THE LABYRINTH
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Indiana State Department of Health Laboratories

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The Life Cycle of an Influenza Specimen Once Received at ISDH Labs

By: Katie Masterson, Jamie Hadley, Kara Hammes, Liz Church, and Judy Kerst

Have you ever wonder how your Influenza specimen goes from a swab in the doctor's office to a diagnosis of a particular type of Influenza? Below you will see how an Influenza specimen is handled when it arrives at the ISDH Laboratory and how the final diagnosis is obtained.

As Influena specimens come into Central Accessioning, the first thing we do is open the boxes and make sure the submitter information is correct and that it matches the accompanying requisition or LimsNet packing list (LimsNet is the web based program that submitting health care facilities are encouraged to use to submit samples for testing at the ISDH Labs). After we confirm the submitter information is correct, the specimens are checked to ensure that the patient ID on the specimen tube matches the patient ID on the requisition. Often times the specimens are wrapped up in paper towels or other absorbent material and the patient ID is not visible. When this occurs, the specimens are placed in the biological safety cabinet (BSC) and unwrapped so that the patient ID can be seen. If the specimens are LimsNet submissions, we go ahead and accession them in immediately. Non-LimsNet submission need to be hand-entered into StarLims (the ISDH Laboratory Information Management System) and are not immediately accessioned in full. Once the specimens are accessioned and labels are printed, a virology staff member comes to pick them up.

When the samples have been accessioned and taken from receiving, they need to be processed before any tests can be run. Before processing can occur, StarLims generated labels are placed on each cryovial and specimen transport tubes are checked to ensure patient ID's match. The vast majority of samples received are swabs in viral transport media (VTM - the type of "packaging" in which the specimens are sent to us) so processing simply involves vortexing each specimen tube for 30-60 seconds in a BSC. The swab is aseptically removed with forceps and the VTM is placed into the labeled cryovial. Once samples are processed, the cryovials are removed from the BSC and placed into the refrigerator until testing can occur.

After sample processing is complete, cryovials are removed from the refrigerator and molecular testing is performed. Samples tested each day include new samples as well as samples (i.e. inconclusive results) to be re-run from previous analytical runs. The first step is to extract influenza RNA (Ribonucleic Acid) from the patient's samples. RNA extractions can be performed by both automated and manual methods. We have two different automated extraction systems that can be utilized depending on the number of samples received. In addition, if a sample was previously run and yielded inconclusive results, the sample extraction is done manually. A "cocktail" of chemicals, called a master mix, is prepared for each influenza type; three seasonal types (A/H1, A/H3, B) and the pandemic (swine-like) type (A/H1N1). After RNA from each sample is extracted, a small volume of each sample is added to each master mix in a tube. A small amount of each sample/master mix is then added to a 96 well plate. The plates are sealed and loaded into

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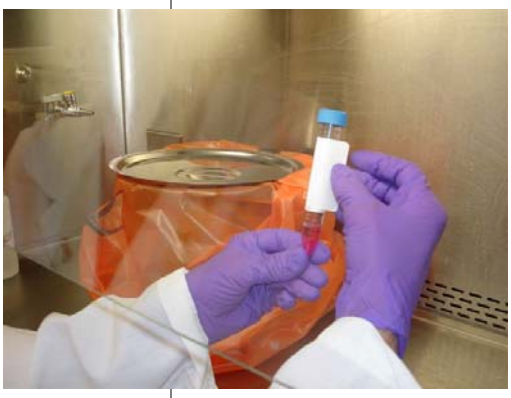

On and Off-site Packaging and Shipping Trainings for Division 6.2 Materials

By: Shelley Matheson

Indiana sentinel laboratorians attended one of three Indiana State Department of Health Laboratory-hosted trainings entitled "Packaging and Shipping: Division 6.2 Materials" last month. One of the October trainings was held at ISDH Laboratories in Indianapolis, while the other two were held at off-site hospital sentinel laboratory locations. The two off-site locations included Community Hospital in Munster and Clark Memorial Hospital in Jeffersonville. Division 6.2 materials are otherwise known as infectious substances affecting humans and/or animals. These courses were facilitated by Shelley Matheson, State Training Coordinator for ISDH Laboratories in conjunction with the Association of Public Health Laboratories

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Inside this issue:

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

Influenza Specimen-continued

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a real-time RT-PCR (Reverse Transcriptase-Polymerase Chain Reaction) instrument for analysis. As the machine runs through a number of heating and cooling cycles a positive sample will generate many copies of a pre-identified sequence of influenza RNA. These copies attach to probes containing a fluorescent dye that can be detected by the real-time RT-PCR instrument. A positive reaction is detected by the accumulation of a fluorescent signal that results in a CT (cycle or crossing threshold), or the number of cycles for the fluorescent signal to exceed the background level. If an acceptable CT value is present, the type of influenza virus will be identified. Results for each sample are analyzed and entered into StarLims. A preliminary report is created and sent for approval.

Jamie and Katie working under the hoods with Influenza specimens.

Once PCR has been run on a specimen it is returned for virus isolation set-up. If less than 30 samples are received in a given week, all samples are set-up in culture. If more than 30 samples are received, only a representative number of samples are set-up: 15 PCR flu-negative samples and 15 PCR flu-positive samples. Both the flu-negative and flu-positive samples are set-up in culture using a variety of cell cultures, including both culture tubes and shell vials. Each sample is manually set-up in StarLims and daily readings for cytopathic effect (CPE) are entered for the culture tubes. Shell vials are not read daily and are used as an initial screening tool only. A FA (fluorescent assay) stain is performed on the shell vials on day 2 post set-up. The flu-positive samples are stained for either Influenza A or Influenza B depending on the PCR results. The flu-negatives samples are stained with a respiratory viral "pool" stain which will detect most respiratory viruses. The FA stains are read and results are entered into StarLims. If the shell vial stains positive with the respiratory "pool" stain, confirmatory testing is needed to identify the respiratory pathogen. Depending on whether CPE is present , the cell culture is either stained for the known respiratory viruses (adenovirus, parainfluenza, influenza, RSV) or set-up for the detection of rhinovirus. It is possible that the CPE present in the culture tubes is not respiratory-like so additional FA stains for other viruses (i.e. Enterovirus) may be necessary. If no CPE is observed in the culture tubes after 14 days, the sample is concluded as "no respiratory virus isolated." Final laboratory reports are generated for all samples, either at the time of virus detection or at the end of 14 days.

Reporting out a flu specimen is a two-step process due to the nature of our laboratory testing. Because each flu sample is tested by PCR and set-up in culture, two laboratory results are provided. Once the sample is tested by PCR, a "preliminary" report is issued to the submitter. The PCR data is hand-entered into StarLims for each sample and a report is generated. After the supervisor approves the run data and ensures that the report information is correct, the lab report is either printed out (non-LimsNet submission) or sent instantly to the submitter via LimsNet. A faxed copy of each "preliminary" PCR report is sent to the non-LimsNet submitters by our administrative staff. Once the virus isolation results are concluded for a given sample, another report is generated and the sample is sent for final approval. This "final" report, which contains both PCR and virus isolation results, is approved and released by the supervisor similarly to the PCR report. LimsNet submitters receive a copy of the final report as soon as it is released by the lab. For non-LimsNet submissions, the final report is mailed to the submitter by our administrative staff.

"Because each flu sample is tested by PCR and set-up in culture, two laboratory results are provided."

Rabies in Bats:

What Indiana Residents Need to Know

By: Rhonda Stidham

On October 9, 2009, a 43-year-old man from Clark County, Indiana, was admitted to Norton Hospital in Louisville, Kentucky. Just 11 days later, he died. The Centers for Disease Control and Prevention (CDC) confirmed the cause of death as rabies. Although the man had not indicated any "exposures of concern," a bat was found to be the source of infection. In Indiana, this is only the second human death from rabies since 1959. However, rabies is a concern for Indiana residents and more education is necessary in order to help prevent future deaths from it.

Rabies – the dreaded disease historically associated with vicious dogs and vampire bats – has existed in most parts of the world since 3000 B.C. The state of Indiana noted its first laboratory-confirmed rabid animal in 1906. Up until the 1940's, dogs were the main hosts of the rabies virus in Indiana. In 1948, the Flurry strain vaccine was introduced for use in dogs and through the 1960's Indiana experienced a steady decline in rabies in dogs with the last rabid dog being diagnosed in 1989. In the 1960's, the skunk replaced the dog as the main host of rabies virus in Indiana and remained so through the 1980's with the last positive skunk being identified in 1998. In 1965, Indiana confirmed its first rabid bat and since then bats have become the primary hosts of the rabies virus in Indiana.

There are several species of insectivorous bats in all areas of the continental United States that are known to be reservoirs for the rabies virus. Almost all of Indiana's 92 counties have in one year or another (since 1965) submitted a bat found to be rabid. However, it is important to note that most bats in the general population are not positive for rabies. According to the CDC, less than 1 percent of bats in the general population carry rabies. The numbers of rabid bats we see in Indiana come from those bats submitted to the lab for rabies testing. These bats are usually either sick or dead or found to be behaving abnormally – unable to fly, found during the day, etc. On average, less than 5 percent of these bats are found to be positive for rabies. Even with such small numbers, it is important to remember that rabies is a serious disease.

If transmitted to humans, the rabies virus is fatal nearly 100 percent of the time unless medical treatment (PEP or post exposure prophylaxis) is given. If PEP is not given within a few days of contracting the virus, death usually occurs from one to three months later. This knowledge helps explain why testing potentially rabid animals in the laboratory is so critical. When someone has been exposed to the saliva of a potentially rabid animal – usually through a bite – it is important to have the animal put down and sent in for rabies testing. Rabid animals can only be identified through laboratory testing and quick and accurate diagnosis is crucial in determining the need for PEP in humans.

When animals are received in the lab, they are prioritized and logged into the database. Bats, raccoons and other wild animals that have bitten someone are tested first, and then dogs, cats and other domestic animals that have bitten someone are tested. All animals involved in non-bite exposures are tested last. Although

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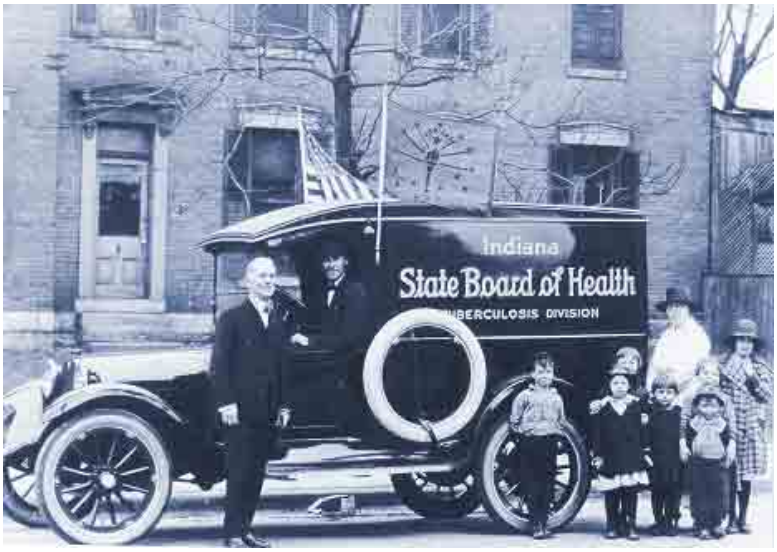


Dr. John Hurty, Indiana State Health Commissioner, 1896 –1922 established the state's first Tuberculosis Control Department in 1915.

ISDH Laboratories Goes "On the Road" with its Hands-On Workshop for Sentinel Laboratories

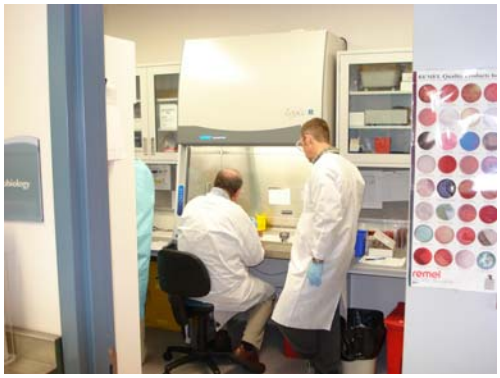
By: Shelley Matheson

Jim Hogan, Ellie Carter and Mark Glazier begin unloading the van to set up the training at White County Memorial Hospital.

Indiana State Department of Health Laboratories (ISDH Laboratories) hosted its first off-site "Hands-On Workshop for Sentinel Laboratories: Biothreat Agents 101" workshop on September 23 at White County Memorial Hospital in Monticello, Ind. Four ISDH Laboratory employees traveled with this workshop in order to make it possible for our laboratory supplies and equipment to go "on the road." Traveling staff included Shelley Matheson, Mark Glazier, Ellie Carter, and Jim Hogan. Matheson is the ISDH Laboratory State Training Coordinator, and Glazier is the ISDH Laboratory Supervisor of Emergency Preparedness and Molecular Virology. Carter is the ISDH Laboratory Program Advisor, and Hogan is the ISDH Hospital Laboratory Training Coordinator. This workshop consisted of ten participants representing nine different Indiana sentinel laboratories. A sentinel laboratory is defined as any laboratory capable of analyzing or referring specimens or samples that may contain microbial agents or biological toxins. In conjunction with reference laboratories, these sentinel laboratories form the nation's Laboratory Response Network (LRN).

Students view suspect agents through microscopes set up in the training facility.

The September 23 workshop provided sentinel laboratorians with an overview of their role as a sentinel laboratory and their role in the presumptive identification of biothreat agents including *Bacillus anthracis, Yersinia pestis, Brucella canis, Francisella tularensis, Burkholderia mallei, and Burkholderia pseudomallei.* With help from Bonnie Waters, White County Hospital's Assistant Laboratory Director, the hands-on laboratory exercises outlined and demonstrated the microbiology of these agents in their microbiology laboratory. The safety implications of handling these suspect organisms were emphasized as well. In addition, select agent policy and packaging and shipping rules and regulations were reviewed with participants. Carter presented "The Role of the Sentinel Lab", Matheson presented "Select Agents, CDC/APHIS Forms, and USDA Permit Information for Sentinel Labs" and "Packaging and Shipping of Category A Infectious Substances," Glazier presented "Agents of Bioterrorism: An Overview of Sentinel Laboratory Protocols."



Mark Glazier supervises a student working under the Bio Safety Cabinet hood.

Ellie Carter works with Students at the Hands-on Wet Lab Training.

The workshop was a successful training experience for all staff and participants in attendance. A pre-test and post-test were given to each participant to evaluate the educational value of the training. We are pleased to announce that there was an average 14 percent increase in test scores between the pre-test and the post-test for participants. According to the participant evaluations, ISDH Laboratories were 100

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Rabies In Bats-continued

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there are several rabies diagnostic tests that can be done, the Direct Fluorescent Antibody (DFA) test has been evaluated for over 40 years and determined to be the most accurate and rapid for routine use.

The first step in performing the DFA test involves obtaining a proper tissue sample from the animal. This is done by necropsy of the head of the animal. With the use of a stainless steel hammer, chisel, forceps, and a scalpel, cross-sections of the cerebellum and brain stem are obtained. Next, impression slides are made using this tissue. After a bath in acetone to fix the tissue, fluorescently labeled anti-rabies antibody is used to stain the slides. The slides are then incubated for 30 minutes in a high humidity chamber. Finally, the slides are then rinsed with phosphate buffered saline (PBS) and mounted with cover slips. The final step is to read the slides under a fluorescent microscope. If rabies antigen is present, the antibody will bind to the rabies virus and produce an antigen-antibody complex which can be seen as apple-green fluorescence under the microscope. This entire process from the start of necropsy to reading the slides takes about four hours to complete with test results usually available within 24 hours of receipt of specimen. As stated earlier, laboratory testing is the only way to know for sure whether an animal is rabid and will prevent the unnecessary worry and expense of getting prophylactic treatment if the animal is found to be negative for rabies. In Indiana, in most cases, the results will be negative, but there are some important points to keep in mind.

In Indiana you have a greater chance of getting rabies from a bat than from any other animal, so it is important to never handle any bat. If you encounter a sick or injured bat, DON'T PICK IT UP. Also, if you awaken to find a bat in your bedroom, it is important to consider that you may have been exposed to its saliva. Because bats have very tiny, razor-sharp teeth, it is possible to be bitten in your sleep and not realize it – especially if you are a heavy sleeper, elderly, very young, or incapacitated in some way. Finally, if a child is known to have found a bat or known to be in the vicinity of a bat, this also constitutes a potential exposure. Unless the child is old enough to fully and accurately communicate what happened when they were near the bat, it is best to err on the side of caution by considering the possibility of an exposure. If you find that you have been exposed or potentially exposed, DON'T PANIC. According to the CDC, rabies is considered an urgent matter, but not an emergency.

The single most important thing you need to do initially is to thoroughly wash the affected area with soap and warm water. This may prevent the rabies virus, if present, from entering your body. Second, if at all possible, trap the animal so that it can be submitted for rabies testing. And finally, it is important to make sure that your home is bat-proofed. Bat-proofing your home simply means finding any and all points of entry –even cracks as small as a quarter-inch by a half-inch - and covering or filling them up. Most people can bat-proof their homes fairly easily, but there are professionals who will do the job for you. They can be found in the yellow pages under "Animal Rescue and Removal" or "Pest Control."

For further information on rabies please visit the following web sites.

http://www.in.gov/isdh/files/CLI_Rabies.PDF

http://www.cdc.gov/rabies/

http://www.in.gov/boah/2337.htm

Shelley Matheson gets ready to give her powerpoint presentation.

On The Road-continued

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percent successful in explaining the concept of Select Agents and USDA Permits as well as discussing the role of the clinical laboratorian as it pertains to the identification of these bio-threat organisms. In addition, the recognition of the microbial characteristics of the primary agents of bioterrorism was effectively taught to participants at the training in addition to the transporting of such agents. One participant commented "Shelley, Ellie and Mark were all very helpful and make themselves available."

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The Indiana State Department of Health supports Indiana's economic prosperity and quality of life by promoting, protecting and providing for the health of Hoosiers in their communities.

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Packaging and Shipping -continued

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(APHL). Subject matter expert, Patricia Payne, Ph.D., MT (ASCP), a consultant to APHL, taught the courses. Dr. Payne has been conducting these courses throughout the United States for over six years.

These intermediate-level, one-day programs provided a comprehensive overview of regulations applicable to packaging and shipping infectious laboratory specimens and cultures. Dr. Payne provided lectures, demonstrations and allowed participants to perform a group hands-on exercise while in class. These instructional tools provided knowledge on complying with international, federal, and local transportation regulations. Participants learned how to properly classify, mark, label and document infectious materials for shipping by land, air and United States mail. Participants were tested on their knowledge of the regulations and received documentation of their attendance and testing. Once signed off on by their employers, participants were certified for packaging and shipping infectious substances for up to three years.

All three October packaging and shipping trainings were overall successful. The Indianapolis training on October 13 drew 23 participants representing 15 Indiana sentinel laboratories as well as two ISDH Laboratories employees, Kara Hammes and Nathan Britt. Collaboration with Arist Sgouroudis, Community Hospital in Munster's Laboratory Director, was vital in the success of the training held at their hospital on October 15. The Munster training allowed convenient access to northern Indiana sentinel laboratorians and attracted 10 participants representing eight Indiana sentinel labs. The southern Indiana training held at Clark Memorial Hospital in Jeffersonville allowed southern Indiana sentinel laboratories a closer commute to an exceptional packaging and shipping training opportunity. The Jeffersonville training on October 27 was made possible through coordination with Clark Memorial Hospital's Laboratory Director, Dave Cooper. The Jeffersonville training drew 12 Indiana sentinel laboratory representatives from seven different sentinel laboratories. Dr. Payne did an outstanding job at all three trainings, and there was a 96 percent passing rate on the certification exams. One Indianapolis participant commented, "A lot of good information – will send another associate when next offered in the area." ISDH Laboratories will be facilitating three encore packaging and shipping trainings in the spring of 2010.