicable diseases. Developed to encompass all aspects of the communicable disease reporting process, attendees learned about the roles of physicians, nurses, laboratorians, and infection preventionists. Offered in all ten Indiana districts (Figure 1), interest and attendance were optimal. Indiana is a home rule state, meaning that each county is free to dictate how diseases are investigated by the local health department (LHD). In each district, a LHD Public Health Nurse presented the investigation from the LHD perspective, which customized the training for each district.

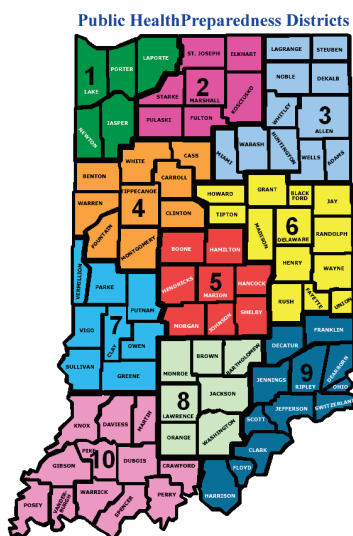


Figure 1: Indiana Preparedness District Map

The training sessions provided attendees details about when to report, how to report, why to report, and what the ISDH does with the data when diseases are reported. Attendees were presented with a basic knowledge of the epidemiology of disease, why reporting is required and how public health policy may be created as a direct result. Additionally, the laboratory component focused on how disease clusters are linked, how immunizations are created or modified to protect against new strains of disease, and how to properly submit isolates to the state laboratory for testing. Finally, the local health department overview, while similar in all districts, was unique to each county during the training sessions. The local health department presentations looked more closely at the investigation of disease, getting samples collected when necessary and issue resolution within each district.

Learning was evaluated using pre- and post-test assessments. Indiana Districts 3, 5, and 9 were completed in 2011. All other district trainings were held in 2012. In December 2011, the ISDH Outreach Team began using classroom-style responders for pre-and post-test assessments and presentations. Over all districts, the post-test scores increased by an average of 34.5%, however with the use of classroom responders, post-test scores were significantly higher (28.3%) than without. Figure 2 shows the percent increase in score from pre-test to post-test by district.

The ISDH Laboratories and ERC are currently reviewing the Communicable Disease Rule (Indiana Code 410 IAC 1-2.3). If significant changes occur in the code, this training will likely recur to highlight those changes.

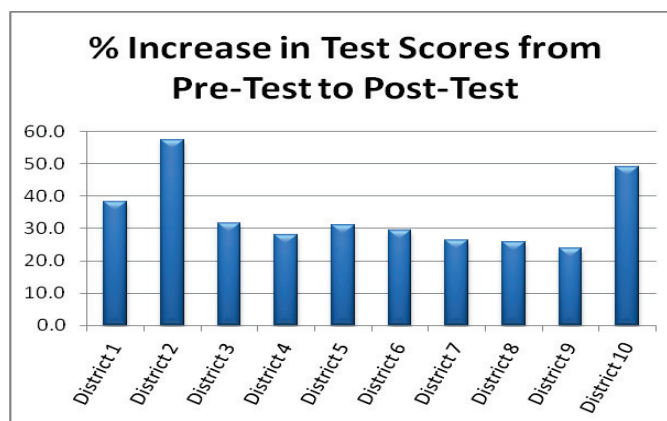


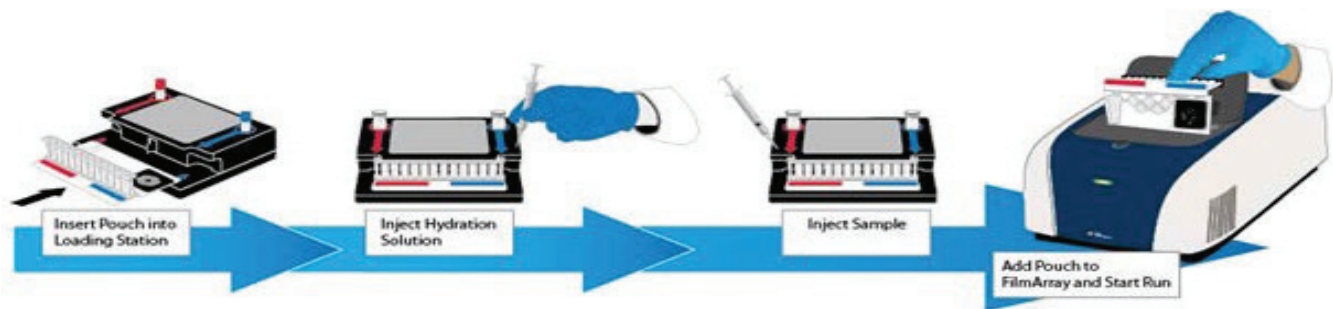
Figure 2: Learning Assessment by District

ISDH Virology is on "Fire"

By Stephanie Dalenberg and Jamie Hadley

Upper respiratory viruses impact everyone and can be particularly hard on the very young, immune-compromised and elderly. With symptoms that include cough, sore throat, headache, muscle ache, fever and fatigue, it is not surprising that diagnosis can be difficult. Since the typical cold and flu season is quickly approaching, the ISDH Virology Lab has been working hard to verify the newest addition to their lab, the BioFire FilmArray instrument. The FilmArray Respiratory Panel (RP) allows an accelerated analysis of nasopharyngeal (NP) swabs in those suspected of suffering from upper respiratory tract infections. This system uses a nested multiplex polymerase chain reaction (PCR) with high resolution melting analysis to detect nucleic acids from 20 different respiratory pathogens, both viral and bacterial.

The FilmArray pouch is a disposable unit that allows the isolation, amplification and detection of nucleic acids in a single NP specimen. Using a portable diagnostic device and the disposable pouch of freeze dried reagents, one specimen can be processed, tested and analyzed in approximately 70 minutes. Prior to the FilmArray RP verification, the previous method used at ISDH for the detection of multiple respiratory pathogens was a multi-step process conducted over two days. The implementation of the FilmArray system significantly shortens the time needed for results and, because it is a closed pouch system, greatly decreases the chance for PCR contamination.



The verification process for the FilmArray RP at ISDH entailed the testing of seven sample pools containing all 20 pathogens; each "pool" was spiked with three to four different respiratory pathogens. Testing was done over an eight day period with two analysts testing the pools in duplicate. All sample pools were run a total of four times and the results, including melting curves of the controls, were compared. Results of the verification were deemed satisfactory and all respiratory pathogens were detected as appropriate.

Implementation of the FilmArray in the ISDH Virology Lab will enable the lab to efficiently and effectively detect an outbreak of respiratory etiology in less than 24 hours. This is important because respiratory pathogens tend to be highly contagious due to the nature of their symptoms. These pathogens can be especially dangerous in institutional-like settings such as nursing homes, child care centers and dormitories where large numbers of individuals live or work within a relatively small space. The use of the FilmArray Respiratory Panel will allow the ISDH to quickly identify the respiratory pathogen(s) present so treatment and decontamination can begin in an effort to mitigate disease transmission and better serve the communities of Indiana.

Jyl Madlem Receives the June 2013 ISDH HERO Award

By Shelley Matheson



of its employees as a HERO.

Jyl Madlem, MS, MT(AMT), Laboratory Program Advisor at the ISDH Laboratories, was awarded the first ISDH HERO (Helping Employees Recognize One Another) Award in June 2013 by the ISDH Commissioner, Dr. William Van Ness. Jyl was presented her award at a surprise gathering of all laboratory employees on June 17, 2013 at the ISDH Laboratories in Indianapolis. Jyl received a \$1,000 Cash Spot Bonus, photo recognition with the Commissioner, and an award presented by the ISDH Executive Board. Each month the ISDH recognizes one



The ISDH HERO Award is an "Employee of the Month" program and was established by Dr. Van Ness in May. Any ISDH state employee may nominate another ISDH state employee to be recognized. The nominated employee must have provided excellent internal or external service beyond expectations, have led a successful project with outstanding results, or have performed above and beyond by taking initiative on a project or issue. The winners are selected by a small committee of Executive Staff members.

Jyl was nominated to receive the HERO Award by her colleague, Dana Greenwood, and was selected, in particular, for her recent development and implementation of a phlebotomy and specimen collection training for Indiana's public health nurses. Through this training, Jyl demonstrated dedication and commitment to improving the quality of laboratory services performed at public health clinics across the state. Jyl created the training using current, up-to-date resources and her own personal experience as a phlebotomist. During the creation of the training, she collaborated with different divisions in the laboratory and with other partners at ISDH, including the immunization and surveillance divisions. Jyl felt these trainings were so important, she spent many long days and many nights away from her young daughters. Over 100 public health nurses across the state have attended these trainings so far.

The results of the pre- and post-test conducted for knowledge of proper technique, site selection, specimen quality, needle selection, etc.; indicate that overall knowledge has increased 44 percent as a result of the trainings. Jyl received very positive comments on the training evaluations and in addition, Dana said that she received a great deal of candid one-on-one feedback from public health nurses. Some comments included, "this training is one of the few trainings I've attended in recent years that was really worth my time" and "I feel so much more confident drawing blood now."

Please join the ISDH Laboratories in congratulating Jyl on her accomplishments.

Kudos to the Food and Dairy Microbiology Laboratory

By Tom Cronau



On August 27, 2013 the ISDH Laboratories received recognition from bioMerieux, Inc. at the 127th Annual Meeting of the Association of Analytical Communities (AOAC) International conducted in Chicago. The recognition was for the participation of the ISDH Laboratories as study collaborator in a first action Official Method (OMA) approval for VIDAS UP *Listeria* (LPT) and VIDAS *Listeria monocytogenes* Xpress (LMX). bioMerieux remarked that it was a first for the industry, and for AOAC, to gain approval for two methods within just a three month period.

bioMerieux credited the good work of the ISDH Laboratories and the other collaborators for the success of the project. Dr. Hesham Elgaali, Supervisor of the Food and Dairy Microbiology Laboratory (FDML) represented the ISDH Laboratories at the presentation. The staff of the FDML includes Zach Beals, James Kirkman, Fatima McClain, Valrie Westmoreland and Mardene Wade.

In this era of ISO 17025:2005 Laboratory Accreditation, it is imperative that the regulatory public health laboratories at the Federal and State level use only validated, approved methods for screening and confirmation of the presence of pathogenic organisms such as *Listeria monocytogenes*. The AOAC International provides a rigorous method validation process that is available to industry, academia and government to evaluate proposed new methods as well as improvements or revisions to current methods. It is beneficial to the ISDH Laboratories to participate in these studies and maintain an up-to-date knowledge of the current and future technology and methodology associated with food microbiology.

The LAByrinth

The LAByrinth is published quarterly by the editorial staff of Indiana State Department of Health Laboratories.

Production Managers: Kirsten Long B.S., Michael Cross B.S

Editorial Board: Judith Lovchik, Ph.D. D (ABMM), Dave Dotson, M.S., Tom Cronau, M.S., Lixia Liu, Ph.D., Phil Zillinger

Director: Judith Lovchik, Ph.D., D (ABMM)

www.in.gov/isdh/24567.htm

Indiana State Department of Health Laboratories

550 West 16th Street
Indianapolis, IN 46202
Phone 317-921-5500
Fax: 317-927-7801

