

Wyoming's Lab Loop

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Foodborne Illness

Active surveillance by the health department is necessary to monitor the prevalence of foodborne illness in WY. It allows health department epidemiologists to assess trends over time and respond to outbreaks. Surveillance data can also be provided to regulatory agencies, industries and consumer groups for decision making.

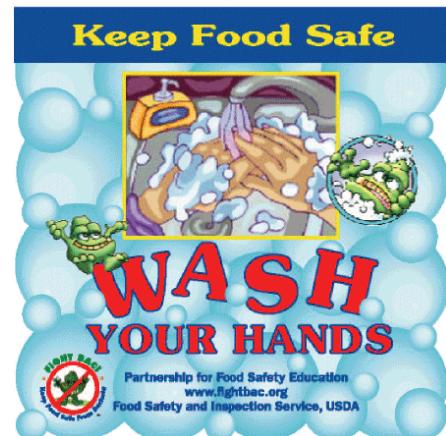
Clinical laboratories are the foundation of active surveillance; the health dept depends upon local hospital laboratories reporting cases and submitting isolates to the public health laboratory. Although most foodborne infections are sporadic, the serotyping and the use of pulsed field gel electrophoresis are important in providing epidemiologists with information to detect and investigate outbreaks and provide early intervention. The development of **PulseNet**, a CDC-initiated nationwide electronic information system involving all 50 state health laboratories, has been a major innovation in sharing molecular typing information on cases of *E. coli* O157:H7, *Salmonella*, *Shigella*, *Listeria*, *Campylobacter* and tracking multi-state outbreaks. Reportable disease isolates submitted to the public health laboratory are serotyped and their PFGE pattern is determined.

FoodNet is an important network providing information on nationwide food-borne illness prevalence involving data from 10 states; **see MMWR April 11, 2008/57 (14); 366-370**. The incidence per 100,000 population were reported as follows: *Salmonella* (14.9%), *Campylobacter* (12.8%), *Shigella* (6.3%), *Cryptosporidium* (2.7%), STEC O157 (1.2%), STEC non-O157 (0.6%), *Yersinia* (0.4%), *Listeria* (0.3%), *Vibrio* (0.2%), and *Cyclospora* (0.03%). The highest group incidence per 100,000 population for *Salmonella* (62.1), *Shigella* (27.8), *Campylobacter* (24), and STEC O157 (3.7)

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DID YOU KNOW?

Foodborne disease accounts for 76 million illnesses, 300,000 hospitalizations, and 5,000 deaths in the United States each year.

Foodborne Illness

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infections was among children aged <5 years. Although significant declines in the incidence of certain foodborne pathogens have occurred since 1996, these all occurred before 2004. Comparing 2007 with 2004–2006, the estimated incidence of infections caused by *Campylobacter*, *Listeria*, *Salmonella*, *Shigella*, STEC O157, *Vibrio*, and *Yersinia* did not decline significantly, and the incidence of *Cryptosporidium* infections increased. In 2006 the CDC reported 623 laboratory-confirmed foodborne disease outbreaks involving 16,904 cases; of which 54% of outbreaks were viral and 36% were bacterial in origin.

DID YOU KNOW?

WPHL PulseNet lab also provides analysis for *Campylobacter*, *Salmonella*, and *Staphylococcus* isolates from the Wyoming State Veterinary Laboratory in Laramie.

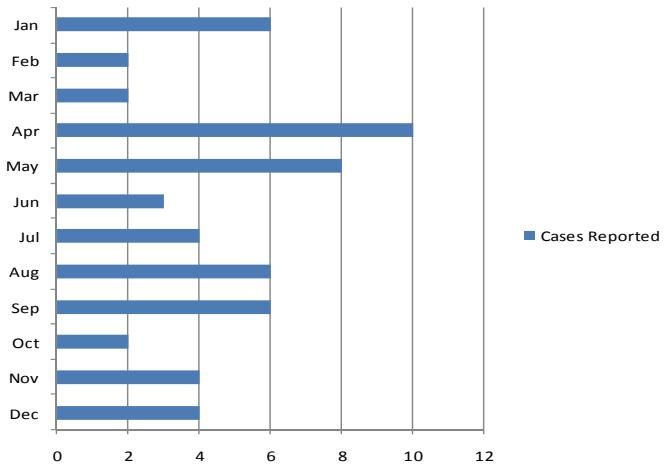
Incidence of Salmonellosis by County Wyoming, 2007

County of Patient Residence	N	Incidence rate / 100,000 population
Albany	3	9.4
Big Horn	3	26.2
Campbell	3	8.9
Converse	4	33.2
Fremont	2	5.6
Goshen	2	16.0
Johnson	2	28.3
Laramie	11	13.5
Lincoln	20	137.2
Natrona	8	12.0
Park	2	7.8
Platte	1	11.4
Sheridan	9	30.1
Sweetwater	2	5.3
Teton	2	11.0
Uinta	4	20.3
Statewide	78	15.6

Notable Foodborne/Waterborne Outbreaks in Wyoming

Organism	Year
<i>E. coli</i> O157:H7	1998
<i>Salmonella</i> Typhimurium	2002
<i>Salmonella</i> Heidelberg	2003
<i>Campylobacter</i> jejuni	2005
<i>Campylobacter</i> jejuni	2006
<i>Salmonella</i> Muenster	2007

Onset Dates Among Cases of Salmonellosis, Wyoming 2007



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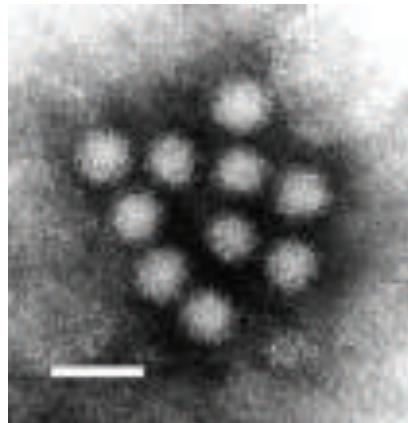
Wanda Manley and Gale Stevens

Norovirus

"Stomach flu", food poisoning, and viral gastroenteritis, all terms that are frequently used to refer to the illness caused by a pesky little virion known as Norovirus. Very infectious and very contagious, Norovirus is responsible for approximately 90% of all non-bacterial gastroenteritis around the world. Only the common cold is reported more frequently than viral gastroenteritis as an illness in the U.S.

A member of the *Caliciviridae* family of RNA viruses, Norovirus is responsible for almost 50% of all food-borne outbreaks in the U.S. There are 5 genogroups of Norovirus that have been identified and are referred to as: Genogroups I, II, III, IV, and V with Genogroups I, II, and IV seen in human Norovirus outbreaks, Genogroup II is by far the most common. Each genogroup also contains different genetic clusters or genotypes within it and therefore, it appears, immunity is strain specific and lasts only a few months, so re-infection is likely in a person's lifetime.

Transmission of Norovirus is through the fecal-oral route usually through contaminated foods, liquids, surfaces, person to person contact, and even through aerosols produced from expelled vomitus, that contaminate surfaces. A CDC study of eleven outbreaks in New York lists the suspected modes of transmission as person to person in seven outbreaks, foodborne in two, waterborne in one, and one unknown source. Shellfish from contaminated water sources and salad ingredients are the most common



Norovirus by electron microscopy. Note the lace-like appearance of individual virus particles.

foods implicated in Norovirus outbreaks, and foods other than shellfish are usually contaminated by food handlers afflicted with the virus. Therefore, people infected with suspected Norovirus should not handle or prepare any kind of food products while symptomatic and for up to 3 days afterward. It is also noted that certain environments make up a large percentage of outbreaks with daycares, nursing homes, cruise ships, and hospitals being especially susceptible.

Symptoms of Norovirus

DID YOU KNOW?

CDC estimates that 23 million cases of acute gastroenteritis are due to Norovirus infection, and it is now thought that at least 50% of all food-borne outbreaks of gastroenteritis can be attributed to Noroviruses.

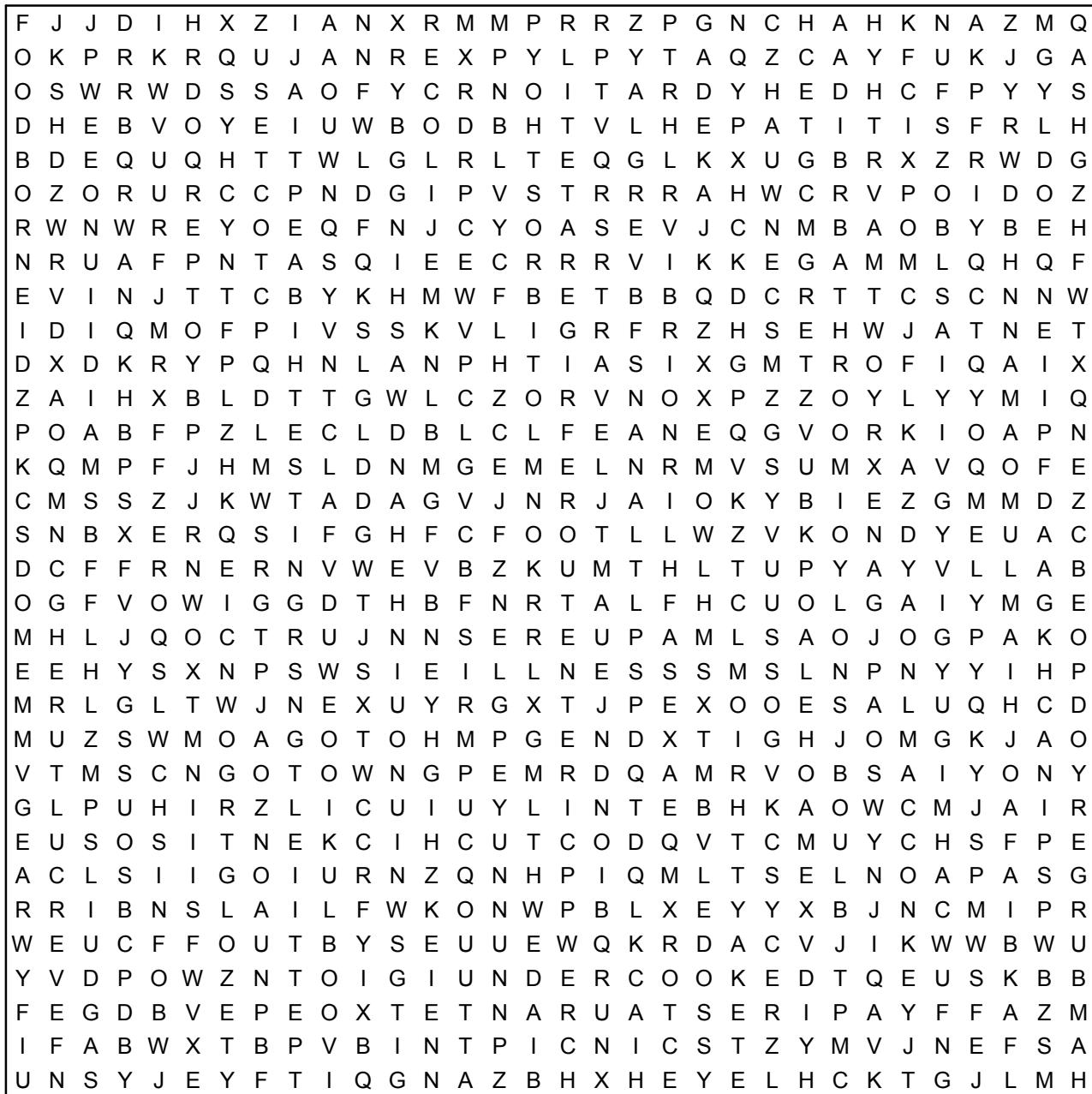
usually appear 24-48 hours after ingestion of the virus or sometimes as early as 12 hours. Onset is usually sudden with symptoms including nausea, vomiting, diarrhea, and abdominal cramps, usually accompanied by a low grade fever, chills, muscle aches, and lethargy. In most cases the symptoms are self-limiting with improvement in 1 to 2 days. It is estimated there are around 300 fatalities from Norovirus a year in the U.S. with the young and elderly being at greatest risk due to severe dehydration. Currently, treatment due to Norovirus infection consists of fluid replacement. Patients may pass Norovirus from 3 days after infection up to 2 weeks, therefore it is important to practice good hand washing and other hygienic practices to prevent further spread of the virus. Clean contaminated areas with bleach based cleaners, especially toilets and sinks, and wash any clothes and linens in hot water with detergent. Any shellfish should be thoroughly cooked and all fruits and vegetables carefully washed before eating.

Stool samples from patients who are symptomatic for diarrhea and vomiting for several days that are negative for routine bacterial gastrointestinal pathogens may often be positive for Norovirus. The WPHL provides Norovirus testing for samples from clusters and outbreaks at the request of the Dept of Health Infectious Disease Epidemiology Section. Testing is

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Word Search

DON'T EAT THAT!



ACHE
CAMPYLOBACTER
DIARRHEA
FEVER
HANDWASHING
INTESTINES
POTATOSALAD
RESTAURANT
SANITIZE
SPINACH
TESTING
UNDERCOOKED

ANTIBIOTICS
CHICKEN
ECOLI
FOODBORNE
HEPATITIS
MACCONKEY
PULSED
ROOMTEMPERATURE
SEQUENCING
SPOIL
TOXIN
VIBRIO

BATHROOM
CULTURE
ENTERIC
GRAMSTAIN
ILLNESS
PICNICS
RECALL
ROTTEN
SHIGATOXIN
STOOL
TRAVEL
VOMIT

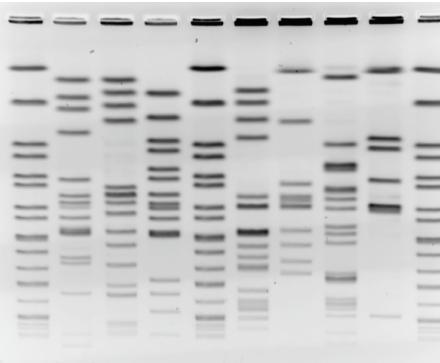
BOTULISM
DEHYDRATION
EPIDEMIOLOGY
HAMBURGER
INFECTION
POISON
REFRIGERATE
SALMONELLA
SORBITOL
STORAGE
TYPHUS

PFGE and PulseNet

PFGE Protocol:

Pulsed field gel electrophoresis is a standardized molecular subtyping method, or finger-printing, of foodborne disease-causing bacteria. Briefly, the protocols involve the following steps: a bacterial organism is isolated and identified from a human clinical specimen, veterinary specimen, food, or water. It is combined with agarose to form a plug, for ease of handling. The plug is washed in a lysing agent to break the cell wall, the plug is then washed to remove the cellular debris. A slice (1mm x 8mm) is cut from the plug and incubated with a restriction enzyme. The restriction enzyme cuts the DNA at known intervals (for example, Xba I cuts at the nucleotide sequence "T/CTAGA" which is found in multiple locations on the bacterial chromosome). The result is 10 to 15 DNA fragments of various lengths. The first part of the process (making the plug, lysing the cell wall, and cutting the DNA) takes ~ 8 hours. The slices are loaded on a comb and agarose is poured around it to form a gel. The gel is loaded into an electrophoresis box, running conditions are programmed, and the gel runs for 18-19 hours. Due to the large molecular weight segments of DNA, a computerized system with multiple electrodes surrounding the gel box is necessary to force the

DNA through the gel. The smallest fragments move the fastest and they migrate towards the bottom of each lane. The following morning, the gel is stained, rinsed, and a UV light image is taken. The protocols are specific to each organism, but all protocols follow this general procedure.

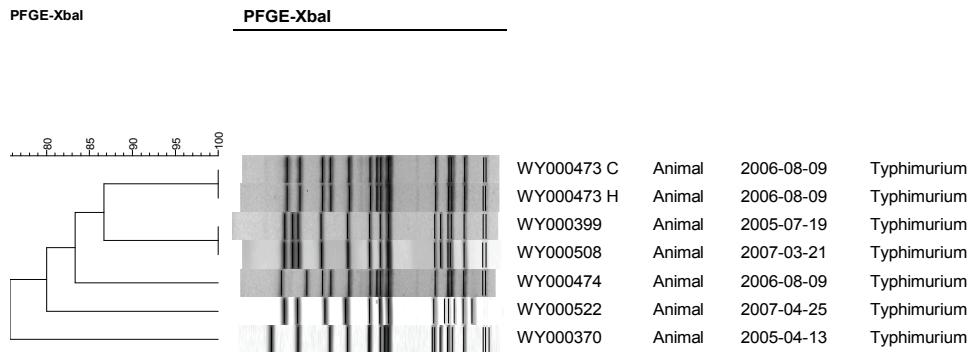


Salmonella PFGE tif image

The image is converted to a tif file and loaded into BioNumerics™ software. Each lane in the gel is normalized against a global standard, the bands of DNA are marked, and the patient demographics are attached. Now the PFGE pattern from each isolate can be com-

pared against the appropriate local Wyoming database for that species or serotype. The PFGE pattern is highly specific and can differentiate minor genetic differences within a single species such as *E. coli* O157:H7 so that isolates unique to a single event can be identified as different from other isolates of the same species. The patterns are uploaded to the PulseNet server at CDC in Atlanta. All PulseNet certified laboratorians can perform searches in this database, looking at all other states PFGE patterns and demographics. No names of patients are transferred out of the state.

A dendrogram is built which displays the relationship of the PFGE pattern of the new entry to all of the other isolates in the WY database and the PulseNet database. A report is generated and sent to the Wyoming foodborne illness epidemiologist. No PFGE reports are sent back to the submitter of the original patient specimen. Each report



Dendrogram of Salmonella Typhimurium in animal isolates.

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PFGE and PulseNet

(continued from Page 5)

contains the names and demographics of all related PFGE patterns in Wyoming.

PulseNet



PulseNet is a national network of public health and food regulatory agency laboratories coordinated by the Centers for Disease Control and Prevention (CDC). The network consists of: state health departments, local health departments, and federal agencies (CDC, USDA/FSIS, FDA). All PulseNet participants use the same protocols, so all patterns that are uploaded to the national PulseNet databases can be compared to each other.

CDC PulseNet has large national databases for *Campylobacter*, *E. coli*, *Salmonella*, *Shigella*, and *Listeria*. They also have smaller databases for *Yersinia*, *Clostridium*, *Vibrio*, and *MRSA*. WPHL maintains databases and certifications for *Campylobacter*, *E. coli*, *Salmonella*, *Shigella*, *MRSA*, and *Yersinia pestis*. WPHL also has smaller databases for Group A *Streptococcus*, Group B *Streptococcus*, *Bordetella*, and non pathogenic *E. coli*. These organisms do not have official PulseNet protocols,

but most organisms are fairly predictable and can be analyzed by PFGE. WPHL also has a catchall database for small numbers of isolates from multiple other organisms that were all analyzed under one protocol.

The PulseNet national databases allow the public health laboratories in the individual states to perform real time comparisons, which leads to rapid detection of outbreaks. The introduction of PulseNet has significantly reduced the period of time between initial detection of a possible outbreak and determining the source and spread of the outbreak. In large multi-state outbreaks involving many people this can have significant impact on preventing new cases and removing contaminated products from public consumption. PulseNet also has a WebBoard that is open to

any participating laboratory, regardless of certification status. This is usually where clusters of similar patterns are first noted. The WebBoard also encourages participants to share ideas about equipment, vendors, new protocols, and trouble shooting methods.

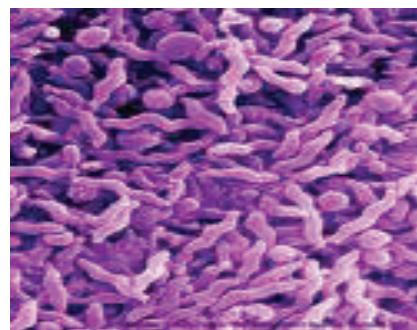
CDC has database managers in Atlanta that respond quickly to each posting of a new cluster. The state labs usually do 30 or 60 day searches, but the CDC database team looks at the entire database. The CDC database managers also talk to the foreign counterparts of PulseNet USA. The other components of PulseNet International are PulseNet Canada, PulseNet Europe, PulseNet Latin America, PulseNet Asia Pacific, and PulseNet Middle East.

Norovirus

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DID YOU KNOW?

Campylobacter is the most common bacterial cause of diarrheal illness.



Campylobacter jejuni

accomplished through the use of real time-PCR (Polymerase Chain Reaction) system which is the most rapid, sensitive and specific method. The WPHL uses the Cepheid SmartCycler along with an ASR (Analyte Specific Reagent) Norovirus kit, which is optimized for use on the SmartCycler platform. The ASR kit includes primer and probe sets that detect GI and GII Norovirus since these are the most common types seen in viral outbreaks. The real-time platform is a major advance in PCR technology with Norovirus RNA extracted from the stool added to the primer-probe mix in a closed reac-

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Summarization of WPHL Spring Traveling Workshop: Antimicrobial Susceptibility Testing for Laboratorians

Wyoming Public Health Laboratory, in conjunction with American Society for Clinical Laboratory Scientists – Wyoming Chapter and the 4 State National Laboratory System Consortium Grant, hosted a traveling workshop for Wyoming laboratorians. This intermediate level workshop provided an overview of the Clinical and Laboratory Standards Institute (CLSI) document, "Performance Standards for Antimicrobial Susceptibility Testing", focusing on antibiotic usage, selection, and reporting. Antimicrobial Stewardship was emphasized, stressing the importance of the microbiologist's role within the health care team, the need for accurate and timely antibiotic reporting, and striving for quality patient outcomes.

The workshop traveled to Sheridan, Thermopolis, Douglas, Rock Springs, and ended in Cheyenne. Fifty-nine participants attended the workshop, reaching 30 Sentinel Laboratory facilities in the state. Participants not only included laboratorians, but Medical Laboratory Technician Students, Infection Control Practitioners, and Physician Assistants.

One more installment of this workshop will be offered through i-Link, a computer/internet based program. The i-Link allows participants to attend anywhere there is a computer with internet access. The i-Link will take place July 16th @ 1:00 PM with all information needed to participate to follow soon! Please contact Gale Stevens at 307.777.3375 with any questions.

Workshop for Sentinel Laboratory Managers Held in Saratoga

In March of 2007, all available lab managers in the state met for a 2 ½ day workshop in Saratoga. Of the 35 facilities in the state, 22 labs were represented. This workshop served many functions.

Based on the criteria list for sentinel laboratories provided by the American Society for Microbiology, all joined in discussion to determine at what level their lab functions within Wyoming's Laboratory Response Network. So far, 20 laboratories function at the ADVANCED level, one at the BASIC level, and one facility not performing microbiology procedures does not fit either criterion. The laboratories not able to attend the workshop will be designated as ADVANCED or BASIC through site visits sometime this summer.

The attendees had the opportunity to meet one of Wyoming's most important partners in emergency response, The 84th Civil Support Team (CST). CPT. Wyatt Kramer and MAJ. Priom Srinual (CJ) represented the CST and talked about their duties and responsibilities on the team, the functions of the team, and presented their mobile laboratory for tours. During the workshop, chemical response for Wyoming was discussed along with preparedness issues, continuance of operations planning, and Hospital Incident Command Systems (HICS) pertaining to laboratories. The workshop ended with an exercise practicing HICS and turning in the paperwork for Year 5 Bioterrorism Community Laboratory Capacity Building Grant.

Norovirus (continued from Page 6)

tion vessel that significantly reduces the potential for contamination of the lab with PCR-amplified DNA product.

Stools are the most common type of sample tested, although vomitus and foods can be tested in some instances but neither is typically used in routine testing for Norovirus infection.

J	D	I	H	X	Z	I	A	N	X	R	M	P	R	Z	P	G	N	C	H	A	K	N	A	Z	Q		
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Wyoming's Lab Loop

Information Available in this Issue:

- Foodborne Illness
- Norovirus
- PFGE and PulseNet
- Antimicrobial Workshop Summary
- Sentinel Lab Managers Workshop

What's Coming Up?

Event	Location	Date
Antimicrobial Susceptibility Testing Education	iLink Webcast	July 16
Micro in the Mountains	Breckenridge, CO	August 13-15
IMSS http://imss.asclsregionviii.org	Jackson, WY	Sept 17-21
Intermediate Microbiology Wet Workshop	TBA	October
USDA Recalls & Public Health Alerts for Meat, Poultry, Egg: http://www.fsis.usda.gov/Fsis_recalls/		

DID YOU KNOW?

Recent evidence suggests that people with blood type O are at the greatest risk of severe infection with Norovirus.

FDA Recalls, Withdrawals, and Alerts:

<http://www.fda.gov/opacom/7alerts.HTML>

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