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THE LABYRINTH

Indiana State Department of Health Laboratories Newsletter

What Will the GHOST Project Do for Indiana?

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

The Indiana State Department of Health Laboratories (ISDHL) and two other state public health laboratories recently joined the CDC/Association of Public Health Laboratories (APHL) GHOST program. GHOST? No, no relation to Casper or any other spooky beings. But yes, we are indeed looking for "GHOST" or quasi-species of hepatitis C virus (HCV) present in patient's serum through this project. For you laboratory trivia fans, "ghost" is a word also used in the clinical laboratory profession by hematologists looking at red blood cells (RBCs) when they find a cell without hemoglobin on a blood smear. However, this GHOST project is an acronym for Global Hepatitis Outbreak and Surveillance Technology. Using next generation sequencing (NGS), genotyping of the virus can reveal different quasi-species (ghost) or multiple HCV genotypes, sometimes present in the same infected patient[(1)].

Hepatitis C virus (HCV) is a positive-stranded RNA virus belonging to the *Flaviviridae* family. This family of viruses also includes yellow fever virus, West Nile virus, Zika virus, and dengue virus, also known as arboviruses because they are generally transmitted to humans from mosquito bites. HCV, however, is in a separate class (*Hepacivirus*) from these infamous flaviviruses because it is blood-borne or sexually-transmitted with an affinity for liver cells (hepatocytes). HCV infections are a significant cause of hepatitis (inflammation of the liver) in the United States, which can progress to cirrhosis or hepatocellular carcinoma if untreated. HCV hepatitis can be treated with antiviral agents and depending on the virus genotype and the genotype of the host's interleukin -28B (IL28B) gene, some will clear the virus from their liver[(2)]. With respect to IL28B gene, certain genotypes result in a stronger immune response to a HCV infection and may result in the infected individual clearing the HCV infection without treatment[(3)]. It is therefore important for physicians to know the genotypes of HCV circulating in their patients to ensure they prescribe the most appropriate antiviral drug[(4)].

ISDHL was selected as a GHOST site in 2017, along with Colorado and Iowa. In addition to the CDC sequencing protocol, the GHOST project was developed with a web-based system to serve as one-stop-shop bioinformatics tool for sequenced fragment assembly and phylogenetic analysis of sequenced assembled data. This analysis tool is aimed at minimizing the need for laboratorian inputs during HCV sequenced data assembly by providing all needed bioinformatics quality control checks to ensure reliable results. Through this CDC GHOST analysis tool, raw sequence data is sent from the public health laboratory directly to the CDC. Subsequent HCV transmission patterns and links are generated automatically from the CDC site. Public health laboratories are provided technical expertise and resources to genotype HCV-positive specimens from their own populations.

An important advantage offered by the GHOST program is its ability to connect every HCV recovered from one patient to its origin. This is possible because of the ability to identify and differentiate a single nucleotide or a DNA single digit change, which occurs as the virus passes from one person to another. More nucleotide changes occur and accumulate in the virus genome as a virus strain circulates through a host population, and analysis of these changes creates unique "fingerprints" for evaluated viruses. Because of this, scientists can construct a "tree" of HCV recovered from patients and relate each back to its parent virus and trace the spread of a virus through a population.

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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.

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This diversity of HCV, resulting from these nucleotide changes, is detected in the Hypervariable Region (HVR) of the gene (E2 gene) that allows the virus to attach to its host cell. Through the GHOST program, the genetic sequences of this HVR are evaluated and can identify relationships between viruses isolated from different infected individuals. The CDC has developed this evaluation method by designing amplification primers upstream of the HVR for NGS sequencing using the Illmina® Miseq platform. This platform provides genotyping with the power to differentiate subtle differences in genotype particles and to identify multiple HCV genotypes within the same infected patient.

Routine serological testing for a HCV infection is available in many clinical laboratories, where detection of antibodies to HCV in a patient's serum by Enzyme-Linked Immunosorbent Assay (ELISA) is the main test performed. ELISA is useful for detecting patients who have been previously exposed to the hepatitis virus, but there is a limitation. Antibodies to HCV produced in the infected patient's blood do not disappear even after all virus particles are cleared. Therefore, the ELISA test cannot differentiate actively infected patients from those patients who have cleared the virus from their blood system. In comparison, NGS not only confirms actively-infected patients, but can also detect if a patient is infected with 1+ HCV genotypes and provides a valuable tool for HCV outbreak investigation.

So what can the "GHOST" project do for Indiana? Just as Casper was the "friendly ghost," the GHOST program is making complex genetic analysis more user-friendly, while increasing the rate of detection. This results in increased accuracy of identifying circulating HCV types in the population and also reducing the time of disease outbreak investigation for many public health departments through the nation[(5)].

An Illumina® Miseq next generation sequencing platform.

This image is provided by: https://phil.cdc.gov/Details.aspx?pid=22175

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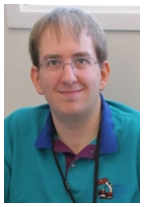
Laboratory Response to Synthetic Cannabinoid "Spice" Contamination with a Rat Poison



By Aaron Bolner
ISDH Chemist

The use of synthetic cannabinoids — often referred to as spice, K2, and fake weed — has been on the rise since their introduction in the early 2000s. Synthetic cannabinoids are dried plant material, often appearing similar to tea leaves or herbs, sprayed with a chemical mixture, packaged, and sold as a legal alternative to marijuana. The sellers of these products are constantly altering formulas to stay ahead of the laws and to enhance potency, sometimes with lethal effects.

In March 2018, a cluster of Illinois residents, primarily in the Chicago area, were treated by emergency personnel after exhibiting unexplained bleeding. Epidemiologists in Illinois conducted interviews with patients and found a link to synthetic cannabinoid use. The number of cases has since increased dramatically, with more than 230 cases spread across at least 10 states and at least five fatalities. New cases continue to be identified.

To determine the cause of these illnesses, blood samples from three of the affected patients were sent to a private laboratory. They confirmed the presence of the long-acting anticoagulant, brodifacoum, which was banned by the Environmental Protection Agency (EPA) in June 2017. Prior to its ban, brodifacoum was sold over-the-counter as the active ingredient in mouse and rat baits. It is highly toxic, and the typical concentration in commercially-available poisons was between 0.001 and 0.005%. The compound is a vitamin K antagonist, so fortunately vitamin K administration is an effective antidote for brodifacoum poisoning. The down side is that brodifacoum is a persistent compound, and treatment requires several months of regular vitamin K doses to fully counteract the poison.

The Indiana State Department of Health (ISDH) sent out a press release on March 28, 2018, to alert Indiana residents to this issue. In early April 2018, two cases of otherwise unexplained bleeding occurred in Indiana. Samples of synthetic cannabinoids sold under the names "Scooby Snax," "AK-47," "Matrix," and "OMG" were collected and sent to the Indiana Poison Center and the Indiana State Police (ISP). The ISDH Chemistry Division was contacted and asked if the samples could be tested for the presence of brodifacoum. Using a method previously developed by Food Chemistry Laboratory Supervisor Pradip Patel to test food contamination with rat baits, the ISDH Chemistry Laboratories were capable of performing the necessary analysis. ISP handed off the samples using their evidence handling procedures, to ISDH Laboratories.

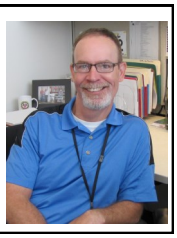
The dry samples were extracted with a mixture of organic solvents and analyzed using High Performance Liquid Chromatography (HPLC), a C-18 column and UV detection at 254 nm. Each of the "spice" samples received were positive for brodifacoum. Initial results were so high that the HPLC's detector was saturated and the solutions need to be diluted to make an accurate concentration assessment. The amount of brodifacoum detected ranged from approximately 150 to 300 times the concentration found in conventional rat baits, such that consumption of even a small amount of affected product could lead to serious complications. The ISDH Epidemiology Resource Center received notes of appreciation from Illinois and CDC for its efficient work on this project.

ISDH continues to monitor the situation and is providing information on the outbreak. See https://www.in.gov/isdh/27788.htm for more information.

Playing a Part in Indiana's Arboviral Surveillance Program

By Mark Glazier

Biological Preparedness, Lab Outreach, and Logistics Division



Every year after a long winter, Hoosiers look forward to the coming of spring and warmer weather. The warm, wet Indiana spring also brings with it an unwanted guest, the mosquito! Unfortunately, the mosquito's "bark" can be much worse than the initial pain and discomfort of its bite. When a mosquito bites a person, it can transmit West Nile virus (WNV), the leading cause of domestically acquired arboviral disease in the United States. According to the Centers for Disease Prevention and Control (CDC), there were 2,002 West Nile virus disease cases in the United States in 2017, with 64% being neuroinvasive, the more severe and potentially deadly form of the disease. Over the past five years, there have been 75 cases of WNV neuroinvasive disease and 11 deaths in Indiana.

To reduce the number of human infections in Indiana caused by West Nile virus and other arboviruses of public health importance, the Indiana State Department of Health (ISDH) has an active mosquito surveillance program. Generally, from late May through October, the ISDH Vectorborne Epidemiology/Entomology Division collects mosquitoes from across Indiana using the gravid trap method. Mosquitoes collected from each trap location are meticulously counted, speciated and divided into sample pools of up to 100 mosquitoes. Samples are then delivered to ISDH Laboratories (ISDHL), where all samples are tested for WNV and St. Louis Encephalitis virus (SLE) by real-time Reverse Transcription Polymerase Chain Reaction (rRT-PCR) assays. Samples collected from certain northern Indiana counties, where disease incidence is higher, are tested for Eastern Equine Encephalitis virus (EEE).

Prior to the start of the 2018 mosquito surveillance season, the ISDHL Assay Development section validated an rRT-PCR assay for detection of La Crosse virus (LACV) in mosquitoes. Starting in June 2018, mosquito pool samples containing an Aedes species are tested for LACV, as well as WNV and SLE. Squirrels and chipmunks are known hosts of LACV, with the tree hole mosquito (Aedes triseratus) as the primary vector. According to the CDC, the virus has also been found in Aedes albopictus mosquitoes in Texas. Even though there are only approximately 70 human LACV cases each year in the U.S., there were 175 neuroinvasive LACV cases in Ohio from 2007-2016. Because cases of LACV appear to be under-reported in Indiana, ISDH believes mosquito testing will help to provide a better picture of the true incidence of LACV disease in the state.

The number of WNV positive samples varies from year to year, and is largely affected by the amount of precipitation during the spring and summer, and the temperatures throughout the season. Figure 1 shows the total number of mosquito pool samples received at the ISDHL and the number of WNV positives detected over the past 4 surveillance seasons. ISDHL did not detect SLE or EEE in any samples collected from 2014 to 2017.

Year	Total # of samples tested	Total # of mosquitoes tested	Total # of WNV positive samples	# of counties with at least one positive sample
2014	2,459	195,978	142	54
2015	2,352	187,254	374	83
2016	2,149	142,523	194	58
2017	2,142	149,083	500	90

Figure 1

The ISDHL received its first mosquito samples of 2018 during the first week of June, and as of the writing of this article, has detected WNV in 163 samples. It seems likely the number of positive samples will continue to increase if the above average temperatures of May and June persist throughout the summer. As mosquito activity increases this summer, it's important to avoid mosquito bites by using insect repellent, and remembering even a water-filled container as small as a bottle cap can become a mosquito breeding ground.

Everything You Didn't Know You Wanted to Know About Food

By Megan Teachout
Food and Dairy Microbiology Supervisor

Have you ever sat around a table discussing who has jurisdiction over your food? This commonly happens in the ISDH Food Microbiology Laboratory when we are receiving a new sample from Food Protection, training new staff, or applying for an FDA grant. Spend a day with us, and you'll realize it's not as easy as it seems.

Who has jurisdiction over my food?

The U.S. Department of Agriculture (USDA) primarily oversees meat and poultry products, while the U.S. Food and Drug Administration (FDA) oversees dairy and non-meat products; but what about overlapping products? Here are a few examples:

- Raw produce is USDA; processed produce is FDA.
- Whole egg in shell is FDA; once it's cracked it is USDA.
- Domesticated poultry is USDA; wild poultry is FDA.
- Opened-face meat sandwich is USDA; closed-face meat sandwich is FDA.
- Raw milk used to make Grade A dairy products is FDA dairy; raw milk used to make Grade B dairy products is FDA food.

In general, we ask ourselves two questions to quickly narrow down the possibilities: 1) Is it more than 3% meat? If so, it should be USDA regulated. 2) Is it milk, cream, sour cream, or cottage cheese? If it is any of these, it should be a Grade A dairy product regulated by the dairy side of FDA. If neither question is true, we search through the extensive list of products in FDA laboratory methods to confirm it is regulated by the food side of FDA.

Why does it matter to the Food Microbiology Laboratory who regulates our food?

Well, it tells us what tests are required, what methods to use, and who can perform the test. FDA and USDA have specific requirements so our results can be used to make decisions at the federal and state levels. The table below summarizes some of the key regulations that we must follow.

	FDA Grade A Dairy	FDA Food / Grade B Dairy	USDA Meat
Certified analysts	X		
Accredited lab		X	X
Specific tests by product type	X		X
Specific methods	X	X	X
Must be same as federal lab	X		X
Audited by federal assessors	Every 3 yrs.		Every 2 yrs.

What is the difference between Grade A and Grade B?
Grade A dairy products must adhere to specific processing, storing, and testing regulations so it is deemed safe to drink. Grade B, or manufacturing grade, dairy products such as ice cream and cheese can be made from Grade A or Grade B milk. Here in Indiana, plants making Grade A milk are inspected and tested monthly, while Grade B plants are inspected once per quarter.

What does that code on the side of a milk jug mean?
There's always a number in **-**** format. This tells you exactly where that milk was processed. The first two numbers indicate the state (Indiana is 18) and the last part is the specific plant within that state.

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"Everything You Didn't Know You Wanted to Know About Food" (continued from page 5)

What's the difference between whole, 2%, 1%, and skim milk?
First of all, these labels refer to the amount of fat in the milk, where whole milk is about 3.25% fat and skim milk is <0.25% fat. The dairy plants separate fat from the milk until reaching the desired percentage. We test all Grade A products to confirm the amount of fat in each sample.

Where do our meat samples come from?
Typically, the state has jurisdiction of the smaller "Mom 'n' Pop" establishments — determined by the number of cattle, poultry, or pigs processed there. Inspectors collect about 1 pound of ground beef, beef trim, sausage, jerky, ham, etc. for microbiological or chemical tests. The USDA Food Safety and Inspection Service (FSIS) has jurisdiction over the larger establishments that are often the common name brand. Those meat samples would go to the USDA laboratories.

Are complaint samples that come from the public treated any different than surveillance samples?
All samples that come in to the laboratory are received based on the submitter — ISDH Food Protection, Board of Animal Health (BOAH) Meat Division, and BOAH Dairy Division. This is because each of our customers have internal requirements that must be met for their records. BOAH dairy samples must measure 0-4.5°C at time of receipt, cannot be submerged in ice or water, and include one raw milk and/or retail milk temperature control sample per cooler per plant. BOAH meat samples must measure <15°C at time of receipt if it is a raw product and be properly labeled. ISDH Food Protection samples can be food, meat, or dairy products and must arrive sealed and properly labeled. Although the samples are received based on the submitter, all samples are tested using the methods required by the federal agency regulating that sample matrix. Our goal is to have legally defensible results that could be used by the FDA or USDA to take appropriate action.

LACV or No LACV? That is the question!

By Cassandra Campion
Assay Development Supervisor

This winter, the Assay Development team was asked to validate a real-time PCR assay for the detection of La Crosse Virus (LACV) for the Indiana State Department of Health (ISDH) Arbovirus Surveillance Program. LACV is a mosquito-transmitted virus, much like other arboviruses tested by the ISDH surveillance program. ISDH tests for West Nile virus (WNV), St. Louis Encephalitis (SLE), and Eastern Equine Encephalitis (EEE). Similar to WNV and SLE, LACV is also endemic to the state of Indiana; but it is transmitted by the daytime biting mosquito Aedes triseriatus (Figure 1), rather than the dusk/dawn biting mosquito, Aedes aegypti. LACV disease cases primarily occur from late spring through early fall. Historically, most cases of LACV neuroinvasive disease have occurred in the upper-Midwestern states (Minnesota, Wisconsin, Michigan, Iowa, Indiana, and Ohio). More cases have recently been reported in the Mid-Atlantic states (West Virginia, Virginia, North Carolina, Kentucky, and Tennessee) (Figure 2). The most common symptoms of LACV disease include fever, headache, nausea, vomiting, fatigue, and lethargy. However, severe neuroinvasive disease can also occur, most commonly in children younger than 16.

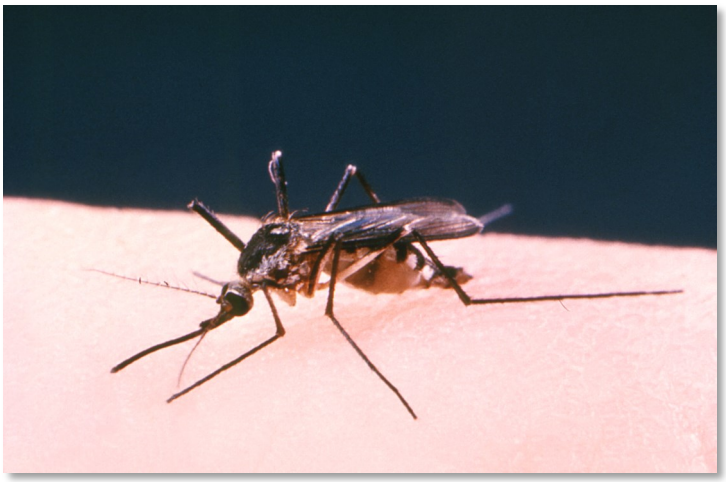


Figure 1: The Aedes triseriatus mosquito

To validate the new LACV PCR assay, the Assay Development team was provided with 50 frozen mosquito pools collected by the ISDH Entomology Division during the 2017 season and a vial of LACV-infected Vero cells from the CDC's Arbovirus Reference Collection (ARC). Given that the mosquito pools provided to the Assay Development team had not previously been tested for LACV, the question arose: how do we validate an assay in which we do not know the baseline prevalence of the analyte?

The first step to tackling this question was to decide if it was necessary to spike the mosquito pools prior to performing the extraction, or if the mosquito pool extracts themselves could be spiked. Because only the real-time PCR itself was being validated, and not the extraction process, it was determined that the simplest strategy was to perform RNA extractions on the mosquito pools and the Vero cells separately, and to spike the mosquito pool extract with RNA from the Vero cells. This strategy also allowed the Assay Development team to spike the mosquito pool extracts to determine the assay's limit of detection without also having to factor in RNA-loss during the extraction process.

For the validation, 10 positive pools and 10 negative pools were used. Positive extracts were spiked with different concentrations of LACV extract to study assay sensitivity. To eliminate the possibility that the positive pools were natively infected with LACV, prior to spiking, each pool extract was split into two; one of which was spiked with a known concentration LACV extract and the other remained unspiked. Therefore, each positive pool assay result was comprised of one spiked and one unspiked portion.

If any negative extracts were found to be positive for LACV, it was assumed the virus was present in the mosquito pool prior to spiking and that mosquito pool was removed from the validation study and replaced with another mosquito pool. The spiked positive extracts were required to show amplification and exhibit a Ct <40. If any un-spiked positive extract was observed to show amplification for LAC, the virus was assumed to be present in the mosquito pool prior to spiking. That mosquito pool was removed from the validation study and replaced with another mosquito pool.

Working on this validation offered new challenges and opportunities for the Assay Development team. While this team has validated and verified a number of real-time PCR assays, this was the first instance of working with a starting material which did not originate from a patient; but came from an environmental source. This was also the first instance of attempting to validate an analyte which is known to be already endemic to the state of Indiana. With the growing focus on mosquito and tick-borne diseases in recent years, the need for new assays which test for diseases endemic to the region are becoming more frequent. Moving forward, ISDH Laboratories and the Assay Development team now have a basic framework to conduct these important validations!

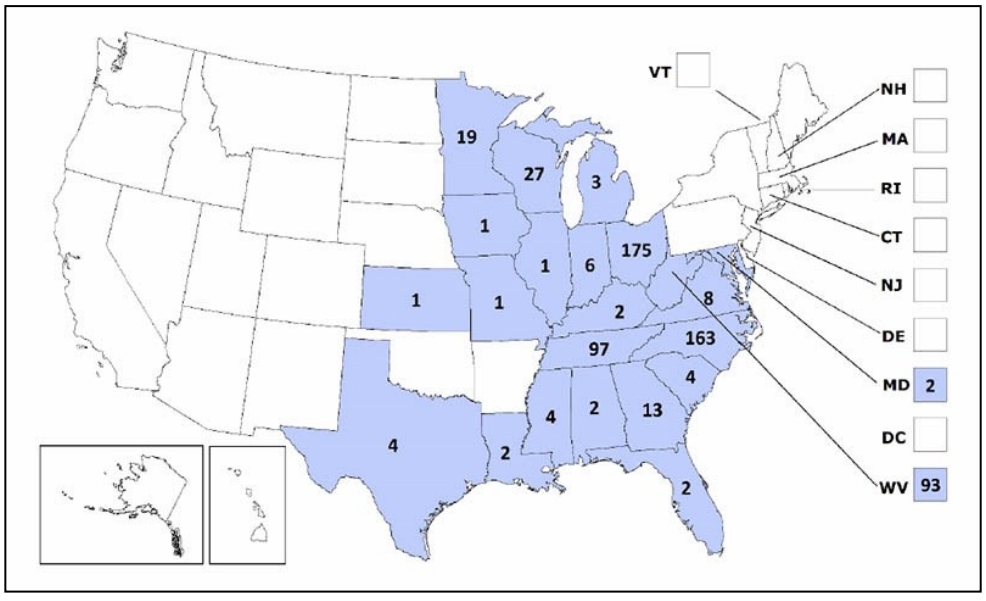
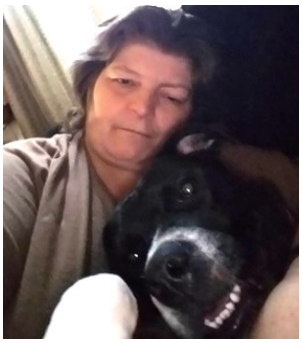


Figure 2: Cases of LACV Neuroinvasive Disease in the United States from 2007-2016

Q & A with Norma Trusler

By Rhonda Stidham



Meet Norma Trusler! ISDH employee since March 2015, just a pup compared to most around here! (Her words, not mine!)

Rhonda: "Norma, how did you come to work for the ISDH?"

Norma: "I have been friends with Bharat Patel for many, many, many years. i have a very familiar Jane-of-all-trades background, so when an opening that he thought suited me became available, he called me. I filled out an application … and POOF! … here I am."

Rhonda: "What is your job position?"

Norma: "My Official Title is Lab Tech 3. I handle the environmental side of the containers area (or maybe I should say Engra handles it, I just work there). If you need to know how to get your water tested, I can get you the correct paperwork and bottle to make that happen."

Rhonda: "Is there anything particular you want to say about your work history?"

Norma: "Before I started here, I spent many years in search of the elusive Monday through Friday, 9-to-5 job with no success. It was really long hours, a lot of weekends, and back breaking physical labor. I came close once, as a copier repair technician. I spent most of my time working in offices, but looking like a well dressed auto mechanic (because of the ink and toner thing). At times, I had to deal with needing to print something while having a non-functioning copier. it was more than a little stressful for the repair person. So alas, no Happy Ever After with that. So finally here I am, ready to settle in to my 8:15 to 4:45 and make this my End of the Trail."

Rhonda: "How has your experience been with your immediate co-workers?"

Norma: "As for my co-workers, I must say that everyone I have had contact with has been very warm and welcoming. They have immediately made me feel like one of the family."

Rhonda: "In what general area do you live and how long have you been there?"

Norma: "I lived most of my life on the southwest side of Indy (Mars Hill neighborhood) but recently moved to the near east side (near Woodruff Place) to help care for my mom."

Rhonda: "Please tell us, Norma, what do you enjoy in your spare time? I know you enjoy trying new restaurants and new recipes. Is there anything else we don't know?"

Norma: "The majority of my spare time used to be spent playing indoor competitive badminton, traveling all around the Midwest going to tournaments. (That was how I became acquainted with Bharat.) I even managed to squeak out a lower level national championship (many years ago). But after several knee injuries and life in general, now it's just occasional club play and helping others learn the game.

I also spend a great deal of time creating Halloween costumes. I've wandered the labs as a geriatric ninja turtle, and popped in to Workingman's Friend as a penguin, but I believe the best creation was the minion. (It may make an appearance in the future.)"

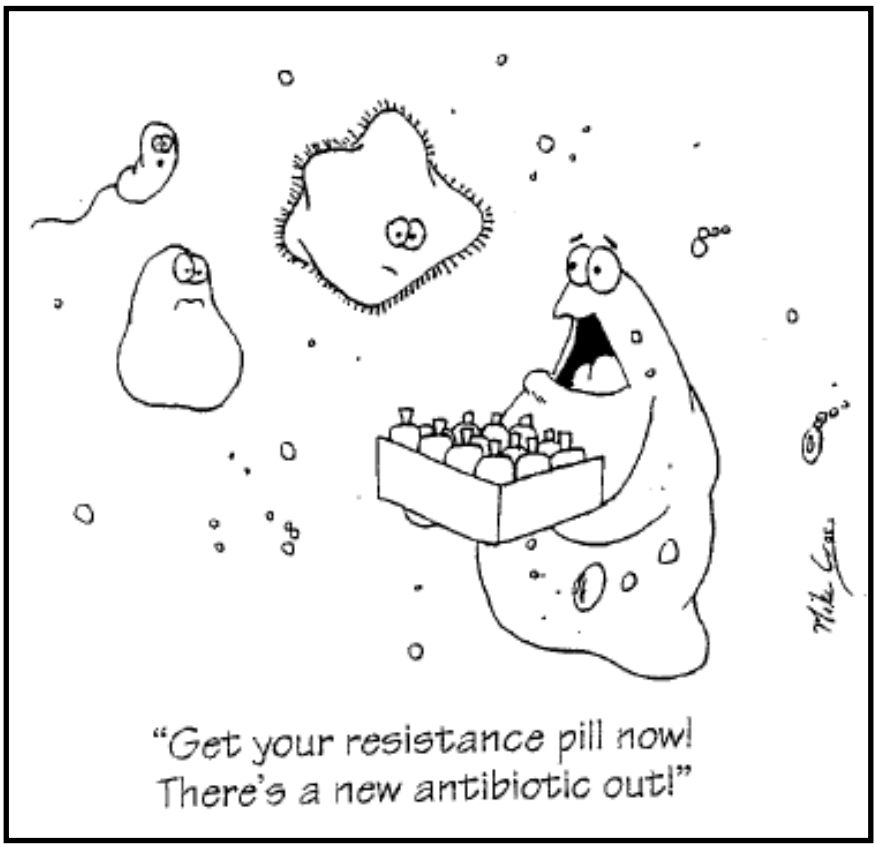
Rhonda: "Is there anything you want to share about your family or animals at home?"

Norma: "A few months ago I got the brainy idea to get a new dog. Ended up with a 5-month-old rescue from the shelter. I don't know if that was the best idea I've ever had (puppies are a lot of work)! Most of my time is now spent with her, when I'm not shopping to replace the devoured portion of my wardrobe (jeans, socks, shoes). Did I mention she was a puppy? We are now in school for both of us to learn proper behavior."

Rhonda: "Well that's a glimpse into the life of Norma, a.k.a. Normal, a.k.a. Emmagene, a.k.a. Nancy, a.k.a. Delores, a.k.a. Abby … in her own words, Norma Trusler has a lot of aliases!"

Cartoon Corner

By Michael Cross



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