



THE LABYRINTH

Indiana State Department of Health Laboratories Newsletter



**Indiana State
Department of Health
Laboratories**

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Public Health Protection
and 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.

Introducing the TB/MAC PCR

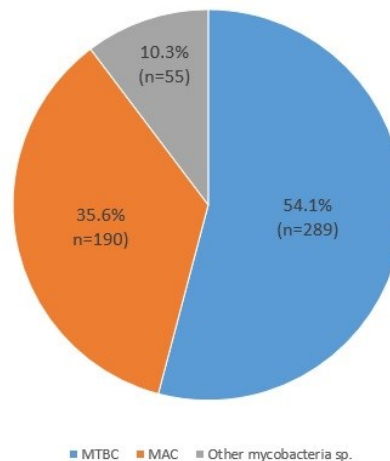
*By Jessica Gentry,
TB Laboratory Supervisor*



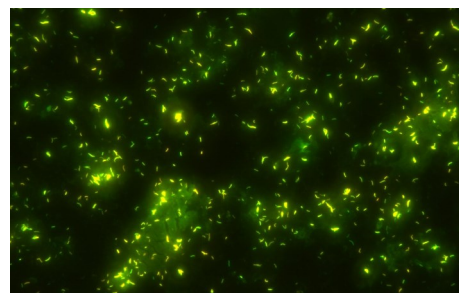
The Indiana State Department of Health (ISDH) Tuberculosis (TB) Laboratory recently added a new Polymerase Chain Reaction (PCR) assay to their existing portfolio of tests. The new TB/MAC PCR assay will be able to detect and differentiate both the

Mycobacterium tuberculosis complex (MTBC) and the *Mycobacterium avium* complex (MAC). The *Mycobacterium avium* complex includes the species *M. avium*, *M. chimaera*, and *M. intracellulare* and is the second most common group of organisms to be isolated at the ISDH TB Laboratory (35.6%, see Figure 1). MAC organisms are commonly found in the environment and can cause opportunistic infection in patients who are elderly or immunocompromised. When these bacteria infect the lungs, they often cause a pulmonary disease with symptoms very similar to that of active tuberculosis. However, MAC patients are not infectious to others and are not considered a public health threat. While both groups of organisms typically respond to antibiotics, different classes of antibiotics are used for MTBC or MAC infections. For these reasons, rapidly differentiating these two types of infections plays an important role in public health.

Figure 1. FY17-18, TB Lab Isolates Received or Recovered



When a patient is under evaluation for TB disease, clinicians typically collect three sputum specimens over a period of 2-3 days. Once these specimens are received in the laboratory, a process of digestion and decontamination is performed in order to liquefy the sputum and kill off the normal flora present (see Figure 2). Next, a smear is made of the processed sediments, which is fixed and stained with an acid fast stain. Organisms that are acid fast, such as mycobacteria, will appear as bright yellow-green fluorescent bacilli. A TB patient who has one or more positive acid fast smears would be considered infectious, and would require respiratory isolation in order to prevent them from infecting others. As MTBC and MAC are virtually identical microscopically, additional testing is needed in order to differentiate these two groups. Generally, smear-positive patients are put into isolation until the MTBC can be ruled out or until the identification of the acid-fast bacillus (AFB) is known.



Tuberculosis under fluorescent microscope

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Introducing the TB/MAC PCR (continued from page 1)

The TB Laboratory has been performing real-time PCR for the detection of MTBC in sputum for many years, as it enables the laboratory to conclusively identify smear positive TB patients rapidly. Until now, however, we were unable to identify smear-positive, MTBC PCR-negative patients until the culture had grown. As mycobacteria are such slowly growing organisms, AFB cultures must be incubated for several weeks (typically 4-5), before an identification could be made.

The new MAC PCR greatly improves the detection time of these organisms, helping to rule out TB infection much sooner. Both of the assays will be used to test both specimens and cultures (see Figure 2).



The MTBC PCR assay is similar to the one previously in use, utilizing the same target of the IS6110 element. This target allows the assay to be extremely sensitive, and also is specific to MTBC. The MAC PCR assay targets the internal transcribed spacer (ITS) region of the mycobacterial ribosome, enabling it to detect the different species in the complex. Another benefit of the new assay is that it now runs on a different instrument platform (QuantStudio Dx), which allows the laboratory to test a larger number of specimens per run, using a 96-well plate format.

We anticipate the new TB/MAC PCR assay will allow the laboratory to continue to detect MTBC infection rapidly, while helping to rule-out suspected TB patients more quickly than before. This will aid in furthering the public health goals of reducing the incidence of TB infection, while also reducing the number of patients unnecessarily treated with anti-TB medications.

Chemistry Lab Analysis Pulls Deadly Product off the Market

By Pradip Patel
Food Chemistry Supervisor



During the first week of April 2018, the Rhode Island/Massachusetts Poison Control Center alerted the Indiana State Department of Health (ISDH) of a patient who appeared to be experiencing symptoms associated with methanol poisoning. The patient arrived at the hospital with a half-consumed 32 oz. bottle of 190 proof non-denatured grain alcohol that had been purchased from an Indiana supplier. Later, the patient died and their physician stated the cause of death was methanol toxicity. ISDH's Food Chemistry Laboratory was contacted and asked if it would be possible to analyze an alcoholic mixture for methanol content. Food Chemistry's copy of the Association of Official Analytical Chemists (AOAC) International's Official Methods of Analysis contained a method for methanol analysis in alcoholic mixture, but the laboratory did not have some necessary reagents on site. Due to the interstate nature of the situation, ISDH Food Protection entered into a joint agreement with the U.S. Food and Drug Administration (FDA) and the U.S. Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF) to investigate the source and nature of the ethanol product, while the laboratory waited to receive the necessary reagents for analysis.



Pictured here: 32 oz. sample submitted for analysis

An interview with the patient's family indicated the patient had ordered the alcohol online and had a history of using alcohol to make consumable tinctures, extracts of plant or animal products, using alcohol as a solvent. Instructions for how to make tinctures using kratom leaves, marijuana, and other similar products for medicinal or recreational use are readily available on the Internet. ISDH Food Protection also found the seller of the alcohol was not registered with any state or federal agency as an alcohol producer; rather, it was registered with the Office of the Indiana State Chemist as an animal feed producer. The bottle of alcohol brought to the hospital was discarded before any state or federal agency could ask for it to be analyzed.

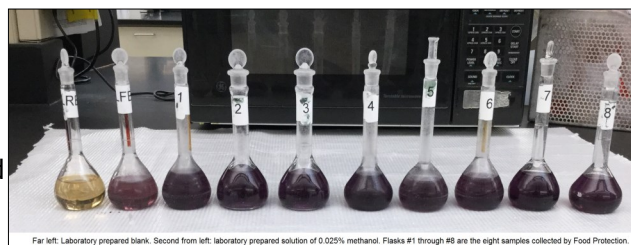


Unconventional distillation unit found at seller's production facility

When reagents were received, ISDH chemist Aaron Bolner performed a qualitative analysis using AOAC method 958.04 to ensure methanol could be detected. Known solutions containing methanol all turned purple, showing the procedure worked as intended. He then performed quantitative analyses with sets of calibration standards and verified when using a visible light spectrophotometer, methanol could successfully be detected at levels as low as 0.025% in a mixture with water and ethanol.

After the laboratory informed ISDH Food Protection of their readiness to accept samples, an on-site inspection, investigation and sample collection was scheduled for the first week of May 2018, with representatives from ISDH Food Protection, FDA and ATF participating. Inspection revealed the production facility had no procedures, documentation, or experience in proper distillation procedures. Furthermore, the distillation unit being used was unconventional (see Figure 3). Samples from seven bulk containers of ethanol, as well as one 32 ounce bottle of finished product labeled as 95% ethanol / 5% water, were collected for analysis.

A portion of each sample was analyzed by the laboratory for three days in the second week of May. The first day's results showed each sample was so high in methanol content it was above the detection limits of our laboratory instrument. Analysis was repeated on the second day using a series of dilutions to determine the level of dilution necessary to achieve results within the scope of the method. Finally, on the third day, quantitative results were obtained. Each sample, including the product marketed as the finished distilled product, was found to contain at least 40% methanol by volume. These results were provided to ISDH Food Protection on May 10, 2018. ISDH Food Protection and its federal partners contacted the seller with these results; within two weeks, the seller agreed to issue a voluntary recall on all of their ethanol products going back to October 2016 and ceased their ethanol operations.



Far left: Laboratory prepared blank. Second from left: laboratory prepared solution of 0.025% methanol. Flasks #1 through #8 are the eight samples collected by Food Protection.

ISDH Herpes and Chickenpox (Shingles) Viruses Testing Moving to Molecular Diagnostics Method

By *Nicolas Epie, Ph.D., TS (ABB),
Virology and Serology Director*



Fig1: Lesions due to Herpes Simplex Virus (HSV-1) (L) and Shingles or Herpes (Varicella) Zoster Virus (VZV)

We have some exciting news for Indiana clinicians and epidemiologists who send specimens collected from patients suspected of Chickenpox, Shingles or other Herpes viruses to the Indiana State Department of Health Laboratory (ISDHL) for testing (Fig 1). Test results will soon be available to submitters in our laboratory information management system (LIMS) faster than before. This is now possible due to the fact that ISDH is moving from our virus isolation testing method to a molecular detection method for Herpes Simplex Virus 1 (HSV1), Herpes Simplex Virus 2 (HSV2), and Varicella Zoster Virus (VZV). This method will deliver faster result turnaround time.

Previously, ISDHL provided diagnostic testing services for HSV1, HSV2 and VZV using a virus isolation method. We had a CDC-approved molecular method for VZV for use in a few highly pathogenic suspected cases, but none was available for HSV1 and HSV2. A virus isolation method requires specimen inoculation on cultured cell lines and virus replication in the inoculated cells shown as cytopathic effects (CPE) when viewed under a microscope (1). Although the cell culture method has always been the classic method for virus detection in clinical and public health laboratories, the method is labor-intensive, time-consuming, and has lower sensitivity. When combined with fluorescent antibody (FA) staining it is, highly specific for the tested virus.

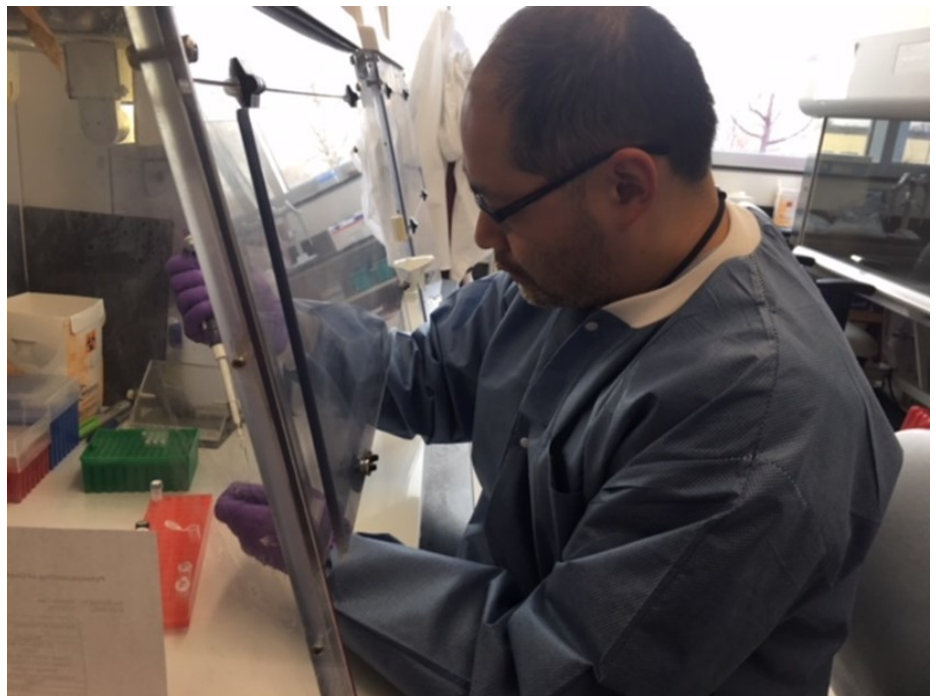


Fig 2: ISDHL Virologist (William Lee) performing real-time PCR at a Molecular Virology Workstation.

Over the past three decades, significant technological advancements in the field of microbiology have been introduced into clinical and public health laboratories. One of these methods now routinely used in many hospital laboratories and clinics is polymerase chain reaction PCR. Real Time PCR relies on the amplification of specific viral nucleic acids present in an infected patient specimen (Fig 2). These nucleic acids are detected in real time, using fluorescent- coupled DNA molecules called probes, in a reaction with short DNA molecules called primers (Fig 3) (2).

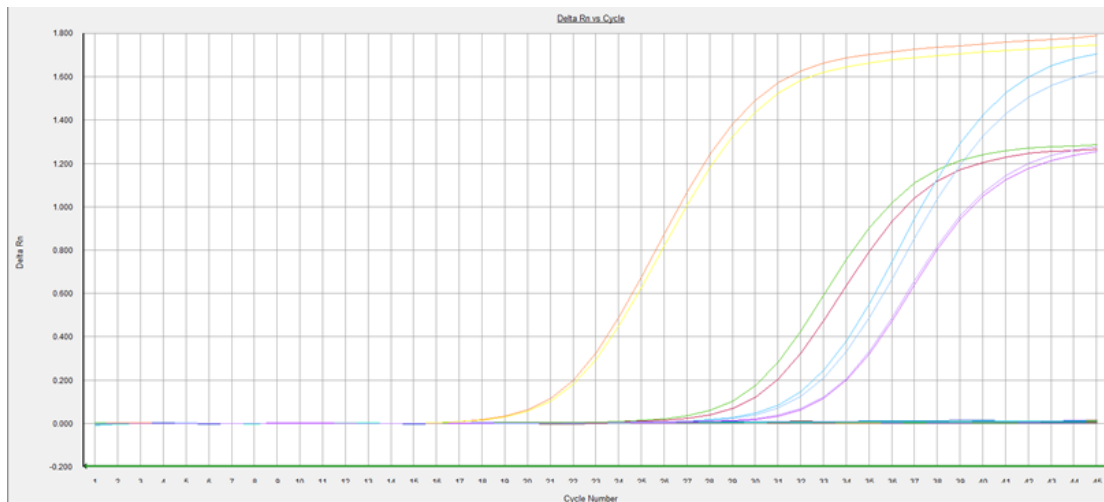


Fig 3: An image of Real Time PCR amplification curves displayed on a Computer screen by Indiana State Department of Health Laboratory (ISDHL) Scientist.

The validation process of a PCR method for HSV1/HSV2/VZV testing is complete and the procedure is now added to our methods of approved tests for patient testing of HSV1/HSV2/VZV. This PCR method will be used for the diagnostic testing swabs of vesicular fluids, skin lesions, genital sites, oral, rectal, ocular sites which collected from patients with suspected HSV/VZV.

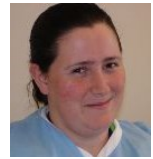
Good News:

Providers who send specimens to ISDHL will now get their results in less than a week. This PCR method for HSV1/HSV2/VZV will detect any of these three related viruses present in a patient specimen, in the same test procedure. To learn more about the HSV/VZV testing at ISDHL, submitters can contact Dr. Nicolas Epie: nepie@isdh.in.gov or Brian Pope: bpopel@isdh.in.gov

1. Yu, X., Seitz, S., Pointon, T., Bowlin, J. L., Cohrs, R. J., Jonjic, S., Haas, J., Wellish, M., and Gilden, D. (2013) Varicella zoster virus infection of highly pure terminally differentiated human neurons. *Journal of neurovirology* 19, 75-81
2. Han, Y., Hou, S. Y., Ji, S. Z., Cheng, J., Zhang, M. Y., He, L. J., Ye, X. Z., Li, Y. M., and Zhang, Y. X. (2017) A novel method of multiple nucleic acid detection: Real-time RT-PCR coupled with probe-melting curve analysis. *Analytical biochemistry* 537, 50-55

Changes to Shiga-toxin producing *Escherichia coli* Testing Protocol

By Jamie Yeadon-Fagbohun,
Enteric Laboratory Supervisor



Last year brought significant changes to the testing protocol for *Escherichia coli* at the Indiana State Department of Health Laboratories (ISDHL). These changes involved a method comparison that was conducted prospectively and a method validation that was completed retrospectively. Shiga-toxin producing *Escherichia coli* (STEC) is a foodborne pathogen that can cause infections in humans. Symptoms of a STEC infection include severe abdominal cramps and diarrhea (sometimes bloody) that begin between 3 to 4 days after exposure, and typically last 5 to 7 days. Occasionally, the infection can progress to a more serious disease known as hemolytic uremic syndrome (HUS), which can lead to kidney failure and possibly death. Due to the nature of the infection, it is important that ISDHL is able to identify and serotype potential STEC specimens in a timely manner.

Changes were made to the *E. coli* algorithm to better follow the recommended guidelines from the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL). A prospective method comparison was completed on March 26, 2018 that looked at the source of material for the initial STEC Polymerase Chain Reaction (PCR) screen and the identification of *E. coli* O157. A comparison for the STEC PCR screen starting material between the current method of an overnight GN broth and the new method of a MacConkey Agar (MAC) plate sweep was completed. Twenty-four specimens from a variety of submitters and original specimen types were chosen. There was 100% agreement reached between the two methods on the STEC PCR screen. Operator variance was also completed and 100% consensus was received. The identification of *E. coli* O157 is completed using a rapid card test. The current method consists of a plate sweep from a MAC or a Sorbitol MacConkey Agar (SMAC) plate and using the Meridian O157 STAT! Card while the new method is to take three colony picks from the MAC or SMAC plate and run them on the Oxoid Latex Card. Twenty-four specimens from a variety of submitters and original specimen types were chosen. There was 100% agreement reached between the two methods on the *E. coli* O157 identification. Operator variance was completed and 100% consensus was also received. Both of the method comparisons were completed and adopted at ISDHL. In addition to saving time, the new changes provide a cost savings for the lab.

In late 2017, ISDHL was informed that Cepheid would be discontinuing the production of the Smart Cycler II instrument including all reagents and service. The ISDHL Enteric laboratory has been using this platform for the testing of *E. coli* isolates for the presence of shiga-toxin by PCR since 2008. The ability to detect shiga-toxin positive isolates is of vital importance due to the clinical importance and the increased severity of disease when shiga-toxin is present.

CDC Enteric Diseases branch, as well as the enteric sections of three state public health laboratories (Michigan, Minnesota and Wisconsin) were contacted to research other methods for the detection of shiga-toxin. All four laboratories were willing to share their standard operating procedures (SOPs) with us and answer any questions we had on their processes. Each of the SOPs shared used different instrumentation for the PCR assay, as well as different extraction methods. We knew we wanted to look at primer and probe sets that would detect the *stx2f* gene variant as it had recently become more widespread in the United States. A comparison of all of the SOPs was completed that looked at the extraction process, including time, cost, primer/probe targets, and the PCR process, including instrumentation. We decided to go with a hybrid of the protocols received for our in-house validation to meet our unique requirements here at ISDHL.

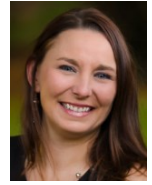
The instrument chosen for the validation was the ABI 7500 fast Dx due to its ease of use and that it is already in use in our laboratory. None of the protocols we reviewed used the ABI 7500 fast Dx but it was similar enough to some of the other platforms, we knew it was possible. A quick comparison between two different heat prep extractions was completed on 5 specimens. The results were very similar, so the shorter, more cost-effective protocol was chosen. A real-time multiplex PCR was chosen that had primers and probes to look for *stx1*, *stx2* and *stx2f* markers in the DNA. The validation was completed using 57 specimens, which included a combination of negative, *stx1* positive, *stx2* positive, *stx2f* positive, and *stx1&2* positive specimens. A variety of specimen types and different serotypes of STEC were chosen. The validation also included operator variance and repeatability studies that compared CT values between runs and analysts.

There was 100% agreement received between the current method on the Cepheid Smart Cycler II and the new method on the ABI 7500 fast Dx. There was also 100% agreement for repeatability and operator variance studies with values that had no difference greater than 2 CTs between runs. The new method has significant cost savings for ISDHL, which is an added bonus to the validation.

The ISDHL Enteric laboratory worked hard on both the method comparison and the validation. We have a better protocol to follow that also saves the state money. We are better able to detect STEC in a timely manner, which benefits public health as a whole.

ISDH Laboratories Conducts Parasitology Workshop for Indiana Laboratories

By Shelley Matheson,
State Training Coordinator



Dr. Ryan Relich

The Indiana State Department of Health (ISDH) Laboratories hosted a first-of-its-kind training, "Those Pesky Parasites: A Hands-on Workshop in Medical Parasitology," Oct. 25-26, 2018, at ISDH Laboratories. The workshop was taught by Ryan Relich, PhD, D(ABMM), MT(ASCP)SM, associate professor with the Indiana University School of Medicine in Indianapolis. As part of this excellent and informative educational opportunity, ISDH Laboratories partnered with the South Central Association for Clinical Microbiology (SCACM) to provide nine hours of P.A.C.E. continuing education credits.

Fourteen laboratorians were present from ten different hospitals, including laboratorians from Indiana, Illinois and Wisconsin. This workshop spanned two days and provided an overview of medical parasitology. Day one included instruction on parasitological terminology, safety practices, regulatory considerations and specimen handling. Methods for detection of gastrointestinal, blood and ectoparasites were discussed, including immunoserological, microscopic and molecular methods.

Laboratory exercises were demonstrated, encompassing processing and examination of fecal specimens, blood and tissue parasites, adult helminths and arthropods.

Dr. Relich reviewed pre-analytical considerations for the diagnosis of parasitic infections, gave an overview of common parasite detection methods for the identification of parasitic protozoa and helminths, and summarized identification of ectoparasites. During the hands-on portion of the workshop, Dr. Relich guided participants as they performed wet-mount, permanent mount, and modified-acid-fast stain examinations, and supervised participants as they examined prepared peripheral blood smears for the presence of microfilariae, *Babesia*, and plasmodia. This two-day workshop concluded with an overview of the identification of lice, ticks and other macroparasites. The workshop was very well-received by participants. Pre- and post-tests were given, and a 76% overall increase in learning was demonstrated.



Thus far, this parasitology workshop is one-of-a-kind, the only one we've offered in Indiana, and the only one that has been conducted in the nation. The ISDH Laboratories' Outreach and Training Team plans to continue this type of workshop in the future, focusing on emerging infectious diseases and the educational needs of Indiana clinical laboratories. Information gathered from course evaluations indicated 100% of attendees agreed Dr. Relich's parasitology workshop was extremely well-structured and organized, and confirmed an excellent overall speaker rating.



Participation was appropriate for those who attended, and the training contributed significantly to their overall knowledge of parasitological identification. Comments received on course evaluations included, "Great presentation and well-presented; a lot of information given intelligently but still easy to understand." Another one stated, "Very good speaker. Nine hours is a long time to sit through lectures but I was very interested in the talk the whole time! This was a great refresher course for me."

Employee Spotlight: ANGELA LUCAS

*By Stephanie Dalenberg,
Serology and Immunology Supervisor*



Please provide a brief background on yourself.

I grew up in Indianapolis, attending public elementary and high schools, and Catholic home-schooling during my middle school years. After completing high school, I went to Purdue University. I later transferred from Purdue University to Indiana University-Purdue University Indianapolis (IUPUI), where I finished my bachelor's degree in biology with a minor in chemistry. After graduating from college, I worked at Dow Agrosociences in the Input Traits-Discovery Department, running protein tests on corn samples, consisting primarily of ELISAs and Western Blots. I also completed assay validations and extraction optimization on unexplored tissue types. I applied to the ISDH Laboratories in 2016 and began working in the Division of Virology & Serology under Dr. Nicolas Epie and Stephanie Dalenberg.



What is your current position and what do you do?

I'm currently a microbiologist 2 in the Serology Laboratory. I perform testing on serum specimens for sexually transmitted diseases, along with other outbreak organisms, such as Zika and Mumps viruses. For the ongoing hepatitis A outbreak, I am the lead analyst working with ISDH Epidemiology and the Centers for Disease Control and Prevention.

What are some highlights of your job? |

I thrive on completing checklists and organizing data. I also deeply enjoy discovering new ways that I can be more efficient in my job. Being able to process specimens with a relatively quick turnaround time is a fun challenge for me, as each day we have projects that are started and completed, which feels good. I enjoy knowing that each result that goes out brings life-giving information to either provide someone peace of mind or enable them to address and treat an illness.

What are your future plans or dreams? Is there a special job or career you hope to one day attain?

In the future, I'd like to race a car over the salt flats and join the Bonneville 200 MPH Club. I'd like to attend the Race of Gentlemen in New Jersey, see the Mooneyes Japan Car Show and participate in the El Diablo Run in Mexico. I'd like to go to Germany and spend a year researching my family genealogy. I would love to have children, attend the Olympics, and sing with Art Garfunkel. I only wish I could have seen the Beatles or Rage Against the Machine in concert. I'm a great scientist, and I'm meticulous. So I enjoy my work, and I'm good at it. I get along well with different types of people, and I try to keep a cool head, so I think I could be an effective leader once I have more experience. However, work/career is not my only goal; it won't be the culminating apex of my life experience. I want my life to be filled with experiences.

Any favorite hobbies you want to share?

I play the flute professionally and teach music to middle and high school students. For the past eight years, I have been on the board of directors of the Phillips Music Guild of Indianapolis. I have had amazing experiences playing with the Indianapolis Symphonic Band, the Indianapolis Chamber Winds, the Athenaeum Orchestra, the Scottish Rite Orchestra, the Metropolitan Youth Orchestra, multiple churches, performing in Hilbert Circle Theater and playing in the backup band for Cathy Morris (electric violinist). I love renovating American Foursquare homes, preparing taxes, long road trips and have a serious weakness for Andes mints, Smarties and Tootsie Rolls.

Anything you would like to share about your family or pets?

I have a husband, Andrew, a stepson, Wyatt, and two dogs; Frank is a pit bull and Adie is a Pomeranian. I enjoy weekly lunches with my mother and am engaged in a project to scan all of my grandparents' photos. My grandparents are 92 and have amassed thousands of photographs, from as far back as the 1860s, which I'd like to preserve digitally for our whole family to enjoy. My mother's family owns a vineyard in Rushville, Indiana. She has 12 siblings, so many hands make light work as we annually plant, pick and prune to sell those grapes to Indiana wineries.

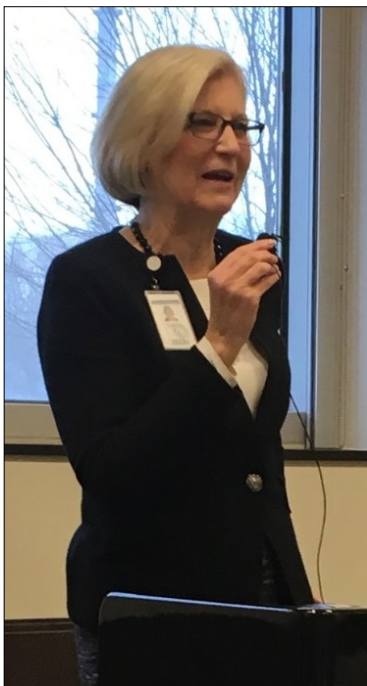
Health Commissioner Visits ISDH Laboratories

*By Chris Grimes,
Director of Quality Assurance / Training and Support Services*



It was near standing room only Jan. 29 in the ISDH (ISDH) Laboratories' cafetorium for the first 2019 visit from the State Health Commissioner Kris Box, M.D., FACOG. Dr. Box focused outside ISDH in 2018, and she successfully visited all 93 local health departments (LHDs). Through this experience, she learned how the LHDs work, how they are funded and what challenges they encounter. She is continuing her efforts with the LHDs this year through monthly meetings of the newly formed local health advisory council. According to Dr. Box, 2019 is the year to focus on the internal workings of the agency, including ISDH Laboratories.

The visit from Dr. Box allowed her to honor the 40 years of service Ray Beebe has given to ISDHL. She presented him with his 40 years of service medal.



Dr. Kris Box

Dr. Box thanked ISDH Laboratories staff for their strong work ethic and their efforts to get the job done no matter the obstacles. This will help the state of Indiana address emerging issues, such as the opioid crisis or the resurgence of tuberculosis (TB). There is a corresponding need to more quickly diagnose TB in patients, since most healthcare providers don't necessarily think of TB when a patient comes in with a cough.



Honoring Ray Beebe, a long time ISDH employee of 40 years.

About The LAByrnth



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