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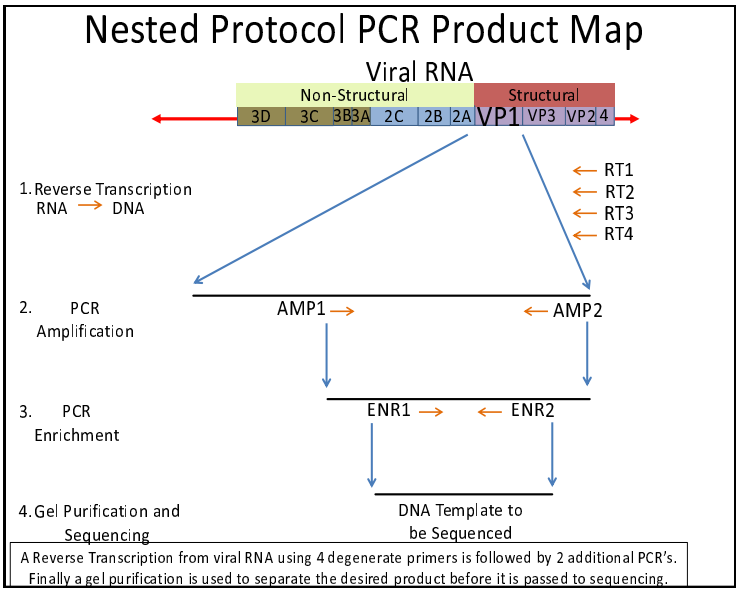
The LAByrinth

Indiana State Department of Health Laboratories Newsletter

Updating Picornavirus Typing by Sequencing with Nested PCR

By Edward Simpson

Recently the Indiana State Department of Health (ISDH) Molecular Test Development laboratory completed the validation of a new method for identification of picornaviruses by Viral Protein 1 (or VP1) sequencing. The picornavirus family is comprised of a number of human, animals, and plant viruses. The main pathogens of interest to public health organizations are Enterovirus, Rhinovirus and Poliovirus. Enteroviruses in particular are responsible for around 10-15 million or more symptomatic infections a year and are the major cause of asymptomatic meningitis in children.[1] Viral Protein 1, a structural protein used in the construction of the capsid shell of the virus has long been a target for identification of picornaviruses. In this new protocol, a multi-step nested PCR process replaced the previously used one-step Reverse Transcription and amplification PCR before sequencing. This resulted in higher sensitivity while maintaining the specificity of the assay.



The primers and conditions obtained from the source paper[2] enhance selectivity of the various reactions of the protocol. Rather than Reverse Transcription and amplification in one reaction, a separate RT reaction with more discriminating primers is done. Next, two consecutive reactions, for amplification and enrichment respectively, are performed. Additionally the PCR products from the enrichment are resolved on an electrophoresis gel. The added gel purification step increases cleanliness of the DNA used in the final sequencing reaction. Unwanted products are separated, the desired PCR product is excised and DNA extracted.

(continued on next page)

Our Mission:

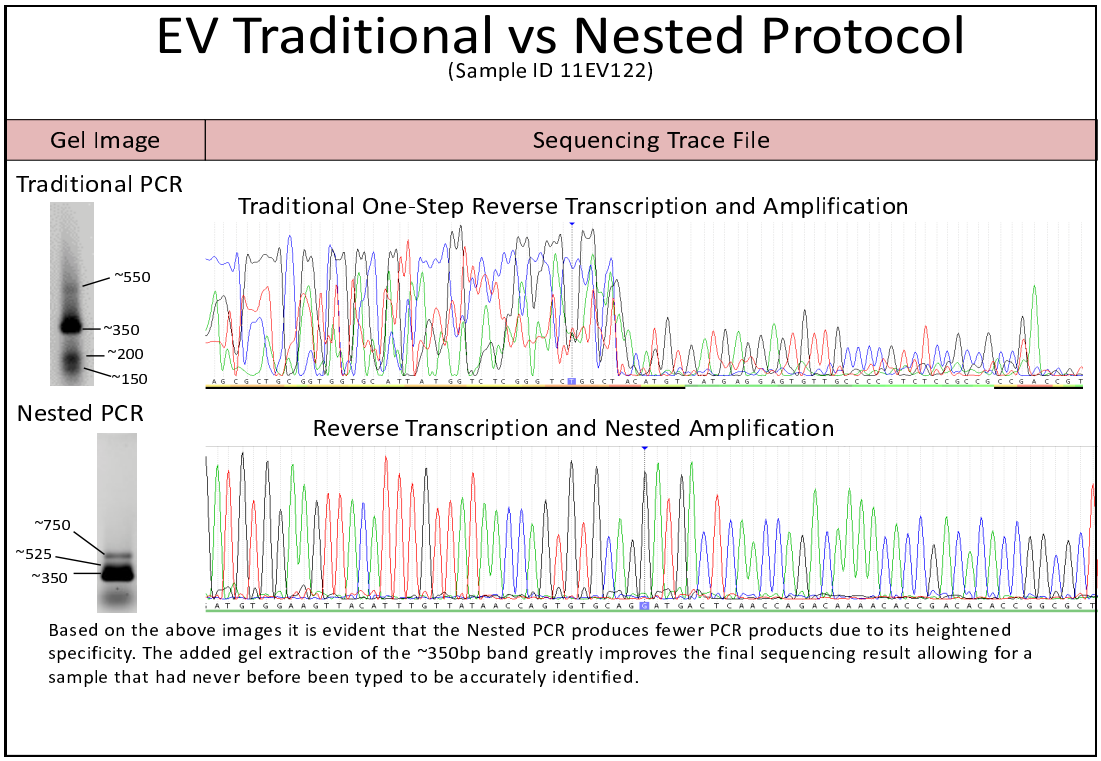
The Indiana State Department of Health Laboratories partners with other public health agencies to provide timely and accurate information needed for surveillance and outbreak investigations to protect and improve Hoosier health.

Inside this Issue:

Updating Picornavirus Typing (*continued*)

These improvements mean less non-specific amplification, which was a significant problem in the older protocol that resulted in more repetition of work and an inability to obtain some sequences. The products are finally sequenced by Dye-Termination or "Sanger" type sequencing. Analysis involves comparing the sequence to both BLAST and local databases for matches with high homology (75 percent or greater) and eliminating similar but lower scoring matches after extensive evaluation.

The validation was completed using 55 previously identified specimens (positive for picornavirus VP1 target by Real-Time PCR). Twelve viruses were sequenced with the new protocol that had been untypable when using the old, giving a 21.8 percent increase in sensitivity and no change in specificity. This new protocol may eventually allow ISDH laboratories to test for picornavirus presence directly from patient specimen avoiding the burden of culturing first. Another development that will enhance the success of this and many other sequencing assays is the purchase of a new sequencer, the ABI 3500. Replacing the much older 3100 will allow faster run times, longer sequences and a more controlled environment.



In an effort to foster co-operation for the bettering of public health and increase public health surveillance, ISDH Laboratory has traditionally provided submitters and the CDC with as much information about specimens as possible. Knowing the strains of pathogens in circulation improves awareness of threats to public health in Indiana and leads to prevention of disease. With the improved sensitivity of this assay, the ISDH hopes to strengthen the collaborations with our public health partners and bolster public health surveillance capabilities by successfully identifying a higher percentage of patient specimens with picornaviruses.

1. Non-Polio Enterovirus Infections. Article from CDC.gov
2. *Sensitive Semi-nested PCR Amplification of VP1 Sequences for Direct Identification of All Enterovirus Serotypes from original clinical specimens.* W Allen Nix Et. Al. J. of Clinical Micro. Aug 2006 p.2698-2704

New Year Brings Increase in Norovirus Illness

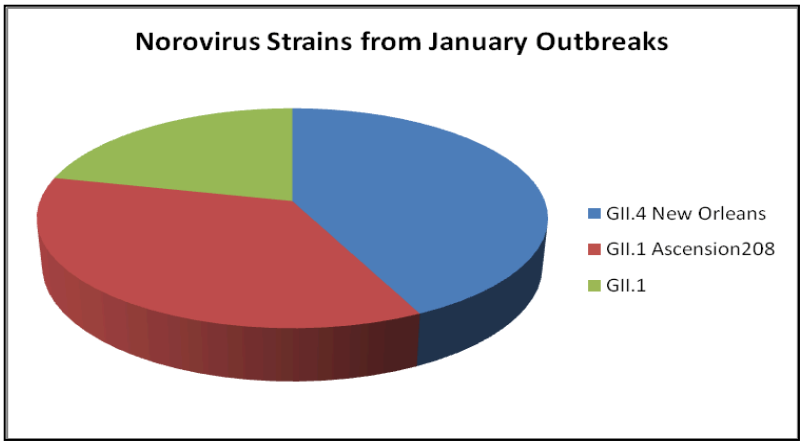
By Mark Glazier

Wash your hands!

As the holiday season ended and the new year began, it wasn't long before the ISDH was dealing with two large foodborne outbreaks in northern Indiana. With dozens of people ill and two restaurants potentially implicated, rapid identification of the causative agent was imperative. The stool specimens received for both outbreaks were set up in bacterial culture and tested by real time reverse transcriptase polymerase chain reaction (RT-PCR) for norovirus. The initial seven specimens received from both outbreaks were tested for norovirus genogroup I (GI) and genogroup II (GII) by RT-PCR; five of the seven specimens tested positive for norovirus GII.

In the United States, Centers for Disease Control (CDC) estimates that more than 50 percent of all foodborne disease outbreaks are attributable to noroviruses. Most norovirus outbreaks are likely caused by direct contamination of food by a food handler immediately before its consumption, as was the case with both outbreaks described above. These types of norovirus outbreaks have frequently been associated with the consumption of cold foods, including sandwiches, salads and bakery products.

While the diagnosis of norovirus as a cause of foodborne outbreaks has improved with the use of real time RT-PCR, the use of genetic sequencing of norovirus strains from clinical samples has greatly helped in epidemiologic investigations. Sequencing data allows cases to be linked to each other and to a common source, as well as providing information to differentiate outbreaks that were mistakenly connected. Sequences can be entered into CaliciNet, a database recently developed by the CDC that serves as a national surveillance network for norovirus sequences. CaliciNet helps to determine links between multi-jurisdictional outbreaks, detect possible norovirus contamination of food and identify new strains as they emerge.



In January, the virology laboratory received 66 specimens for norovirus testing from five potential outbreaks. At least two of the samples that were PCR positive from each outbreak were sequenced to determine the norovirus strain (results are summarized in graph). Samples from three of the outbreaks matched norovirus strain GII.4 New Orleans. According to the CDC, variants of the GII.4 genotype (strain) have been the most common cause of norovirus outbreaks over the past decade.

As the most common cause of gastroenteritis in the United States, it is impossible to eliminate norovirus infection. However, there are some simple ways to reduce the risk of getting norovirus. The easiest way to stop the spread of norovirus is to practice proper hand hygiene; wash hands with soap and water, especially after using the bathroom and always before eating and preparing food. It is also important to carefully wash fruits and vegetables, and cook oysters and other shellfish thoroughly. Hopefully by following these simple steps, you won't be a part of the next norovirus outbreak!

ISDH Labs Provide Methamphetamine Analysis to Support County Health Departments

By Pradip Patel

Methamphetamine abuse is a growing problem in Indiana and clandestine drug labs are found throughout the state. Production of methamphetamine occurs in homes, apartments, hotel and motel rooms, and mobile homes. When a drug lab is seized, law enforcement agencies remove chemicals and drug making equipment from the site, however, residual contamination often remains because the hazardous chemicals that are used when manufacturing these drugs can contaminate the property.

Without proper cleaning, it's possible to be exposed to potentially dangerous chemicals. New occupants moving into former drug labs would be unaware of the contamination problem. For this reason, local county health departments may need to condemn the property until the property owner can prove that the property is no longer contaminated.

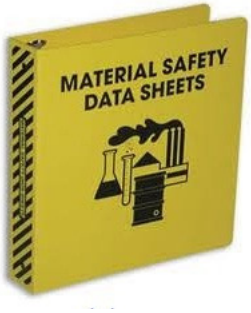
Sometimes the county health department may be unsure about the extent of the contamination and whether or not to the property should be condemned. At this point the health department must either condemn the property or send samples to an out-of-state lab to test for residual contamination. Testing a single residence could cost the county thousands of dollars.

In a customer satisfaction survey sent out by the ISDH Chemistry Laboratories, one county health department suggested the ISDH Labs provide this service. In response, the laboratory reviewed the current literature and developed a method for the analysis of methamphetamine residue. The method has a detection limit of 0.5 ppm for methamphetamine which is the required detection limit in Indiana. This method can also be used for trace analysis of amphetamine, ephedrine and pseudoephedrine.

County health departments requiring methamphetamine residue testing should contact Robin Bruner, Chemistry division director at 317-921-5559 or by email at rbruner@isdh.in.gov for questions or for further information.

Department of Health MSDS Update

By Mary Robinson



As safety coordinator (SC) for the ISDH Laboratories, an Indium Standard Material Safety Data Sheet (MSDS) was left on my desk recently. Have you ever seen a chemical product name and wonder just exactly what was in it? In laboratories, identification of chemical compound components is critical for safety. Most laboratories develop collections of MSDS reports for each chemical compound received. An MSDS includes information about a compound's composition, requirements for safe storage, use and disposal. As it turns out, this information is required by Occupational Safety and Health Administration (OSHA) regulations. As stated in the *OSHA Hazard Communication Standard, 29 CFR 1910.1200, paragraph (g)(8)*: "The employer shall maintain in the workplace copies of the required material safety data sheets for each hazardous chemical, and shall ensure that they are readily accessible during each work shift to employees when they are in their work area(s). (Electronic access, microfiche, and other alternatives to maintaining paper copies of the material safety data sheets are permitted as long as no barriers to immediate employee access in each workplace are created by such options.)."

Since 1995, MSDS information at ISDH Labs has been provided through the use of an internal electronic database, MSDS ExPress. Incoming MSDS reports are sent to the SC who enters the information into the database. Access to the database is provided to all laboratory employees via their PC. A specific MSDS can be accessed by searching lab area, product name, vendor name, CAS number or reference number. Detailed information on the products HMIS/NFPA hazard codes, properties, composition (% by CAS number), vendor contact information, user-defined characteristics, required protective equipment, copy of original MSDS and additional notes can be viewed.

Benefits of the database are eliminating the cost and hassle of maintaining hard copy MSDS paperwork (over 2,000 MSDS reports are currently entered into the database). Previous to the use of the MSDS ExPress software, ISDH Laboratories MSDS documents were stored in binders which required three shelves in a book case. In addition, the Indiana Health and Forensics Science Laboratories are a multi-agency building. Each agency provides building management with MSDS information for the products used in their agency. Electronically providing this information simplifies this process.

Today there are many options available for MSDS management including hard copies, internal electronic databases and an abundance of options via the internet. It would be interesting to learn how other facilities are addressing this requirement. If you would like to share your facility's MSDS management experiences please e-mail Mary Robinson at mrobinson@isdh.in.gov.

Note: For those who are curious, the Indium Standard is composed of indium, nitric acid and water. Interesting facts about indium (symbol **In**, atomic number 49): It is a rare, very soft, malleable chemical element discovered in 1863 and its primary source is zinc ore. Pure indium metal gives a high-pitched "scream" when bent. It can be used in the production of alloys, germanium transistors, thermistors, photo-conductors, mirrors, photocells, and in solders.

Improve your Wellness

By Charles Hostetter

Walking may be the easiest way to burn calories, strengthen bones and muscle and prevent disease. It's simply a great way to increase your energy level while reducing stress. In addition, walking reduces cholesterol levels, lowers blood pressure and promotes intestinal regularity.

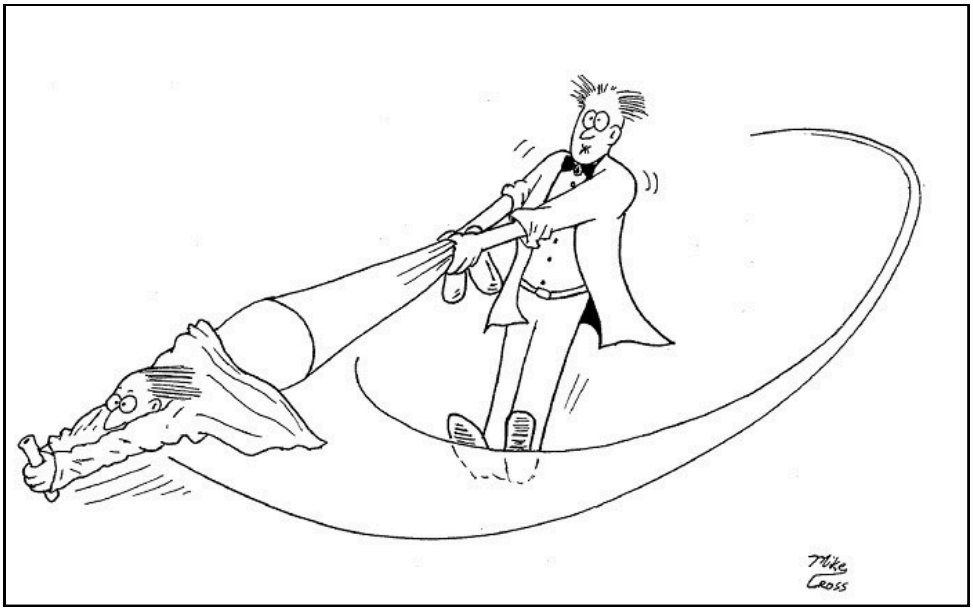
Studies have consistently shown that walking can extend your lifespan by several years. For instance, researchers found that people who walk approximately 20-25 miles per week outlive those who don't walk by several years. Another interesting walking fact is that people who walk an extra 20 minutes a day lose, on average, seven pounds of body fat over the course of one year.

Running is another great exercise with the same health benefits mentioned above. One hour of jogging can burn approximately 500 calories, while an hour of brisk running can burn over 1,000! A 30 minute jog three times a week, along with a healthy diet, is a great regimen for weight control.

Experts recommend at least two-and-a-half hours of moderate activity per week, such as brisk walking or jogging. So, get those comfortable shoes on and find a friend or loved one to join you for a stroll around your neighborhood or a jog through the park. While at work, take a 15 minute break and do some walking – use the stairs for added benefit! Here at the ISDH Labs, we have a weekly 30 minute lunch walk. It is always important to check with your doctor before starting an exercise program, especially to ensure your goals are set at the appropriate pace for your health.

Microtoon

By Mike Cross



In the days before centrifuge machines were invented.

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