Idaho Bureau of Laboratories

Clinical Forum

Volume 6, Issue 1 Spring 2013

State of Idaho

Bureau of Laboratories

2220 Old Penitentiary Rd

Boise, ID 83712-8249

Phone: 208-334-2235

Fax: 208-334-4067

E-mail: statelab@dhw.idaho.gov

IDAHO DEPARTMENT OF HEALTH & WELFARE

PFGE and Outbreak Investigations

Amanda Bruesch, M.S. and Ellen Zager Hill, M.S.

Laboratory data from molecular techniques performed on isolates obtained through culture, such as serotyping and subtyping, can aid in the inclusion or exclusion of cases during an outbreak investigation. Prior to molecular techniques, to determine if patients with similar enteric infections were part of a foodborne outbreak, for example, interviews were the main source of information. If most or all patients mentioned the same or a similar food item, then the most likely source of the outbreak was thought to be this item. Collection of information about food consumption and other epidemiologic data are still essential to an outbreak investigation. Cases identified through regular review of laboratory data generated by molecular techniques, such as pulsed-field gel electrophoresis (PFGE), can enhance the ability to determine if a cluster of similar infections during a defined time frame is indeed an outbreak. Laboratory data from molecular subtyping has helped to more rapidly identify potential outbreaks and focus outbreak investigative efforts to patients with similar infections. Public health laboratories across the US use the same protocols, making results comparable across jurisdictions and enabling more complete outbreak investigations.

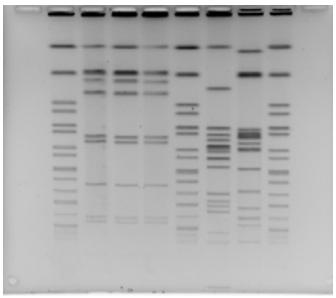

Figure 1: An image of a PFGE gel showing the 'fingerprints' for multiple *Salmonella* isolates.

Bacteria of the same genus and species (*e.g.*, *Escherichia coli* or *Salmonella enterica*) isolated from multiple people within a defined timeframe have the potential to have been exposed to the same source of infection. However, this level of identification provides very little information on

(continued on page 3)

2012-2013 Influenza Update

Lisa Smith and Dr. Christopher Ball

From the beginning of the flu season in October through March 2013, IBL tested 703 specimens using the CDC developed real-time PCR assay. Of those, 89.3% (628/703) were positive for influenza. The first sample arrived early, on October 9, 2012, and the testing peaked with over 200 samples in January (Figure 1). This season, Influenza A:H3 was the most abundant subtype detected, comprising 71.2% (447/628). Influenza B (28.2%; 177/628) and A:H1N1pdm09 (0.6%; 4/628) made up the remainder of the samples tested (Figure 1 inset). IBL forwarded 54 specimens to CDC for strain-typing this season. All of the strain-typed A:H3 specimens and over half (61.5%; 8/13) of the Influenza B strains matched this year's vaccine. A total of 45 specimens were submitted for antiviral resistance testing and all were susceptible to both Oseltamivir (Tamiflu®) and Zanamavir (Relenza™).

Most samples (593/703; 84.4%) sent to IBL included rapid influenza diagnostic test (RIDT) results.

(continued on page 5)

Inside this issue:

Risk Attribution Study by Submission Demographics

Katey Anderson, Ashley Machado, Joanna Lewis, Dr. Christopher L. Ball

Clinical diagnostic testing for infections of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) began at IBL in 2005. In 2007, the practice of sample pooling was adopted to realize cost savings and reduce sample turn-around times. Over the last several years, IBL has worked to optimize their sample pooling procedures to include information gathered at the time of sample submission[1] . More recently, IBL staff conducted a study to examine the statistical significance of certain patient-provided information in the prediction of positive samples. The study explored the impact associated with select patient-provided demographics and the risk of testing positive for *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* (CT/NG). The intent of the project was to further optimize the pooling protocol using a risk-based stratified pooling design to realize additional cost savings and efficiency.

A database of CT/NG testing data was compiled and analyzed using SAS software. Specimens included urine, cervical, and urethral samples obtained from both men and women submitting samples for STD screening. Additional sample characteristic data, available on the self-reported STD/Infertility Prevention Project (IPP) Submission Form (Figure 1), was collected on submitter demographics and characteristics, including *Reason for Visit*. The patient-provided *Reason for Visit* information was analyzed to determine the risk(s) associated with select demographic characteristics and *Reason for Visit* categories.

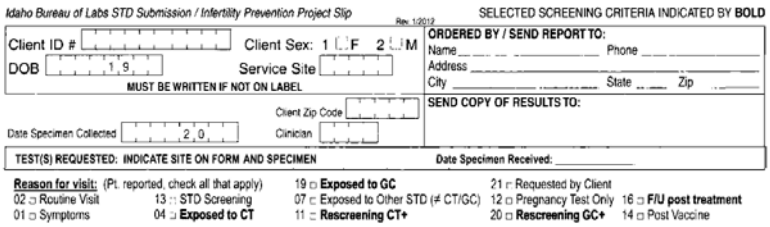
Idaho Bureau of Labs STD Submission / Infertility Prevention Project Slip Rev. 1/2012
SELECTED SCREENING CRITERIA INDICATED BY **BOLD**
Client ID # Client Sex: 1 F 2 M
DOB 1 9 Service Site
MUST BE WRITTEN IF NOT ON LABEL
ORDERED BY / SEND REPORT TO:
Name Phone
Address
City State Zip
Client Zip Code
SEND COPY OF RESULTS TO:
Date Specimen Collected 2 0 Clinician
TEST(S) REQUESTED: INDICATE SITE ON FORM AND SPECIMEN
Date Specimen Received:
Reason for visit: (Pt. reported, check all that apply)
02 Routine Visit 13 STD Screening 19 **Exposed to GC** 21 Requested by Client
01 Symptoms 04 **Exposed to CT** 07 Exposed to Other STD (≠ CT/GC) 12 Pregnancy Test Only 16 **F/U post treatment**
11 **Rescreening CT+** 20 **Rescreening GC+** 14 Post Vaccine

Figure 1: The STD/Infertility Prevention Project (IPP) Submission Form collects submitter demographics and characteristics.

The recent work by IBL staff has shown that samples submitted for CT/NG screening have differing likelihood of testing positive when certain demographic variables are considered using odds ratios. The following three odds ratio charts depict the relative odds ratio for each category. The dot represents the individual odds ratio while the

(continued on page 4)

Electronic Test Results Available to your Lab Online

Sharon Matthies

Idaho Bureau of Laboratories' test results are distributed via paper or electronic copies to clients (e.g. hospitals, health districts, and other state agencies). Additionally, electronic messaging is sent to CDC and the Idaho Department of Health and Welfare (IDHW) Bureau of Communicable Disease Prevention. IBL provides electronic reports to clients via Web Portal and plans to implement Electronic Transmission for Orders and Results (ETOR) in the near future.

Web Portal

The web portal software functions like email, featuring an inbox for new messages and a file cabinet for long-term archiving. Clients log in to a secure URL online to check the status of samples received and to access final test results. Since it's backed up on a daily basis, the portal can serve as a standalone storage option, or a client may wish to download test results to their server as PDFs. The download option allows clients to add meaningful information to the file name, sort files however they'd like (e.g., type of test, client name), download results to medical records software, and create process folders such as "to be re-

(continued on page 6)

PFGE and Outbreak Investigations

continued from page 1

relationships between cases. Serotyping is a method of characterizing antigens present on the cell surface and flagella of the bacteria associated with gastrointestinal illness (e.g., *Salmonella, Shigella,* and *E. coli*). Serotyping provides another level of information about the similarities or differences of the bacterial isolates. For example, perhaps two patients have the same serotype of *Salmonella* isolated from their stool sample within the same month. If this is a rare serotype, then this level of information might be enough to say that the cases are likely linked to a common risk factor. The epidemiologic investigation may also support that hypothesis, with both patients having a common exposure. However, if it is a very common serotype, then additional subtyping will help determine how related those two isolates might be. PFGE, a molecular tool used by public health laboratories, is a method by which the bacterial genome from a sample is cut into fragments and separated on an agarose gel to produce a 'fingerprint' for the organism. These fingerprints can then be compared between cases and environmental samples in the cluster to determine if they are related genetically. Identical or very similar patterns generally mean that the isolates are very closely related, whereas dissimilar patterns indicate isolates come from different sources.

The Idaho Bureau of Laboratories (IBL) is part of PulseNet, a network of public health and food regulatory (USDA, FDA) laboratories that perform PFGE on certain enteric bacterial pathogens. The electronic test results, or fingerprints, are uploaded into a national database for comparison with other patterns submitted from across the country. The US database is curated by CDC and examined daily for matching fingerprints. Without this national view, matching cases, even just one per state, would not likely be linked together. These multistate linkages greatly aid in the ability to jumpstart an epidemiological investigation and find a common source of exposure for all of the PFGE matches.

Figure 2: Raw chicken breasts, such as pictured above, were found to be the source of the *S.* Heidelberg that infected at least 37 people in 9 states, including 2 in Idaho. *Image provided courtesy of Debora Cartagena (CDC).*

In June 2012, Idaho epidemiologists with the Bureau of Communicable Disease Prevention and the IBL PulseNet lab were alerted to a single case of *Salmonella* Heidelberg in an Idaho resident that matched case fingerprints from submissions from seven other states. By August, this particular PFGE cluster had grown to 37 cases from 9 states, including 2 from Idaho. Food histories taken from these cases determined that a majority had exposure to a particular brand of frozen chicken breasts. Samples of the implicated frozen chicken breasts collected from the homes of outbreak-associated patients were analyzed with PFGE and found to contain the outbreak strain of *Salmonella*. The PFGE data also helped to differentiate this cluster of *S.* Heidelberg from another cluster of *S.* Heidelberg which occurred earlier in 2012 associated with a different food item.

In the absence of PFGE and PulseNet, the association with chicken may not have been made for the two Idaho cases. PulseNet has repeatedly proven its worth by linking cases across local and state jurisdictions providing molecular data to support and confirm the findings of epidemiologic investigations.

There are over 16 years' worth of archived human, animal, and environmental sample data in the PulseNet database. Statistical analysis yields informative trend data for unique serotypes and subtypes. When certain serotypes or fingerprints increase in frequency across multiple jurisdictions, PulseNet staff at CDC query the database to see if they can find past examples which match the outbreak strain in question. These historical results provide hypotheses to jumpstart current investigations. This history and cooperation between federal food regulatory agencies has helped to streamline outbreak investigations and improved food safety by enabling recalls of tainted food products, enhancing animal importation regulations, and improved food processing practices to minimize risks for contamination.

The success of the PulseNet program is dependent upon high rates of enteric sample submission from clinical laboratories in each state. While Idaho does not mandate submission of clinical samples to IBL, voluntary submission of clinical samples testing positive for any of the reportable pathogens that PFGE can be performed on (particularly *Salmonella, Shigella* and Shiga toxin-producing *E. coli* (STEC) isolates) will greatly enhance outbreak detection in Idaho. IBL receives samples for about 90% of all reported *Salmonella* cases, about 60% of all reported STEC cases, and about 75% of all reported *Shigella* cases reported state-wide. Increased submission of STEC and *Shigella* samples may improve the ability to detect clusters of Idaho cases involved in local, state and national outbreaks. It is only through dedicated efforts of clinical laboratories in the Idaho Sentinel Laboratory Network who voluntarily submit all *Salmonella, Shigella* and Shiga toxin-producing *E. coli* isolates or highly suspicious stool samples that clusters can be adequately detected.

Please contact the Molecular Epidemiology Lab at IBL for more information or for any questions you may have at (208) 334-2235 extension 267.

Risk Attribution Study by Submission Demographics

continued from page 2

whiskers on either side represent the relative 95% confidence interval. The solid line at 1 is representative of even odds.

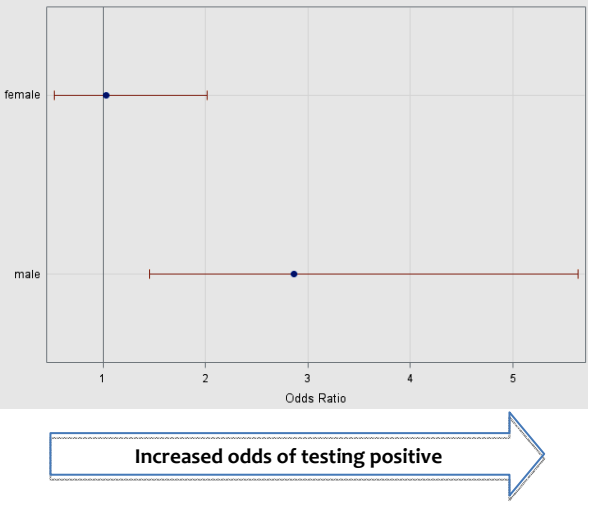

Figure 2: Males had nearly 3 times the odds of testing positive for CT/NG than women.

Of the 46,752 samples included in the study, 4,000 tested positive for a positivity rate of 8.56%. Females comprised 65.6% of these positive samples while males made up the remaining 34.4%. Despite their number, however, females did not exhibit increased odds for testing positive. Males on the other hand, had nearly three times (2.9) the odds of females of testing positive for CT/NG.

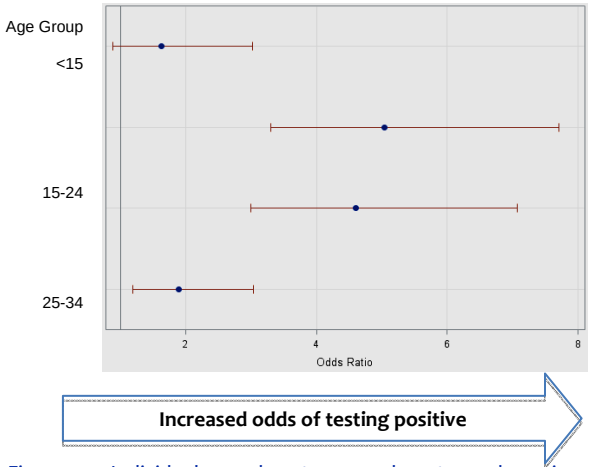

Figure 3: Individuals aged 15 to 24 and 25 to 34 have increased odds of testing positive for CT/NG.

Likewise, individuals aged 15 to 24 and 25 to 34 also have an increased odds of testing positive (5.0 and 4.6, respectively).

Nearly eighty percent (78.5%) of patients in the dataset had at least one *Reason for Visit* selected. The *Routine* category was the most commonly selected option, making up 62.6% of the samples.

'*STD Screening*' was the second most abundant category, at 31% of the samples. Of the positive samples, 'Routine,' 'STD Screening,' and 'Exposed to CT' were the most commonly reported *Reason for Visit*. 'Exposed to CT' was the *Reason for Visit* that had the highest rate of positivity. 'Routine,' 'Pregnancy,' and 'Exposed to Other' had the lowest positivity rates.

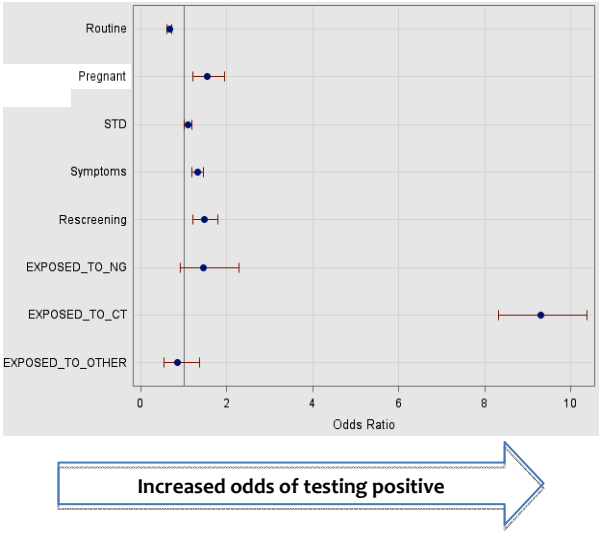

Figure 4: 'Exposed to CT' was the *Reason for Visit* that had the highest rate of positivity for CT/NG; 'Routine,' 'Pregnancy,' and 'Exposed to Other' had the lowest positivity rates.

Certain selected *Reason for Visit* categories had statistically significant odds ratios as well. Samples where Routine was selected as *Reason for Visit* had the lowest odds for testing positive (OR 0.8 p<0.0001), while those samples submitted as Pregnant (OR 1.5 p <0.0001) or Exposed to CT (OR 9.3 p<0.0001) had higher odds of testing positive.

The work conducted at IBL suggests that certain demographic and patient-supplied submittal characteristics are more likely to test positive for CT and NG than others. These include the *Reason for Visit* categories and demographic characteristics like gender and age group. IBL staff used the information gathered in this study to increase testing efficiency strategies and more effectively realize cost-savings.

References

[1]Lewis, J., Lockary, V., & Kobic , S. (2011). Cost savings and increased efficiency using a stratified specimen pooling strategy for Chlamydia trachomatis and Neisseria gonorrhoeae. *Sexually Transmitted Diseases, 38*(12).

Word Find

answers on page 7

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

CHLAMYDIA
CLIENTS
DEMOGRAPHICS
E COLI
EPIDEMIOLOGY
FINGERPRINT
INFLUENZA
NEISSERIA
OUTBREAK
PFGE
PULSENET
RISK ATTRIBUTION
SALMONELLA
SCREENING
SEROTYPING
SHIGELLA
STRAIN TYPING
SURVEILLANCE
VACCINE
WEB PORTAL

To be added or removed from the Clinical Forum email list

statelab@dhw.idaho.gov

2012-2013 Influenza Update

continued from page 1

According to CDC, RIDT accuracy and predictive values are dependent on both influenza prevalence and assay sensitivity and specificity. CDC notes that RIDTs tend perform best when prevalence is moderate to high and assay specificity is ≥98% and sensitivity is ≥90%[1]. We were curious to see if the RIDT data collected by IBL supported these observations. For this analysis, data were grouped in 2 month blocks to represent the early, mid and late flu season (Figure 2). To estimate influenza prevalence we averaged the reported Region 10 positivity rates for MMWR weeks 41-14 from FluView[2]. We then compared PCR results to the reported rapid test results to calculate RIDT positive and negative predictive values (PPV and NPV). Using the PPV and NPV, we can infer the RIDT sensitivity and specificity for strains circulating in Idaho this year. According to CDC, if prevalence is moderate (~20%) and RIDT specificity is ≥ 98%, then PPV should range between 86-93%[1]. As shown in Figure 2, when our average Region 10 positivity rate was >14%, then the PPV was >93%. This would suggest that the specificity of the RIDTs used in Idaho this season was good.

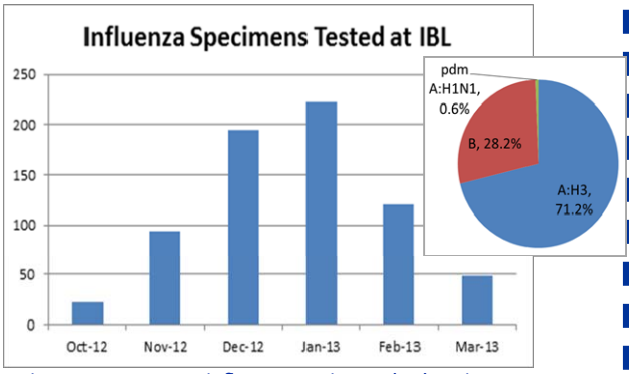

Figure 1: IBL 2012-13 influenza testing and subtyping summary.

When flu prevalence is moderate (~20%) and RIDT sensitivity is ≥ 90%, then the NPV should range between 97-99%[1]. As shown in Figure 2 the calculated NPVs never approached this range. During the peak months of December and January the NPV was 70.6%, which suggests

(continued on page 7)

Electronic Test Results Available to your Lab Online

continued from page 2

Figure 1: The web portal functions like email and includes an inbox for new messages and a file cabinet for long-term storage.

viewed by co-worker before discussion with patient."

All 7 health districts in the state began receiving test results via the web portal between June 2011 and May 2012. Seventeen additional clients have begun accessing the portal since spring 2012. The clients range from hospitals, private laboratories, and veterinarian offices to other state agencies.

When clients were asked in August 2012 regarding their favorite aspects about the web portal, replies included the following:

- "It is so easy! I just love getting my labs this way! You folks are the best!"
- "It is fast and easy…The other nice thing is that the copies are much clearer and more readable than the faxed copies we used to get. This system saves on money and paper!"
- "Easily accessible."

The web portal is secure and meets HIPAA requirements. When accessed externally, the portal uses SSL security to encrypt traffic from the user's browser to the web server. The encryption is the same as the method used for online banking, PayPal, and so forth, and can be verified by looking for the "lock" symbol in the lower right corner of the browser window. The traffic from the web server to the application server is encrypted and protected behind the IDHW firewall. The setup is structured and encrypted to the same level as other HIPAA-regulated applications.

ETOR

Electronic Transmission for Orders and Results (ETOR) is a pilot project that allows two-way communication between clients and IBL. A client can submit a test request electronically through software customized by the client rather than a paper form. When they submit the corresponding sample, such as a vial of serum or a bottle of well water, it contains a barcoded label identifying the client and providing a unique client-generated number for that specific sample. That number is then used to import the client's electronic test request into IBL's database system. Because the information isn't being manually keyed into IBL's system, data entry time is reduced. Once tests are completed, an electronic message is sent from IBL to the client, utilizing the identifying information to provide test results and comments. Eastern Idaho Public Health District is currently in the software-testing process of this pilot project with IBL.

If you are interested in learning more about receiving electronic test results, contact Sharon Matthies at MatthieS@dhw.idaho.gov.

"The web portal is secure and meets HIPAA requirements…. The encryption… can be verified by looking for the 'lock' symbol."

Rachel Beukelman, Microbiologist Senior

Rachel has been with IBL since September 2009, first as a lab technician, then as a microbiologist. She studied Biology at the [Albertson] College of Idaho, where she received her Bachelors of Science degree. She looks forward to expanding her horizons in her new role as Microbiologist Senior.

Rachel lives in Boise with her husband, 2 cats and 4 chickens. She enjoys collecting record albums, going to concerts, and attempting to learn French, German, and Spanish in her spare time.

Ashley Machado, Microbiologist

Ashley moved to Boise almost five years ago for college and is now happy to call Idaho her home after previously living in Hawaii and Pennsylvania. She received her B.S. in Biology from Boise State University and was previously a student intern and did temporary summer work for IBL. She is very excited to be a part of the IBL team once again.

Ashley has recently started the process of working towards getting her private pilot's license and enjoys golfing, skiing, traveling, cooking, and entertaining friends in her free time.

Word Find Solution

(Over,Down,Direction)

CHLAMYDIA (15,9,NE)
CLIENTS (5,29,NE)
DEMOGRAPHICS (18,17,E)
E COLI (11,25,E)
EPIDEMIOLOGY (30,2,S)
FINGERPRINT (21,20,SW)
INFLUENZA (22,28,N)
NEISSERIA (24,1,S)
OUTBREAK (9,22,SW)
PFGE (25,19,SE)
PULSENET (22,13,NW)
RISK ATTRIBUTION (10,30,N)
SALMONELLA (19,23,NW)
SCREENING (1,21,E)
SEROTYPING (18,5,W)
SHIGELLA (12,6,W)
STRAIN TYPING (1,9,SE)
SURVEILLANCE (1,12,NE)
VACCINE (3,29,N)
WEB PORTAL (13,8,SE)

2012-2013 Influenza Update

continued from page 5

that the RIDT sensitivity could have been low in Idaho this season. The most likely explanation for the suspect NPVs, however, is that very few RIDT negative specimens were submitted to IBL for confirmation. In order for us to truly evaluate the effectiveness of RIDTs in Idaho, more rapid test negative samples need to be submitted for confirmation. To address this issue, we hope that our ILI sentinel labs will increase the number of RIDT negative samples submitted, particularly in early, late or off season when prevalence is low. As a reminder, IBL performs influenza surveillance year round and we are happy to receive clinically suspicious samples at any time.

Thank you to all the sites that participated in the influenza surveillance program this year. Through your voluntary efforts, we gained information that helps us track seasonal influenza subtypes, strains, resistance patterns, and rapid test performance characteristics. We hope to see continued laboratory support for influenza surveillance during the upcoming flu season.

References

[1]http://www.cdc.gov/flu/professionals/diagnosis/rapidlab.htm

[2]http://www.cdc.gov/flu/weekly/FluViewInteractive.htm

	Oct-Nov (MMWR		Dec-Jan (MMWR		Feb-Mar (MMWR	
	Region 10 Mean Positivity 15.4%		Region 10 Mean Positivity 31.8%		Region 10 Mean Positivity 14.3%	
	RIDT +	RIDT -	RIDT +	RIDT -	RIDT +	RIDT -
PCR +	75	5	317	5	132	14
PCR -	2	1	8	12	9	13
PPV	97.4%		97.5%		93.6%	
NPV	16.7%		70.6%		48.1%	

Figure 2: 2012-13 rapid test performance characteristics as compared to PCR based confirmatory testing.