Idaho Bureau of Laboratories

Clinical Forum

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State of Idaho

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IDAHO DEPARTMENT OF HEALTH & WELFARE

Lab Spotlight: Reference Bacteriology Laboratory

Lisa Smith

The Reference Bacteriology lab at the Idaho Bureau of Laboratories offers a variety of testing services to hospitals, doctor's offices, commercial laboratories, and district health departments in an effort to facilitate patient care and provide laboratory confirmation for disease surveillance and control. This laboratory plays a critical role in identifying rare bacterial strains, performing difficult or unusual testing, and characterizing bacterial outbreak isolates.

The Reference Bacteriology laboratory uses a variety of methods, from conventional biochemical testing to rapid molecular methods like polymerase chain reaction (PCR) testing in order to provide isolate identification, serotyping of enteric bacterial isolates and invasive *Neisseria meningitidis* and *Haemophilus influenzae,* and diagnostic testing for pertussis. Additionally, there is coordination of testing between this lab and the molecular epidemiology lab to perform a variety of molecular tests, which are used to verify and complement biochemical method identification and characterize isolates for epidemiologic use. The Molecular Epidemiology lab performs pulsed field gel electrophoresis (PFGE) on all *Salmonella*, *Shigella*, and toxigenic *E. coli* for use in outbreak detection and characterization. *Salmonella* isolates are also serotyped using a molecular method which provides rapid turnaround time and improves the ability to serotype difficult isolates. Reference isolates received and tested in the Reference Bacteriology lab are also subjected to ribosomal RNA gene sequencing for definitive identification.

This lab performs 37 different procedures and completes approximately 3,600 tests annually. Some types of tests are requested

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Whoop There It Is: *Bordetella pertussis*

Joanna Lewis

Whooping cough, caused by *Bordetella pertussis,* is a highly contagious respiratory illness nicknamed because of the characteristic "whooping" noise that follows a prolonged coughing fit. These coughing fits can last up to 10 weeks and are most serious for unvaccinated persons and infants. The side effects in infants can include pneumonia, apnea, and convulsions. Up to 57% of infected infants will require hospitalization due to pertussis[1]. In teens and adults, the side effects are more likely related to the cough itself: weight loss, temporary urinary incontinence, and possible rib fractures due to vigorous coughing fits.

The current acellular pertussis vaccines are rated as 60-90% effective against moderate to severe pertussis[1]. New research (including the California outbreak in 2010) sugggests that immunity to pertussis after the last scheduled dose of the vaccine (around age seven) waned during the five years following the shot, increasing the

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Inside this issue:

Pulsed-Field Gel Electrophoresis (PFGE): Outbreak Detection and Characterization

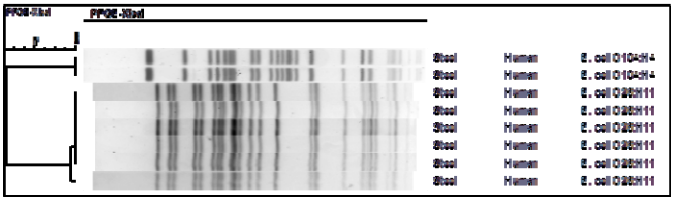
Amanda J. Bruesch, M.S.

Idaho Bureau of Laboratories (IBL) has performed PFGE on enteric bacterial isolates such as Shiga toxin-producing *E. coli* (STEC), *Shigella,* and *Salmonella* since 2001. We have been an active participant in CDC's PulseNet program – a network of labs across the country and around the world who perform PFGE using standard protocols to aid in outbreak detection and data comparison. Participation in this network has not only allowed Idaho to identify cases of enteric illness that belong to nationwide clusters and include case patients from Idaho in these outbreak investigations, but it has also afforded Idaho the ability to detect clusters of enteric illness that originate in Idaho that have then been linked to a common source. In addition to outbreak detection, PFGE provides information to help characterize an outbreak and include or exclude case patients. Below are case studies that demonstrate the utility of PFGE data and highlight IBL's contribution to outbreak detection and characterization.

Outbreak #1:

During the height of the German *E. coli* O104 outbreak in the spring and summer of 2011, an Idaho resident with recent travel history to Germany came down with a STEC infection. This resident happened to be a cook at an Idaho summer camp which was currently being attended by residents from multiple states. IBL received this patient's sample as well as news that a number of campers were exhibiting symptoms and having samples taken for testing. It was concerning that this person's STEC might be the German O104 strain that was causing significant morbidity and mortality. Because Idaho does not have serotyping reagents available to identify an *E. coli* O104 and could not rely on our ability to serotype the patient's isolate to determine if it was part of the German outbreak or not, multiple molecular methods were utilized to characterize the strain of *E. coli*. We were able to provide the information to our State Epidemiologist to allow them to investigate the case in a timely manner.

Below is an image of the German E. coli O104 strain's PFGE banding pattern in lanes 1 and 2. The remaining lanes in the image are that of the sentinel case of STEC from the patient in Idaho and the subsequent six cases that were confirmed by IBL. Isolates that are identical strains will exhibit identical banding patterns by PFGE. Isolates that are not identical strains will exhibit different banding patterns. As you can see, by using standard methods and being able to directly compare data with other PulseNet labs, we were able to conclusively say that this patient's STEC isolate was not the same strain as the German O104 strain and that the Idaho outbreak consisted of multiple patients with the same strain of *E. coli* as evidenced by the identical patterns among all case patients.

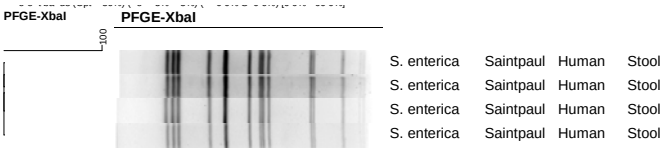


In addition to PFGE data, IBL performed a PCR to detect the Shiga toxin genes present in the bacterial isolate. We determined that the Idaho residents' STEC isolates contained the *stx1* gene while the German strain was previously determined to contain the *stx2* gene. This was another piece of evidence that allowed us to confirm that we were not dealing with cases of the German O104 strain of *E. coli*. The Idaho patients' isolates were identified as *E. coli* O26:H11 and had matching PFGE patterns as shown above, indicating that they were all sharing the same strain of *E. coli*.

Outbreak #2:

In the spring of 2008, our enteric bacteriology lab noticed that there were a large number of *Salmonella* isolates that were serotyping as *Salmonella* Saintpaul. While this is not a new serotype to our lab, it is not one of our most common serotypes. Routine PFGE testing was being performed on all *Salmonella* isolates, and all of these isolates were also showing identical patterns upon analysis. As of this time, there was not a national published notice regarding any clusters of *Salmonella* Saintpaul. Idaho was one of the first states to detect this cluster, upload our PFGE patterns to CDC's national database for comparison to other states' patterns, and post an announcement to other PFGE labs that we were seeing this trend.

Below is an image of the banding pattern for the outbreak strain of *Salmonella* Saintpaul that eventually became associated first with red Roma tomatoes and then jalapeno and Serrano peppers grown and packed in Mexico. There was much contention over the source of the contamination. However, jalapeno peppers from a distributor in McAllen, Texas as well as irrigation water and Serrano peppers from a farm in Mexico all tested positive for the outbreak strain. The warning regarding red Roma tomatoes was lifted following discovery of the positive peppers, as no tomatoes ever tested positive for the outbreak strain of *Salmonella*.



It is with continued support from our clinical lab partners that we are able to perform this testing. Without submission of enteric bacterial isolates for serotyping and PFGE, we would not be able to serve this role of outbreak detection and characterization. Thank you to all of our clinical lab partners for your contributions to this important testing.

Emergence of Drug Resistant TB

Brian Deis, M.S.

Tuberculosis is a serious and pervasive disease that affects 12 million people and accounts for nearly 1.7 million deaths each year globally[1]. Although TB infection rates have been steadily declining in regions with high standards of living, 9 million new cases still arise each year in predominately low and middle income countries. This trend demonstrates that early detection and proper treatment are vital to saving lives and curtailing the spread of this epidemic.

Although great strides have been made to control TB, the emergence of drug resistant strains threatens to undermine these gains. Multi-drug resistant (MDR) TB, resistant to at least two 1st line drugs, and Extensively Drug Resistant (XDR) strains of TB, resistant to additional 2nd line drugs, began to appear in the 1990s. Currently they account for approximately 5% of TB cases and 10% of TB related deaths worldwide[2]. In 2007, Totally Drug Resistant (TDR) TB cases began to appear in Europe, and later in the Middle East, and Asia. TDR strains, as the name implies, are resistant to all 1st and 2nd line drugs. Although reports of TDR are relatively rare and tend to be regionally isolated, a global emergence would be disastrous.

Drug resistance arises as a result of spontaneous genetic mutations in the TB bacteria from single drug therapies. Additional drug resistance can be conferred when a new drug is used to replace an ineffective one. The emergence of MDR, XDR, and TDR TB emphasizes the critical importance of a concurrent multi-drug treatment regime for the prescribed duration, yet, the WHO estimates that only 20% of TB patients receive proper treatment[1].

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Bordetella pertussis

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chance of catching pertussis by about 42% per year on average[2]. This could explain the increase in cases among children aged 7-10 during the year 2010 (27,550 total cases reported in the US, the highest since 1959!)[3]. Further research is needed to determine the need for a new or modified pertussis vaccine.

In Idaho, pertussis continues to cause serious illness, especially in the early fall and winter. The Bureau of Communicable Disease Prevention and Control reported a rate of 16.8/100,000 population for 2010 with an increase to 19.7/100,000 for 2012 (preliminary data through September 23, 2012)[4]. Compared to neighboring states, Idaho has had less impact from pertussis than Washington where an epidemic was declared in April 2012. Cases in Oregon were projected to be nine times higher than the same time last year, and cases in Montana were five times higher[5]. The Idaho Bureau of Laboratories (IBL) tested 361 pertussis specimens in 2010, with a positivity rate of 10.8% and has tested 312 specimens this year (as of September 28, 2012) with a positivity rate of 8.4%[6]. Most of these specimens were submitted from clinical labs and health districts.

IBL uses a two-step PCR algorithm for detecting pertussis: the first PCR amplifies a conserved region of a high copy number insertion sequence specific to several *Bordetella* species; the second is a PCR reaction specific to the pertactin gene which codes for a membrane associated adhesin protein found in *Bordetella pertussis*. Thus, a specimen would have to amplify in both reactions to be called *Bordetella pertussis.* Pertussis like illness can be caused by other *Bordetella* species, including *B. bronchiseptica, B. parapertussis*, and *B. holmseii.* IBL does not currently differentiate between *B. pertussis* and the other species that can cause disease, but a molecular method to speciate *Bordetella* in clinical samples is in consideration for the future. Culture-based methods are the gold standard for speciation of clinically relevant *Bordetella* species but, unfortunately, culture has low sensitivity if specimens are collected late in disease progression when many seek medical care[7].

Pertussis continues to persist despite public health efforts to effectively vaccinate Idahoans against this disease. Through partnerships with clinicians, nurses, physicians, and epidemiologists, the testing performed at IBL helps us learn more about this disease and how it continues to impact the health of Idaho's communities.

References

[1]Centers for Disease Control and Prevention. (2000). *Guidelines for the Control of Pertussis Outbreaks.* Retrieved from http://www.cdc.gov/vaccines/pubs/pertussis-guide

[2]Klein, N.P. MD, PhD, et al. (2012). Waning Protection after Fifth Dose of Acellular Pertussis Vaccine in Children. *New England Journal of Medicine, 367*, 1012-1019.

[3]Centers for Disease Control and Prevention. (2012). *About Pertussis*. Retrieved from http://www.cdc.gov/pertussis/about

[4]Table 1: Idaho Statewide Pertussis Data. Bureau of Communicable Disease Prevention and Control (September 23, 2012)

[5]Idaho Department of Health and Welfare. (2012 Oct). Spotlight on Pertussis: How does Idaho Incidence Compare? *Health and Welfare Disease Bulletin*, 19(3) (Proof).

[6]Idaho Bureau of Laboratories. Unpublished data. 2010-2012.

[7]Centers for Disease Control and Prevention. (2012). *Diagnosis Confirmation.* Retrieved from http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-confirmation.html

Bacteriology Laboratory

continued from page 1

and performed once or twice a year while others have volumes in the hundreds. For example, the Shiga Toxin Immunoassay (EHEC) was run over 380 times in the 12-month period ending in August 2012. The EHEC EIA assay tests for a toxin that is found in some *E. coli* strains including *E. coli* O157, the culprit in the spinach foodborne outbreak of 2006. Human bacterial isolates of *Salmonella* and *Shigella* are also frequently submitted for identification by biochemical and serological testing, including molecular serotyping.

An XLD plate showing bacterial growth. This media is selective for *Salmonella* and *Shigella*. *Shigella* colonies look red while *Salmonella colonies produce H_2S, which produces a black center in some colonies.* This plate shows *Citrobacter* colonies, which can sometimes be confused for *Salmonella*.

The bacteriology lab tested over 250 specimens in the 12-month period ending in August 2012 for *Bordetella pertussis*, the bacterium that causes pertussis or whooping cough. These results are used in direct patient care and prophylaxis.

Addition of substrate step in the EHEC EIA procedure to detect Enterohaemorrhagic *E. coli*. Up to 94 samples can be run at once in this enzyme immunoassay procedure.

Test of cure samples are also frequently submitted from the health districts that follow cases of reportable diseases such as *Salmonella*, toxigenic *E. coli*, *Cryptosporidium,* and *Giardia*. A negative result may be required for children to resume activity in childcare or for foodservice workers to go back to their jobs.

In addition, this laboratory provides reference diagnostic testing for private sector labs that may not have the capability to fully identify disease agents of public health significance. Recently, an isolate was submitted for confirmation of *Vibrio,* a bacterial infection ususally caused by eating undercooked seafood. Through biochemical methods and genetic sequencing, it was determined not to be *Vibrio* at all but *Gallibacterium anatis*, a bacterium normally associated with chickens.

Some testing is seasonal; for example, more enteric disease specimens are submitted in the warmer months. Other tests are submitted throughout the year with occasional outbreaks occurring. With this in mind, there is a need to balance workload and supplies to be able to meet the demands of testing at any time.

The testing performed in the bacteriology laboratory serves various agencies and labs throughout the state of Idaho. Through the methods and procedures, staff accurately and efficiently detect diseases that are both rare and of high consequence to maintain the health of Idahoans. IBL would like to thank the clinical laboratories for submitting samples to help further characterize isolates and to help with the health department's efforts to identify and reduce the incidence of diseases. For more information on samples and sample types accepted, view the Sampling and Submission Guide on the StateLab website.

If the culture ferments any of the lactose or glucose, the reaction produces acid and the media changes to a yellow color. Gas production is noted in some of the tubes by cracks, bubbles or movement of the media up from the bottom of the tube. The third tube on the left shows H_2S, which is typical of *Salmonella*.

Bacteriology Word Find

answers on page 6

R R B B B S O W T K I G D J O B T N A T S I S E R J E Z I H
L A T X E N L Q A F N A A Q O A P I S D O D R G I P L X J A
U C J O S Y H T M I Y O C R Z S P H B E P J T I Y R X G B E
Q Q J E A F Q N H P W M D Q V Y J Z A Y Y R R T X L H Z W D
V P K L F Q V S Y V Y E D U D N R J E O R E O J E J Z Q S A
E T M O L T A G B Q T D L Y Z J G A I X J R E O Z X A N C F
R L P F C W P Z V E E R X T N Q J P L N E A L G Y S A O H Z
K E D T D I L E L Q F V J I O Y S Z R S G R B N H I Q P M L
P A T N M R M L Y J I Q J W X Z N M C Q Z D I A M Q R F F Z
D M A C Q F A C U B Z X J T B N G O E O Z J T M P H C J T O
H H C Y A E L T R U T B R D G E Q Q L A I F P K S Q E D Y A
B C D O I B V D J V Y G U T X J W N X O T P E R N X L N E O
Z R U C Z B I L N T A D Y H E L W F P Q C S C Q N F X Q D U
W P A X X U I L K I V O Q D G C B O F C H E S N C A C N W T
G H A E C C N K L D Q G N Y Y E M A H G H S U F I B T I H F
L D N Y N G Z I O A H I A Q Z O P X C X J Q S G Y I Y M J C
V H O S L D G L W S G I U Q N P A O W T H F E P M S W P R T
D S R W I Q E S S K Y I M A J G R Y A N E S T N R C S G S P
S Y W U P G E R N G C J E L L Z O Y Q I H R F B G B L G A H
H V S D I F K Q U I H H P F I X X Q O A B Q I Y H E P O G Z
O P G K I M J D J P T W U F C Z Y Z H T F B S A F L T E O M
I I T R E B L A A I H C I R E H S E U S O U T B R E A K M P
J M H L G L Q V H I M H A R G W M Y C M P L A T E W K I E C
P K U L T P P E B G G B Q T M E A G Q A C L C H Q S A Q X R
R G U P R A J P U U M W X Y R F L P H R I U V V F U A Z I W
U K S H I G A T O X I N U R W E X L S G E B Y I W M I G C F
M S P Z Q W O C F D B V W B B V P D V I R V C O W Y Z M O T
P D V R F L Q B U Y V W Q T T L F L L F K R V D Q Y C B V U
R J B X C A V Y H Q I F F E I T J K S U D Y W C U M D B C F

BACTERIA
BORDETELLA
BRAENDERUP
COLONY
COUGH
ESHERICHIA ALBERTII
GALLIBACTER
GRAM STAIN
HAND WASHING
LOOP
MANGO
MEXICO
OUTBREAK
PAROXYSMAL
PERTACTIN
PLATE
POMONA
RESISTANT
SEROTYPE
SHIGA TOXIN
SUSCEPTIBLE
TURTLE

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Emergence of Drug Resistant TB

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Unfortunately, inadequate infrastructure and limited healthcare resources in regions where TB is common have given rise to drug-resistant TB. Often, healthcare workers do not have reliable access to multiple drugs, and many patients aren't able to routinely visit healthcare providers for a protracted treatment regime. Furthermore, treatment costs for drug resistant TB can be significantly higher than that of conventional TB.

Lack of resources affects patient outcomes and impedes the ability to adequately screen and detect new TB cases. Ideally, a culture and drug-susceptibility test (DST) should be done for every suspected TB case. Traditional culture-dependent DST methods are highly sensitive and reliable, although due to cost and limited laboratory capacity, routine culturing and drug-susceptibility testing occurs in only 22% of countries globally while 41% of countries have no culturing or DST program[2]. Many countries rely on less reliable methods of diagnosis including microscopy and x-ray.

In addition to improving patient access to reliable healthcare and improving laboratory operations worldwide, simplified, lower-cost diagnostic and screening methodologies must be developed. Pyrosequencing technology is one approach that is gaining popularity. This sequencing method is optimized to detect gene mutations responsible for drug resistance[3,4]. Pyrosequencing can analyze large numbers of samples in a fraction of the time of traditional testing with less cost.

(continued on page 6)

Solution to Word Find

(Over,Down,Direction)

BACTERIA(17,14,SE)
BORDETELLA(16,1,SW)
BRAENDERUP(1,12,SE)
COLONY(21,13,NW)
COUGH(8,27,NE)
ESHERICHIA ALBERTII(18,22,W)
GALLIBACTER(11,17,NW)
GRAM STAIN(20,26,N)
HAND WASHING(2,11,NE)
LOOP(27,19,SE)
MANGO(24,10,N)
MEXICO(29,22,S)
OUTBREAK(21,22,E)
PAROXYSMAL(17,16,S)
PERTACTIN(17,27,NW)
PLATE(21,23,E)
POMONA(19,13,SW)
RESISTANT(25,1,W)
SEROTYPE(20,8,NE)
SHIGA TOXIN(3,26,E)
SUSCEPTIBLE(23,16,N)
TURTLE(11,11,W)

Emergence of Drug Resistant TB

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Development of new drugs and therapies is crucial to curb the spread of this disease. Several new drugs are under development but are not yet ready for clinical trials. Currently, the FDA is fast-tracking Bedaquiline, an MDR drug being developed by Johnson & Johnson. Bedaquiline would be the world's first MDR specific drug and the first new TB drug in more than forty years. In addition, the non-profit vaccine developer, Aeras, will receive funding to expedite their TB research.

Ultimately the control and eradication of TB will require that all patients regardless of their socio-economic situation have access to reliable detection and treatment. This will require the collaborative efforts of governments, researchers, and healthcare providers.

References

[1]World Health Organization (2011). *Global tuberculosis control*. Retrieved from http://www.who.int/tb/publications/global_report/2011/gtbr11_full.pdf

[2]Gandhi, N.R., Nunn, P., Dheda K., Schaaf, H.S., Zignol, M., Van Soolingen, D., Jensen P., & Bayona, J. (2010, May 22-28). Multidrug-resistant and extensively drug-resistant tuberculosis: A threat to global control of tuberculosis. *The Lancet*, *375*(9728), 1830-1843. doi:10.1016/S0140-6736(10)60410-2

[3] Garza-González, E., González, G. M., Rentería, A., Cruz-Pulido, W., Rivera, G., & Bocanegra-García, V. (2009, Aug 7). A pyrosequencing method for molecular monitoring of regions in the *inhA*, *ahpC* and *rpoB* genes of *Mycobacterium tuberculosis*. *Clinical Microbiology and Infection, 16(6), 1469-0691. doi:* 10.1111/j.1469-0691.2009.02932.x

[4]Engström, A., Morcillo, N., Imperiale, B., Hoffner, S., & Juréen, P. (2012, Mar 29). Detection of First- and Second-Line Drug Resistance in *Mycobacterium tuberculosis* Clinical Isolates by Pyrosequencing. *Journal of Clinical Microbiology*, 50(6), 2026. doi:10.1128/JCM.06664-11

Upcoming Teleconferences

November 7, 2012 11:00 am Mountain Time

"Laboratory Efficiencies Initiatives (LEI): Public Health Capacity"

November 8, 2012 11:00 am Mountain Time

"TB 101"

November 13, 2012 11:00 am Mountain Time

"NAAT, IGRA and Genotyping: Using Nontraditional TB tests well"

November 20, 2012 12:30 pm Mountain Time

"*Neisseria gonorrhoeae*: Epidemiology, Laboratory Identification and Resistance Detection in 2012"

November 27, 2012 11:00 am Mountain Time

"Susceptibility Testing of *M. tuberculosis:* an update"

December 4, 2012 11:00 am Mountain Time

"Select Agent Regulatory Update"

December 6, 2012 11:00 am Mountain Time

"Laboratory Quality Control Based on Risk Management"

December 12, 2012 11:00 am Mountain Time

"Transportation Regulations affecting Infectious Substances"

Teleconferences will be held at Idaho Bureau of Laboratories. Contact Dave Eisentrager (eisentra@dhw.idaho.gov) to register.