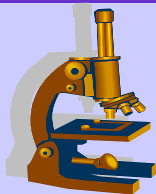
January/February 2011 Issue No. 12



The LAByrinth

Indiana State Department of Health Laboratories Newsletter

Advancing DNA Sequencing Technology

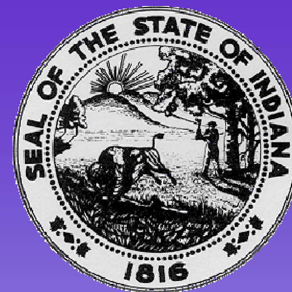
By Robert Pawlak

Next generation DNA sequencing technologies are rapidly entering molecular diagnostics arena, but should the traditional sequencing methods be retired?

Since the structure of the DNA double helix was discovered in 1953, and the subsequent advent of modern DNA sequencing methods in the mid-70s, tremendous progress has been made towards deciphering the genetic makeup of prokaryotic and eukaryotic organisms. The genome for the bacteriophage *phi X174* was the first to be sequenced in 1977 by Frederic Sanger and his colleagues at the University of Cambridge, England. Sanger used, novel at that time, the "DNA sequencing with chain-terminating inhibitors" method published by his group in the PNAS journal the very same year. Only 18 years later, a complete genome of *Haemophilus influenzae* was published in Science; not a small task considering it is 1,000 times larger in size than that of the viral genome of *phi X174*. Since then, more than 900 prokaryotic genome sequences have been completed and many more projects are currently in progress. The turn of the millennium brought what many scientists consider the apex of all genome sequencing efforts - Craig Venter's group from the TIGR consortium published "The Sequence of the Human Genome" article in the February 2001 issue of Science. This inundation of readily available genome sequence data, deposited in the GeneBank and other public databases by scientists around the world, revolutionized many areas of the life sciences. Some of these include: human genomic research and clinical molecular diagnostics, identification of etiologic agents in microbiology laboratory practice, microbial taxonomic and evolutionary studies (phylogeny), as well as the field of forensics- just to name a few.

For over a quarter of century, Sanger's "chain termination method" served as the workhorse for all DNA sequencing projects, only recently to be challenged by the next generation sequencing technologies. These novel methods offer many advantages over the chain termination strategy, including higher throughput, very high accuracy, technical simplicity, ease of automation, and reduced labor & cost. For example, pyrosequencing, the next generation sequencing

(Continued on page 2)



Indiana State Department of Health Laboratories

Gregory N. Larkin, M.D.
State Health Commissioner

Loren L. Robertson, M.S.
Deputy State Health Commissioner

Judith Lovchik, Ph.D
Assistant Commissioner & ISDH Lab Director

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:

DNA Sequence Technology	**1-3**
Proficiency Testing in the ISDH Laboratories	**3**
Shiga-Toxin Producing E. coli	**4-5**
Pesticide Analysis in Drinking Water	**6-7**
ISDH Dairy Lab Certification	**8**
Employee Spotlights	**9**

Advancing DNA Sequencing Technology (continued from page 1)

method recently implemented by the ISDH Molecular Test Development Laboratory for the detection of mutations conferring microbial drug resistance, can process the same number of samples in half the time and at one sixth the cost of traditional sequencing. Pyrosequencing relies on a cascade of enzymatic reactions sequentially adding nucleotides to the nucleic acid chain synthesized along the DNA template. Addition of a nucleotide complementary to the sequencing template, results in generation of a luminescent signal that is registered by the pyrosequencer as the match for the nucleotide. Unlike Sanger's method, pyrosequencing does not require expensive fluorescent primers, gel polymers and capillary arrays to resolve sequencing products, significantly bringing down the cost of the assay. Since pyrosequencing generates short sequences, data analysis is not as involved as with long and complex reads of traditional sequencing, assuring faster turn-around times. This is an important factor in the ISDH molecular diagnostics laboratory. Due to the method's inherent accuracy, pyrosequencing really shines when it's applied to the detection of the "single nucleotide polymorphisms" (SNPs). SNPs are point mutations resulting from single nucleotide changes at defined positions in a genome that frequently serves as a biomarker for hereditary predisposition to diseases, microbial drug resistance, and phylogenetic studies. For example, *katG315* and *rpoB* point mutations confer *Mycobacterium tuberculosis* resistance to the antibiotics isoniazid and rifampin respectively, and H274Y SNP confers pandemic influenza resistance to antiviral drug oseltamivir (Tamiflu) – all three tests are currently performed at the ISDH Laboratories. To achieve the level of accuracy offered by pyrosequencing, the chain termination method would have to be performed multiple times with redundant sequencing runs for each clinical sample. In addition, single nucleotide changes need to be located in long stretches of the sequence complicating data analysis and delaying reporting of the results.

So are we ready to switch sequencing-based molecular diagnostics entirely to pyrosequncing? Probably not yet, or at least not all the way. One drawback of this novel method is the inability to provide long sequence reads following a single sequencing run. While chain termination method can reliably sequence an average of 700-1,000 base pairs of DNA on a single pass, pyrosequencing is limited to 40-80 base pairs. Obtaining the same genomic coverage requires multiple overlapping pyrosequences that are later assembled into one continuous long "consensus" sequence – something better left to a genomic research facility, not a clinical laboratory. New pyrosequencing chemistries promise to increase attainable sequence length to an estimated 150 base pairs; however, it is still a far cry from the Sanger method. In our molecular diagnostics laboratory, this sequence length limitation particularly affects microbial identification applications. For example, sequencing of 16S rRNA locus, a genetic target commonly used for bacterial identification, in most cases requires sequencing of at least the first 500 base pairs of the gene since this is the region where most of the interspecies variations or genetic polymorphisms are located. For bacterial genera with interspecies polymorphisms spread over longer stretches of the gene, 1,000 base pairs or even the full 16S sequence (1,500 base pairs) is necessary to obtain proper species designation. Sanger's method easily provides reads of such lengths on one or two sequencing passes while single base pair inaccuracies intrinsic to this technology do not really matter for accurate identification of all but the most closely related species. Another good example of the chain termination method's superiority is Enterovirus (EV) typing. Accurate EV typing requires sequencing of about 350 base pairs of the virus's almost uniformly polymorphic VP1 capsid protein gene; a difficult task to accomplish via pyrosequencer route in a typical clinical laboratory setting but easily done using the chain termination method. Both, 16S rRNA and EV typing are currently performed at the ISDH laboratories using the Sanger method.

While Sanger's chain termination approach still remains the sequencing method of choice for identification of most microbes encountered in clinical laboratory practice, attempts are being made to utilize next generation technologies to applications where sequence length is not critical for microbial identification. This route is only possible for pathogens displaying interspecies variations clustered in short regions of their genomes with the rest of the DNA sequence conserved between species. In such instances, it is perfectly feasible to obtain accurate species ID by pyrosequencing the short, variable regions only, instead of the whole target gene or a significant portion of the target gene. Two such applications of pyrosequencing are currently utilized at the ISDH Laboratories, one for speciation of Mycobacteria and the second for the identification of members of the Nocardia genus. For both genera, it seems sufficient to obtain one or two 30 base pair pyrosequences from variable portions of their 16S rRNA genes for proper species designation thus avoiding the cost, labor, and technical complexity associated with the full-length 16S chain termination sequencing.

As next generation technologies continue to be optimized for increased sequence reads, high-throughput capabilities, and cost efficiency, they may ultimately emerge as universal methods of choice for all sequencing applications in a molecular diagnostics laboratory. For now, however, the 30 year-old Sanger DNA sequencing method via chain termination still offers advantages in the microbial identification arena that will keep it from being retired for quite some time.

Proficiency Testing in the ISDH Clinical Laboratories

By Chris Grimes, QA Coordinator

The ISDH Laboratories encompasses a wide variety of different testing areas. As part of our integral Continuing Quality Improvement, each area is responsible for checking the assay work they do. The most common way to do that is through Proficiency Testing (PT). Our clinical laboratories receive PT surveys sent to us from various outside vendors and sources like the Centers for Disease Control and Prevention (CDC), the Association of Public Health Laboratories (APHL), College of American Pathologists (CAP), Wisconsin State Laboratory of Hygiene (WSLH), and American Association of Bioanalysts (AAB).

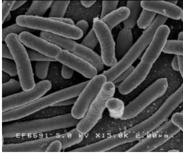
Each of these surveys consists of a series of unknowns - specimens that may or may not contain specific analytes. We receive them and handle them as though they were normally submitted specimens. Once we are done testing the specimens, we send the results back to the vendor or source for evaluation. To maintain our CLIA certification, we have to score well on each of our PT surveys, too. For example, to maintain our CLIA certification, we have to correctly identify at least 80 percent of the specimens in each of the PT surveys for the clinical laboratory areas, such as bacteriology, serology, virology, parasitology, Mycobacteriology, mycology, blood lead, and molecular diagnostics. To meet that 80 percent level, we can only misidentify one out of the four or five specimens we receive for each testing event.

Throughout the entire 2010 calendar year, the ISDH Clinical Laboratories received 247 specimens for proficiency testing and we correctly identified 243 of those, for an overall score of 98 percent for the year. Of course, even when we passed a PT survey event, but still misidentified a specimen; we conducted an investigation to determine what happened and how we could prevent it from happening again in our laboratories.

All of these PT surveys help to demonstrate the diligence and hard work of the ISDH Clinical Laboratory staff. The high level of quality built into our preanalytical, analytical, and post-analytical processes is only possible through the attention to detail and vigilance of the ISDH Laboratories staff members.

Tune in next time for an update on the quality of testing in the ISDH Environmental Labs!!

Shiga-toxin Producing E. coli (STEC): What is it? Where Does it Come From? How Does STEC Work in a Living System? How Can STEC Infections Be Prevented?



This information was compiled by Sean O'Connell of the Tennessee Department of Public Health Laboratories. Originally published in their newsletter from Summer 2010, Volume 4, issue 1. Reprinted with permission granted by Ms. O'Connell.

What is STEC?

Escherichia coli (abbreviated as *E. coli*) are a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others can make you sick. Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness, pneumonia, and other illnesses. *E. coli* 0157:H7 is the most noteworthy because much of its pathogenicity (ability to cause disease) comes from the production of Shiga toxins. These Shiga toxins can be produced by over 100 serotypes of *E. coli* and collectively this group of bacteria are referred to as Shiga-toxin producing *E. coli* or STEC. The Shiga toxins produced by the bacteria are responsible for the condition known as hemorrhagic colitis, the source of the bloody diarrhea associated with STEC infections. Shiga toxin production is also responsible for hemolytic uremic syndrome (HUS), a complication of infection that leads to kidney failure. Shiga toxins derive their name from the bacterial organism from which they were first identified, *Shigella dysenteriae*. *S. dysenteriae* causes Shigellosis (bacillary dysentery), which, like STEC infections, can cause severe, bloody diarrhea. There are two types of Shiga toxin, Stx1 and Stx2, which may be produced by STEC.

Who gets STEC infections?

People of any age can become infected. Very young children and the elderly are more likely to develop severe illness and hemolytic uremic syndrome (HUS) than others, but even healthy older children and young adults can become seriously ill.

What are the symptoms of STEC infections?

The symptoms of STEC infections vary for each person, but often include severe stomach cramps, diarrhea (often bloody) and vomiting. If there is fever, it usually is not very high (less than 101°F/ less than 38.5°C). Most people get better within five–seven days. Some infections are very mild, but others are severe or even life-threatening.

How are these infections spread?

Infections start when you swallow STEC—in other words, when you get tiny (usually invisible) amounts of human or animal feces in your mouth. Unfortunately, this happens more often than we would like to think. Exposures that result in illness include consumption of contaminated food, consumption of unpasteurized (raw) milk, consumption of water that has not been disinfected, contact with cattle, or contact with the feces of infected people. Some foods are considered to carry such a high risk of infection with STEC, or another germ, that health officials recommend people avoid them completely. These foods include unpasteurized (raw) milk, unpasteurized apple cider and soft cheeses made from raw milk. Sometimes the contact is pretty obvious (working with cows at a dairy or changing diapers, for example), but sometimes it is not (like eating an undercooked hamburger or a contaminated piece of lettuce). People have become infected by swallowing lake water while swimming, touching animals and the environment in petting zoos and other animal exhibits and by eating food prepared by people who did not wash their hands well after using the toilet. Almost everyone has some risk of infection.

How Does STEC Work Inside a Living System?

Much of the pathogenicity associated with STEC comes from the production of Shiga toxins. When Shiga toxin is released from a STEC bacterium, it is responsible for the bloody diarrhea associated with STEC infections because it kills cells of the intestine which causes bleeding and swelling. As a result, the body increases permeability of intestinal cell barriers so that important cells of the immune system (neutrophils/PMN's) can reach the *E. coli* infection. Shiga toxin may also use this opportunity to break through the walls of the digestive tract, enter the blood stream and bind white blood cells for transport to locations such as the kidneys or the central nervous system (brain and spinal cord). Once Shiga toxin reaches a target organ such as the kidney, it binds to receptors on cell membranes. The toxin is then brought inside the cell and stops the cell from producing proteins it needs to function. Without the ability to sustain its function, the cell dies. This is the case with HUS which results from damage to the kidney cells. Shiga toxins are also responsible for the changes in the central nervous system that is associated with severe HUS. In coordination with pro-inflammatory factors Shiga toxins cause damage to the endothelial cells that make up the blood-brain barrier. This barrier is extremely important and normally prevents pathogens and toxins from causing damage to the brain itself.

How can STEC infections be prevented?

1. WASH YOUR HANDS thoroughly after using the bathroom, changing diapers, and before preparing or eating food.

2. WASH YOUR HANDS after contact with animals or their environments (at farms, petting zoos, fairs, even your own backyard).

3. COOK meats thoroughly. Ground beef and meat that has been needle-tenderized should be cooked to a temperature of at least 160°F/70°C. It is best to use a thermometer, as color is not a very reliable indicator of "doneness."

4. AVOID raw milk, unpasteurized dairy products and unpasteurized juices (like fresh apple cider).

5. AVOID swallowing water when swimming or playing in lakes, ponds, streams, swimming pools, and backyard "kiddie" pools.

6. PREVENT cross contamination in food preparation areas by thoroughly washing hands, counters, cutting boards, and utensils after they touch raw meat.

Added by Kirsten Long, ISDH Enteric Lab:

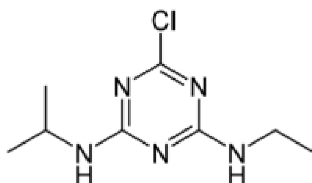
At the Indiana State Department of Health Laboratories we have recently implemented a new standard operating procedure to include testing for the "Big 6" along with E. coli O157:H7. The "Big 6" are the six most common non-O157 STEC reported in the United States according to CDC. They are E. coli O26, O45, O103, O111, O121, and O145. If an isolate is determined to be STEC positive by PCR, but E. coli O157 negative, it will be tested with antisera for the "Big 6" as well. If the serotype is still undetermined, the isolate will be forwarded to the CDC for further testing.

Information available from the
National Center for Zoonotic , Vector-Borne and Enteric Diseases,
Centers for Disease Control and Prevention, Atlanta, Georgia
For more information on STEC visit:
http://www.cdc.gov/nczved/divisions/dfbmd/diseases/ecoli_o157h7/

Pesticide Analysis in Drinking Water

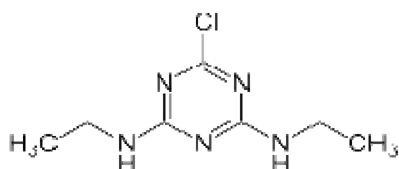
by Mark Starzynski

Two of the pesticides the Indiana State Department of Health Lab's Organic Chemistry Lab are required by the Federal Safe Drinking Water Act (SDWA) to analyze for in drinking water are Atrazine and Simazine. These are members of the family of pesticides known as triazines.



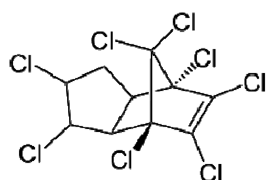
Atrazine

Atrazine is one of the most widely used herbicides in the world, although its use was banned in the European Union in 2004. It is used to kill broad-leaf weeds in Indiana corn crops; however it has been linked to birth defects in humans and is an estrogen mimic in some aquatic life. Atrazine is suspected of causing sterility in three out of four male frogs, and changing one in 10 male frogs into females at levels several times less than the maximum contaminate level (MCL) in drinking water, which is three parts per billion. If the drinking water comes from a surface water source such as a river or reservoir, there can be small amounts of Atrazine present due to runoff from farmer's fields. If the drinking water comes from an underground source, Atrazine is not normally present due to biodegradation. In Indiana, Atrazine has been found in surface water.



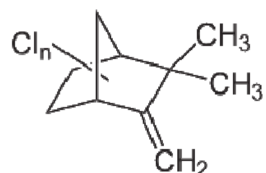
Simazine

Simazine is also one of the most widely used herbicides in the world, although its use is now banned in the European Union. The maximum contaminate level (MCL) in drinking water is four parts per billion. In Indiana, Simazine has been found in Indiana's rivers and streams.



Chlordane

Two other pesticides that ISDH organic chemistry lab tests for are Chlordane and Toxaphene. Chlordane was an insecticide for home use sold as an ant killer with the trade name ORTHO. Chlordane was banned in 1983, except for use on termites. Chlordane was further banned as a termiticide in 1988.



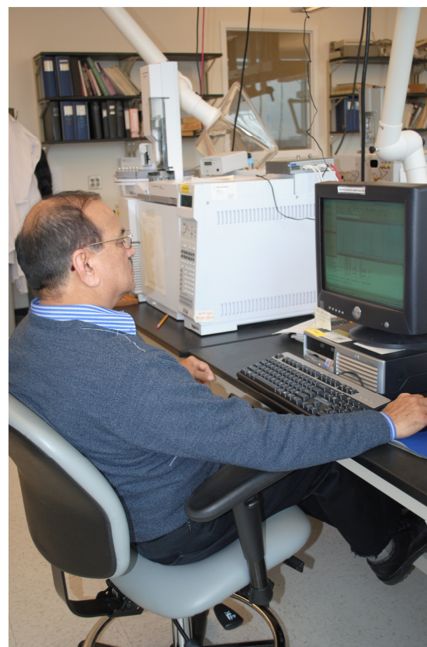
Toxaphene

Toxaphene is a mixture of hundreds of organic compounds. It is a miticide which was used on cattle, mostly in California, during the mange outbreak of the 1970s. It was also used as an insecticide in the Southeastern U.S. for cotton and soybean crops. Toxaphene, as the name suggests, is highly toxic and was banned in the U.S. in 1986. It was one of the first nine compounds to be outlawed in May 2001 by the entire world during the Stockholm Convention on Persistent Organic Pollutants. This law was instated through an international environmental treaty that went into effect in May of 2004.

In Indiana, the Indiana Department of Environmental Management (IDEM) has identified dumping sites that contain hazardous wastes such as Chlordane and Toxaphene which could leach into ground water and our drinking water.

This is just a small sampling of four of the approximately 100 organic compounds that are required to be tested for in Indiana's drinking water. In this way, the Organic Chemistry Lab helps protect your Indiana drinking water.

Samples to be analyzed on a gas chromatograph.



Mohammad Zaman performing calculations for Atrazine and Simazine

Yaqub Masih extracting samples from Indiana's rivers and streams for Atrazine and Simazine.

Types of Pesticides

There are many different types of pests and many chemicals used to kill them.

- *Insecticides* are used to kill *insects*.
- *Herbicides* are used to kill *plants*.
- *Fungicides* are used to kill *fungi*.
- *Algaecides* are used to kill *algae*.
- *Rodenticides* are used to kill *rodents*.
- *Nematocides* are used to kill *nematodes* (worms).
- *Miticides* are used to kill *mites*.

ISDH Dairy Laboratory Passes FDA Certification

By Tom Cronau

ISDH Dairy Laboratory team: From left to right, Louis Douglas, Fatima McClain, Zach Beals, Valerie Westmoreland, Mardene Wade, Hesham Elgaali, and Jim Kirkman.

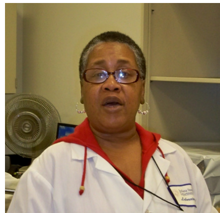
Indiana differs from some states in the manner that it regulates and monitors the dairy industry located within the state. Although it follows the same Food and Drug Administration (FDA) and National Conference of Interstate Milk Shipments (NCIMS) rules and regulations as the other States, Indiana divides the duties and responsibilities between two separate State agencies. In many States, all aspects of the dairy industry including the farms and processing facilities, are inspected and regulated by a department of agriculture. These departments typically include the inspectors, laboratory support, administrators, and dairy lab evaluation officer(s).

In Indiana, the laboratory support is currently provided by the ISDH Food and Dairy Laboratory (FDL) Section of the Indiana State Department of Health Laboratories. The remaining functions/duties including enforcement are performed by staff of the Indiana Board of Animal Health (BOAH). In 1996, all but the laboratory functions of the Dairy and the Meat and Poultry Divisions were transferred to the BOAH from the ISDH. This included the State Dairy Laboratory Evaluation Officer.

In order to provide the required laboratory support to the Dairy Division of BOAH, the FDL must comply with the rigorous Quality Assurance and Control requirements administered by the Laboratory Proficiency and Evaluation Team of the FDA. One feature of this required program that is not found in most certification programs is that all the analysts are individually evaluated for each FDA approved method for the official examination of Grade A milk and other milk products. This is accomplished by an on-site audit every three years and the successful completion of a panel of FDA provided proficiency samples on annual basis.

Just weeks prior to the scheduled FDA on-site inspection in June 2010, two of our staff members resigned to make major career changes. Under the leadership of Hesham Elgaali, Ph.D. and the hard work of his remaining staff, Valerie Westmoreland, Jim Kirkman, Mardene Wade and Louis Douglas, the on-site audit was passed. It was not until two weeks prior to the annual dairy proficiency samples that a replacement was hired. Again, due to the effort of the new employee, Zach Beals, and his coworkers and immediate supervisor, he was able to earn Conditional Approved status until he participates in the next FDA on-site visit. All of the remaining analysts including Dr. Elgaali, have obtained or maintained their FDA status to perform the FDA official examinations of dairy products. It is planned that the second replacement hire, Fatima McClain, who started in February 2011, will also successfully learn the dairy procedures and participate in the annual proficiency samples typically scheduled for mid-September.

Employee Spotlight– Sharon Garrett



Sharon Garrett has been an employee of the Indiana State Department of Health Labs for the past 20 years. She started her tenure in housekeeping, but moved to the Containers lab after six years. At her present position she packages up and ships out empty sample collection containers to all of our submitting clinical labs, local health departments, some hospitals, and private individuals. Her section of the department consists of the environmental and water collection containers and mailers.

Sharon is a native of Indianapolis. She is the granddaughter of a Baptist minister and grew up singing in the choir. She has had a very colorful life outside of work. She has done things such as model for a Hair stylist, won fourth place in the Black Expo dance contest, helped deliver a few babies, and played multiple sports. Sharon is a woman of all trades. She has been a first grade reading tutor at IPS, a customer service/repair person for Sears Service Center, and assembled record players and televisions at RCA. More recently she worked as a bartender. In her spare time she likes to volunteer at the American Legion #249, serving our vets. She says that she has met some of the most interesting people there over the past 14 years.

Sharon has two daughters and is a grandmother of four and great-grandmother of one. She spends a great deal of time with her family and caring for her mother. Sharon would like to start taking dancing lessons, specifically hula dancing, in the not-so-distant future and loves all forms of dance and music.

Employee Spotlight– Karl Leatherman

Karl Leatherman is our Media lab guru. He originally started in the Virology/Immunology labs at the Indiana State Department of Health Labs over 26 years ago after attending classes at IUPUI. The ISDH Media department is where we order most of our plated media, tubed biochemicals, and many other forms of reagents needed for our daily testing in the Microbiology and Chemistry sections of the labs.

When not working, Karl likes to relax at home. He's a laid back guy who just likes to hang out. He is a local member of Society for Creative Anachronism (SCA). This is an international organization dedicated to researching and re-creating the arts and skills of pre-17th-century Europe. This includes forging weaponry such as chain mail and swords & creating authentic costumes and jewelry. It is quite an involved art. Karl helps with the re-enactments at local events and fairs.

The LAByrinth

The LAByrinth is published bi-monthly 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., Robin Bruner, B.S., Lixia Liu, Ph.D.

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

Indiana State Department of Health Laboratories
550 West 16th Street
Indianapolis, IN 46202
Phone 317-921-5500
Fax: 317-927-7801

Check us out!

www.in.gov/isdh/24567.htm