A Year in Review

2009 Laboratory Annual Report

Indiana State Department of Health Laboratory

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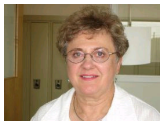
Letter from Laboratory Director



Dr. Judith Lovchik

Dear Friends and Supporters:

The Indiana State Department of Health Laboratories will remember 2009 as the year the Influenza Pandemic finally arrived, after decades of planning. The pandemic proved once again that the future is unpredictable. While the disease produced by the new virus was not nearly as deadly as had been feared, the laboratory had to act without this fore-knowledge. This meant hugely increased specimen testing load, especially at the beginning of the pandemic. Lab staff pulled together as a cooperative team to insure that our epidemiologists and clinicians had the best current information possible. Confident in the ability of our staff to deal with crises, I attended the annual Association of Public Health Laboratories meeting in Anchorage, Alaska early in the pandemic, while many other State Public Health Laboratory Directors skipped the meeting, fearing their labs could not function without them for a week. My confidence in our ISDH lab staff was well-founded, as they functioned at peak capacity without serious incident for the duration of the pandemic.

Regular Epidemiology-Laboratory joint meetings were initiated in 2009 by our Outreach team of Ellie Carter and Shelley Matheson, which have greatly enhanced communications between partners. It was also a year of intense preparation for the Indiana State Laboratory System Improvement Program, which was kicked off in October, after being delayed by the Pandemic.

Another highlight of the year was the visit from the Shanghai Institute for Food and Drug Control in November. You can read about these and other highlights in this, the second Annual Report, put together by former Laboratory Program Advisor Ellie Carter and a new team of editors: Jamie Hadley, Lyndsey Hensler, Liz Church, and Kara Hammes.

Sincerely,

Judith C. Lovchik

Judith Lovchik, PhD, D(ABMM)
Assistant Commissioner
Laboratory Director

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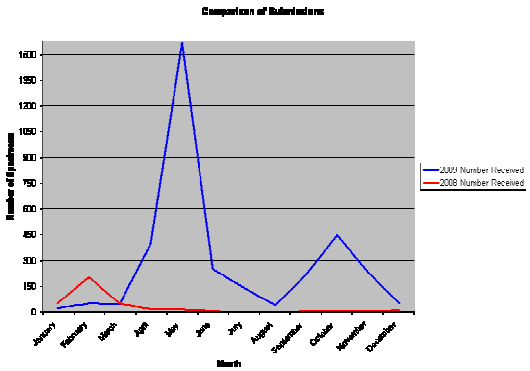
Virus Isolation room with staff working in Biological Safety Cabinets (BSCs)

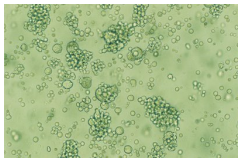
Of Swine and Men...a recap of 2009 H1N1

The virology laboratory faced many new and difficult challenges due to a dramatic increase in the number of specimen submissions for influenza testing, as well as the addition of the H1N1 subtype testing. The lab was trying to deal with the processing and testing hundreds of samples each week with the outbreak of the 2009 Pandemic Influenza. The first challenge was to determine a priority system for specimen testing. Due to a limited number of reagents, equipment and staff, all samples could not be tested as they were submitted; therefore; the lab was forced to become more restrictive in acceptance of sample submissions. For a period of time, specimens were screened and sorted for testing based on specific clinical history. Specimens from patients who had died, were seriously ill, pregnant, less than two years of age or over 65 were considered higher priority and immediately tested for influenza while lower priority specimens were set aside for testing at a later date to prevent reagent and supply shortages. A testing algorithm was also established to conserve reagents and efficiently produce results. Instead of testing each specimen for every possible influenza subtype, testing concentrated on the circulating strain. With help from the Centers for Disease Control and Prevention (CDC), we were able to receive reagents and supplies in a timely fashion and maintain testing volume. Shortly after the outbreak occurred, alternative reagent kits were made available in case of additional shortages. The virology staff quickly confirmed that these new kits produced accurate results for both the detection of seasonal and pandemic H1N1 flu viruses.



Graph of influenza sample submissions 2008 and 2009 by month

In addition to molecular testing by polymerase chain reaction (PCR), specimens sent for influenza testing are typically also set-up for virus culture. This allows the lab to send influenza isolates to the CDC to assist with antiviral resistance monitoring in influenza strains as well as to enable the composition of the annual vaccine to be determined. Throughout the year, the virology staff continued to send influenza isolates to the CDC. Another advantage to virus culture is that it allows for the detection of other circulating respiratory viruses. Due to the large number of samples received during the 2009 pandemic influenza outbreak, virus culture had to be greatly reduced because of the amount of time required to complete this method of testing. The staff was able to further adjust the testing algorithm so that virus isolation was performed only on a representative set of all specimens submitted. Implementing this reduction in virus culture brought to light additional challenges pertaining to our Laboratory Information Management System (LIMS).



Cytopathic Effect (CPE) in virus isolation

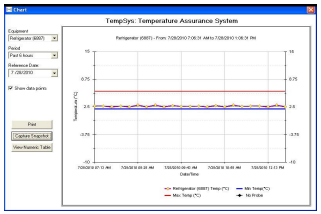
H1N1 continued

The virology laboratory LIMS module in place at the time of the outbreak was designed for normal, routine sample testing, which includes both PCR and Virus Isolation, not pandemic-sized volume. In order to have a system capable of handling the specimen load and meeting testing and reporting demands, ISDH and LIMS staff quickly tested and moved a PCR-only module into production just weeks after the outbreak onset.

Equipment and instrumentation issues during the pandemic were also encountered. The shortage of reagents and supplies, combined with the restrictive nature of CDC's Food and Drug Administration (FDA) approved PCR procedure for novel H1N1 virus, forced the laboratory to use an extraction instrument that is not routinely utilized. Because of this, instrument performance issues were initially encountered. These issues required emergency maintenance to be performed to ensure that the instruments were at their optimal working conditions. Finally, staffing became a challenge as it was immediately apparent that normal working hours were not sufficient to handle the incoming specimen load. The ISDH virology staff worked many long days and weekends to sustain testing and help reduce the backlog. In addition, many other dedicated ISDH staff members worked extended and weekend hours to help with time-consuming tasks such as specimen accessioning, data entry and specimen reporting.

Despite the many challenges we faced during this pandemic, we will be better prepared and able to handle similar challenges in the future. In the words of Confucius, "Success depends upon previous preparation, and without such preparation there is sure to be failure."

Wireless Temperature Monitoring



The data can be monitored by the designated analyst in real time by either a graph of a numeric table

The ISDH Laboratories invested in a wireless temperature monitoring system developed and distributed by TempSys. The system is designed to monitor the air temperature of the various refrigerators, freezers, ultra-low freezers, and incubators that store expensive test kits, reagents and other consumable supplies. This system allowed for storage of the vaccine for the H1N1 prevention efforts in the fall of 2009 because it was critical that the temperature records of the vaccine storage be collected and documented. These units also store irreplaceable clinical specimens that are archived.

Thermometer and TempSys monitor inside equipment.

There are several useful features of the current system, which supports 130 different units located on four floors. The air temperature in each unit is measured and reported every 15 minutes. An e-mail alert system is programmed to notify staff when the air temperature in the unit is out of the acceptable range, even outside of routine work hours. An analyst can even remotely check the temperature status of any assigned equipment.

There are future plans to improve this system by upgrading the sensor units so that they can continue to capture temperature data during a power failure and to replace the server hardware with a virtual server that will reside off site in order to provide better security and reliability.

ISDH Chemistry help Indiana Wildlife

The U.S. Department of the Interior Fish and Wildlife Service and Indiana Department of Environmental Management (IDEM) met with ISDH Laboratory staff on the afternoon of June 9, 2009. The ISDH laboratory chemistry staff was presented with a plaque and letters of appreciation by the U.S. Fish and Wildlife Service in recognition of their work on a U.S. Fish and Wildlife Service project. The findings of this project were summarized in a study entitled "Diagnosis of Contaminant Patterns in Streams and Rivers of National Wildlife Refuges in Indiana" published in the Fish and Wildlife Journal.

Picture at ISDH Labs following award presentation

The U.S. Fish and Wildlife Service undertook an investigation into the levels of contamination and the impacts of contamination on the aquatic life in two watersheds that supply three of Indiana's National Wildlife Refuges (NWR). Among the sources of contaminants are acid mine drainage, effluents from oil and gas exploration, and runoff from agricultural and high-density residential land use areas. The Big Oaks NWR includes portions of a former military base, the Jefferson Proving Grounds, and suffers from the residual contamination of exploded ordnance.

The Patoka River watershed supplies the Patoka NWR while the Vernon Fork of the Muscatatuck River supplies both the Muscatatuck NWR and the Big Oaks NWR.

The number and diversity of aquatic species living in the rivers and streams were catalogued within the National Wildlife Refuges and chemical analyses were completed on the rivers and streams both in the field and in the laboratory. The U.S. Fish and Wildlife Service used the data provided by the ISDH Chemistry Laboratories to plot "hot spots" for each analyte within each refuge. Possible sources of the contaminants were also identified. The data collected will continue to play an important role in future analyses as established baselines for long-term trends in the water quality at all three NWR.

Peanut, Peanut Butter and Salmonelly...*Salmonella* outbreak

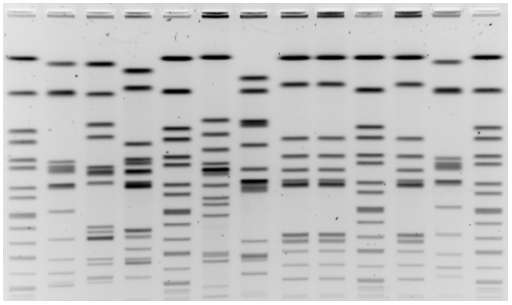
From January to April 2009, the ISDH Enterics Lab, the Pulsed-Field Electrophoresis (PFGE) Lab, and the Food Microbiology Lab helped investigate the nationwide *Salmonella typhimurium* outbreak associated with peanut butter and peanut butter-containing products. During that time, the Food Microbiology Lab participated in the Food Emergency Response Network's (FERN) nationwide effort to quickly identify the source of contamination. Most of the food samples submitted for testing to ISDH were peanut butter crackers. All samples received were negative for the presence of *Salmonella* species. The Food Microbiology Lab also provided testing assistance to the North Carolina Department of Agriculture as they tested production lines for cheese and peanut butter crackers after the outbreak.

Peanut Butter Crackers

Top: Enterics Lab biochemicals set-up for testing
Bottom: Positive for Salmonella

During this same time period, the ISDH Enterics Lab identified 47 specific cases of salmonella from human specimens; eighteen of these were *typhimurium*, the same strain as the nationwide outbreak. These samples were then analyzed using PFGE. This method allowed the comparison of the relatedness of different bacterial strains at the DNA level via the creation of unique DNA fingerprints or patterns. The salmonella DNA fingerprints were then matched against local and national DNA pattern databases, allowing the rapid identification of common source outbreaks and the separation of outbreak associated cases from other, sporadic cases. Ten clinical specimens tested by the ISDH Enterics Lab had the same PFGE type as the national outbreak and were officially linked to it.

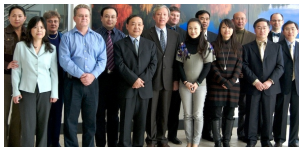
The ability to identify the specific strain of bacteria infecting a patient enables identification of possible sources of the outbreak for further investigation. Clearly, the capability to perform the entire testing process on food or clinical specimens, from isolation and identification to PFGE analysis, is a valuable asset and service provided by the ISDH Laboratories.



DNA bands from a PFGE run

Making connections across continents

On November 10, 2009, ISDH Laboratories were honored to host delegates from the Shanghai Institute of Food and Drug Control (SIFDC), their equivalent of the U.S. Food and Drug Administration (FDA). Their goal for this trip was to visualize laboratory building structures that could accommodate the needs of food microbiology testing for the detection of organic and inorganic compounds in food, drug, and cosmetics products.



Delegation with ISDH Lab staff

The delegates toured the second floor and focused on the Food and Dairy Laboratory area. ISDH Lab staff explained the testing process, the instruments used, and the lighting design of the plating room. The delegates also toured the Chemistry Laboratories and admired the design feature that utilizes movable laboratory benches and ceiling mounted data lines. The delegates were also interested in the rapid detection method of protein digestion in the Food Chemistry area.

Another main focus of the visit was to tour the mechanical and inner workings of the building. The SIFDC group was shown the HEPA and HEGA filter manifold, air handling systems and the electrical and data control boards.

The SIFDC delegates expressed their interest in sending a staff member to the ISDH Laboratories for training in the future. A common interest in creating long term collaboration was established.

Delegation viewing the inner workings of the building

Broadening our technological horizons

The ISDH Laboratories continued to expand implementation and use of the STARLIMS Sunrise Laboratory Information Management System (LIMS) as well as the in-house developed LIMSNet system.

LIMSNet is accessed by hospitals, local health departments and clinics to enter demographic information regarding specimens prior to mailing. LIMSNet increases data accuracy by using edits and drop-down boxes during data entry. Electronic data eliminates paper storage and retrieval at the laboratory. Test submitters enjoy the ability to track specimen status as samples are received and tested. This results in faster access to reports and secure access to patient information.

STARLIMS®
LAB DATA/ENTERPRISE ACTION

STARLIMS collects and stores more information per specimen than previous systems could accommodate. STARLIMS tracks each specimen from arrival to final report and shares data with appropriate divisions and partners.

STARLIMS processes around 2,000 specimens in a typical week, of which 97 percent are received electronically. The following assays are in production and in use: Chlamydia/Gonorrhea, Syphilis, HIV, Hepatitis A/B/C, Herpes, Influenza, and Varicella. Assays soon to be functioning in STARLIMS include TB, Blood Lead, Rabies, and Dairy.

What can the lab do for you?

Bioterrorism Lab Workshop at ISDH Labs

The ISDH Lab's Outreach program had a productive and successful 2009. The ISDH LAByrinth, the lab's bi-monthly newsletter, had its first electronic publication with the March-April 2009 issue. In addition to the newsletter, ISDH also communicated to Indiana Sentinel Laboratories with lab-related updates and announcements of continuing education opportunities as well as urgent lab alerts throughout the year. Particularly during the 2009 H1N1 influenza outbreak, ISDH Laboratory Outreach team functioned as a reliable and important partner within Indiana's laboratory system.

The Outreach team coordinated and conducted two Hands-On Bioterrorism Lab Workshops and four Packaging and Shipping of Dangerous Materials Certification Trainings in Indianapolis. Additional trainings were held outside of ISDH including a Bioterrorism Lab workshop at White County Hospital, a Packaging and Shipping training at Clark Memorial Hospital, and another Packaging and Shipping training at Community Hospital in Munster.

The ISDH Lab Outreach Team was invited to participate in several scientific and public health related meetings and conferences as a way to connect with the wide array of health care workers in the state. These gatherings included the annual and regional meetings of the South Central Association for Clinical Microbiology (SCACM), the Indiana Public Health Week Conference, the Indiana Public Health Nurse Conference, the Indiana Refugee Health Conference, and the American Lung Association in Indiana Tuberculosis Education Program Symposium.

Display for SCACM meeting

The ISDH Lab also hosted several tours for clinical laboratory science undergraduate students, medical residents, and a group from the Hoosier Association of Science Teachers, Inc. We were also able to have Field Epidemiologists visit the lab to learn more about the test performed at the ISDH Lab.

Let's Make a Deal, how we can improve together

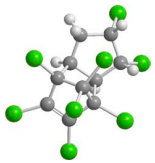
Sixty public health stakeholders and laboratorians from around the State met at the Indiana Government Conference Center on October 23, 2009 to tackle the questions: What is the Indiana State Public Health Laboratory System, what does it do, how well does it perform, and what should it do better?

In its efforts to support public laboratories, the Association of Public Health Laboratories (APHL) has created the Laboratory System Improvement Program (L-SIP) as a tool for state public health laboratory systems to assess their programs and identify areas of improvement.

Indiana became the 20th state to conduct such an assessment as this project was spearheaded by Dr. Judy Lovchik, ISDH Assistant Commissioner and Laboratory Services Director. The initial meeting was accomplished by inviting stakeholders from all over the state and from various disciplines that either contribute to or are impacted by laboratory services in Indiana. An ISDH Lab team will serve as a hub for communication and coordination with the goal that Laboratory System Improvement projects will be a collaborative process where all system partners will be directly involved.

Deadly Disagreement - fish - innocent victims

Diagram of Chlordane



Last August the ISDH Organic Lab was called to action by the Indiana Department of Environmental Management (IDEM) to investigate a criminal fish kill in a neighborhood pond.

According to reports, two neighbors had been feuding over access and rights to the use of a local pond, until one neighbor retaliated against the pond itself. The man went home and returned with a chunk of white material, approximately the size of a baseball, and threw it into the pond. Witnesses stated that an entire section of the pond appeared to die almost immediately. Fish and other organisms began to float to the top and rot. IDEM was alerted and took a sample of the white substance.

This sample was submitted to the ISDH Organic Chemistry Laboratory for testing. The substance was identified as 3 percent Chlordane, a highly chlorinated and toxic insecticide. The use of this man-made mix of chemicals began in 1948 and was sold under the names Octachlor® and Velsicol 1068®. Before 1978, it was applied on lawns, gardens, and crops as a general pesticide, but from 1983 until 1988, it was restricted for use as a pesticide for termites only. Eventually, the Environmental Protection Agency (EPA) recognized it as a cancer risk and all approved uses ceased in 1988. However after decades of use around homes and for agriculture purposes, chlordane persists in the environment and continues to cause contamination concerns.

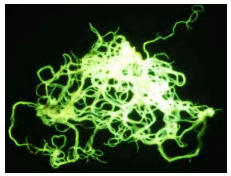
Chlordane chemical

Apparently, the feuding neighbor had stored a large amount of this extremely hazardous chemical at his home for years. Perhaps he did not realize that exposure to this dangerous mix of chemicals can cause significant health risks. Chlordane, a human carcinogen, has been shown to cause acute effects, such as headaches and dizziness, as well as long-term neurological disease. Additional information about chlordane can be found at the websites for the EPA and the Agency for Toxic Substances and Disease Registry.

Tuberculosis Outbreak in Marion County

Tuberculosis, a disease caused by Mycobacterium tuberculosis complex (TB), is one of the world's deadliest diseases. One third of world population is infected with TB. As the result of the TB Control efforts in U.S., a rate of 4.2 cases per 100,000 persons was reported in the United States in 2008. However, don't let your guard down just yet; a TB outbreak has occurred in your backyard-Marion County, Indiana.

The TB outbreak started in May 1, 2009 in a homeless population in Marion County. During May 1, 2009 to July 28, 2010, the ISDH TB Laboratory performed 257 tests that included 126 smears, 7 real time PCR, 138 cultures and four first line drug susceptibility tests for 23 patients. Fifteen out the 23 patients were confirmed cases that had matching genetic fingerprint. These laboratory test results enabled the ISDH TB Control program to promptly investigate the outbreak and assure the TB infection control.



Positive fluorescence staining of a TB organism with cording

In addition to participate in the Marion homeless TB outbreak investigations, the ISDH TB Laboratory partners with the ISDH TB Control program to work towards the goal- Eliminate TB.

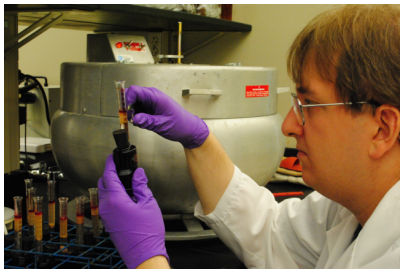
Elevated Blood Lead levels send investigators to the store

In recent years the Burmese refugee population has grown within the United States. Approximately 24,000 Burmese refugees were resettled in the United States between 2007 and 2009. In 2008 alone, approximately 5,000 Burmese refugees were resettled in Fort Wayne, Indiana, and 800 to 1,000 new arrivals were expected each year throughout the next several years.

In the second half of 2008, the Fort Wayne-Allen County Department of Health and the Indiana Lead and Healthy Homes Program began investigating elevated blood lead levels (EBLLs) in children within the Burmese refugee population in Fort Wayne. Concern was raised about the incidence of EBLLs among Burmese refugee children because these children had been resettled in lead-safe or lead-free housing upon reaching the United States or they had been born in the United States to Burmese refugee parents. It appeared as though these children were developing EBLLs after arriving in the United States. An initial investigation examined rice, tea leaves, medicine, cosmetics, spices, and candy but found no causative agent. Therefore, in February 2009, the ISDH Epidemiology Resource Center launched a special surveillance program to determine the prevalence of EBLLs among Burmese refugee children in Fort Wayne and to identify potential sources of lead exposure.

Some products tested in the lead contamination investigation

The ISDH Food Chemistry Laboratory and Metals Laboratory worked together to analyze samples for both of the investigations. Samples of items that are ingested were prepared for testing by the Food Chemistry Laboratory and analyzed using inductively coupled plasma-mass spectrometry (ICP-MS) by the Metals Laboratory. Results of these tests indicated that several traditional Burmese medicinal products contain high levels of dangerous chemicals, including lead. Daw Tway, a digestive aid for young children, was found to have lead levels higher than 450 ppm, which is a sufficient concentration to cause EBLLs with regular use. Additionally, Daw Tway and another digestive aid, Wonotsay or Daw Kyin, along with a chest rub product, Htet-Lin, were all found to have elevated levels of arsenic.

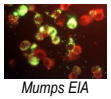


Food Chemistry staff diligently at work

At the conclusion of this study, the ISDH Chemistry Laboratories received a letter of appreciation from the Fort Wayne-Allen County Department of Health's Health Commissioner Dr. Deborah A. McMahan, MD and Amy Hesting, REHS, the Healthy Homes Program Director. Work on this project continues in 2010 with the submission of additional food products.

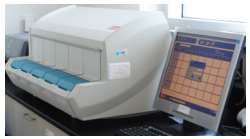
Lab Innovations

- Enteric Lab: Real-Time PCR for the detection of Shiga toxin producing *E. coli* was implemented in April 2009. This assay is in multiplex format and can detect and differentiate between Shiga toxin1 and Shiga toxin2 producing *E. coli*
- Reference Bacteriology Lab: Real-Time PCR for the detection of *Bordetella pertussis* was implemented in December 2009. This test allows for the rapid detection of *B. pertussis* in clinical patient specimens.
- PFGE Lab: A protocol for subtyping of *Neisseria meningitides* was implemented in December 2009. This protocol allows for the lab to subtype and assess relatedness of *N. meningitides* isolates.
- Serology Lab: The Mumps IgM Capture EIA replaced a less specific IFA assay, reducing the number of false positive results for mumps, in December 2009. The same month, testing was completed for the West Nile IgM Capture ELISA validation. This assay replaced an older capture ELISA protocol written by the CDC. The new version results in a faster assay time.



Mumps EIA

- Virology Lab: An Acid Lability Test was implemented in February 2009 which allowed the virus isolation laboratory the ability to differentiate acid stable viruses [i.e., enteroviruses] from acid labile viruses [i.e., rhinovirus]. Real-Time rt-PCR Detection of H1N1 was implemented in May 2009 to allow for the detection of the emerging H1N1 Influenza subtype during the 2009 pandemic. A Measles Virus Indirect Immunofluorescence Assay (IFA) was implemented in December 2009 which enabled the laboratory to detect and confirm measles virus in cell culture.
- Food and Dairy Lab: A Mini-Vidas was upgraded to a full-size Vidas. This new instrument expanded the capacity to conduct the Salmonella surveillance testing of ground beef and beef carcass swabs submitted by the meat inspectors of the Indiana Board of Animal Health. This instrument uses technology referred to as Enzyme Linked Fluorescent Assay to rapidly detect *E. coli O157*, *Listeria*, *Salmonella,* and Staph Enterotoxin.



Vidas machine

- Food Chemistry Lab: A method for the analysis of metals in food products was introduced which eliminated the muffle furnace for sample preparation and replaced it with a block digestion method. The new method reduced the analysis cost in both labor and equipment. It also improved the recovery of heavy metals by eliminating transfer steps. A nicotine analysis was added in May 2009 for the testing of fresh dissolvable tobacco designed to be swallowed. This was added at the request of the Indiana Tobacco Prevention and Cessation Division due to concerns about the concentration of nicotine in the product. The GC/MS instrument measures the quantity of nicotine contained in smokeless tobacco products.
- Chemical Threat Lab: Added three new assays as a Level 2 LRN laboratory that can be used in response to a chemical threat or chemical spill.
- Inorganic Chemistry Lab: Decreased the amount of mercury used in the Chemical Oxygen Demand (COD) analysis and mercury use was eliminated in the Total Kjeldahl Nitrogen (TKN) analysis.

Administration

Clerical

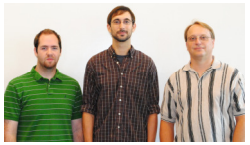
Containers

Serology & Tuberculosis

Microbiology

Virology/EPR

Reference Microbiology



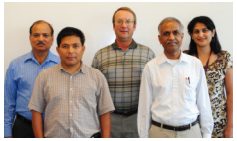
Molecular Development

Food & Dairy

Food Chemistry

Metals

Water Chemistry



Organic Chemistry

Inorganic Chemistry

Blood Lead & Radiology

Thank you to all the contributors for this report:

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