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IDAHO BUREAU OF LABORATORIES
CLINICAL FORUM

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IDAHO DEPARTMENT OF HEALTH & WELFARE

Idaho Influenza Surveillance 2010-2011

Colleen Greenwalt

The Idaho Bureau of Laboratories (IBL) is once again serving as a World Health Organization (WHO) Influenza Collaborating Laboratory providing influenza surveillance on behalf of CDC for the state of Idaho. Virologic surveillance of influenza viral strains that circulate in our communities is crucial. IBL performs surveillance for the following reasons: to detect the frequent genetic and antigenic shifts in these viruses; to rapidly identify the appearance of novel strains; to detect the emergence of antiviral resistance; and to provide the information needed to formulate vaccine components each year.

Each season we ask our partners in the medical community to participate in surveillance by submitting a subset of respiratory specimens to us throughout the season. IBL performs RT-PCR for rapid identification of influenza A and B and identification of subtypes AH1N1, seasonal AH1, AH3, and AH5. Our testing algorithm includes viral culture to obtain influenza isolates for anti-viral resistance studies and for antigenic studies performed at the CDC.

Influenza activity this season thus far has been fairly quiet in Idaho and across the U.S. At IBL, 30 respiratory specimens have been submitted and tested, with influenza detected in only four, all subtype AH3. Nationally, influenza surveillance laboratories located in all 50 states and Washington, DC have detected influenza in about 10% of respiratory specimens, with 41% Influenza A and 60% Influenza B. The predominant influenza subtype being seen so far is AH3, with a few 2009 AH1N1. It is difficult to predict what this season will bring, but weekly updates can be seen at the CDC's FluView web site at http://www.cdc.gov/flu/weekly/.

If you would like more information about the IBL influenza program or would like to receive collection kits, please contact the Virology/Serology section at 208-334-2235 (ext 230 or 228) or e-mail at greenwac@dhw.idaho.gov

IBL Awarded APHL Grant

IBL was very pleased to learn recently that our application for the TB NAAT Expansion Grant was approved by the Association of Public Health Laboratories (APHL). These funds will be used to enhance our TB laboratory services by completing the validation of our in-house Nucleic Acid Amplification Testing (NAAT) for the identification of *Mycobacterium tuberculosis* complex directly from clinical specimens and to develop molecular assays for detection of drug resistance markers in *M. tuberculosis.* These new assays should enable IBL to more rapidly identify this important pathogen.

In this issue

16S Gene Sequence Analysis Aids Infectious Disease Diagnoses

Vivian Lockary, MT, MPH, and Amanda Bruesch, MS

Over the last two decades traditional phenotypic bacterial identification schemes have gradually been supplanted by genotypic, especially 16S rDNA gene sequence based methods[1]. In 2005, the Idaho Bureau of Laboratories (IBL) verified and began using partial 16S rDNA gene sequencing for bacterial identification to increase the accuracy and timeliness of our reference bacteriology reporting. The 16S rDNA gene product is the rRNA for the small subunit of the ribosome of prokaryotes. The gene contains conserved and variable regions that allow for genotypic identification of many bacterial species. The chromatogram shown below (Figure 1) exemplifies the data collected by the sequencer when reading a strand of DNA.

Recently, IBL utilized this method to identify organisms that could not be recovered by traditional microbiological culture. The process starts with the amplification of 16S rDNA gene fragments from primary samples by PCR. The amplified product is then sequenced using our ABI310 genetic analyzer. The resulting chromatograms, normally sequences of ~450-500 base pairs, are analyzed in Bionumerics and compared to multiple public databases for identification and consensus.

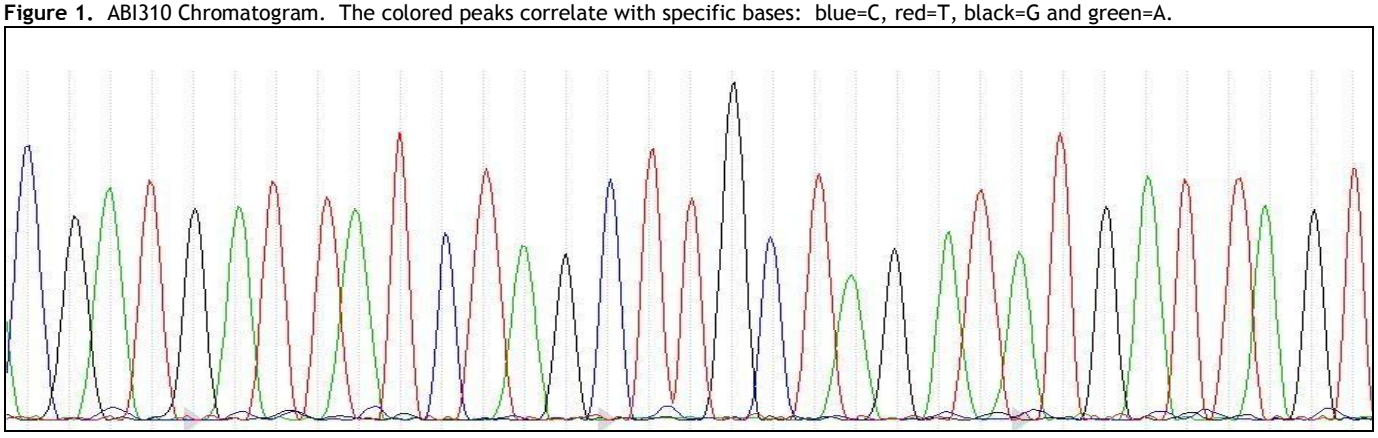
The first sample was an aortic valve tissue from which *Streptococcus mitis* group was identified by 16S rDNA gene sequencing but was not recovered in culture. The mitis group includes species we think of as viridans streptococci, traditionally associated with subacute bacterial endocarditis[2]. Antimicrobial resistance continues to increase worldwide among isolates of *Streptococcus pneumoniae* and other species of streptococci, increasing the importance of identification and accurate antimicrobial susceptibility testing results for guiding therapy[3].

The second sample was a surgical specimen from the left clavicle of an eight-year old with what was thought to be non-bacterial osteomyelitis, as the lab had been unable to culture anything. 16S rDNA gene sequencing identified *Nocardia cyriacigeorgica* which, in this case, changed the course of patient treatment. *N. cyriacigeorgica* is one of the most frequent human nocardial pathogens, at least in areas where actinomycotic mycetomas are relatively rare[4].

IBL has found partial 16S rDNA gene sequencing to be a very valuable part of our reference bacteriology program. Examples of some unusual organisms identified from isolates submitted to IBL's molecular lab include: *Chryseobacterium indologenes* from a sputum culture, *Rothia aeria* from a sputum culture of a CF patient, and *Bacillus cereus* group from an arm wound. *C. indologenes* is a gram-negative, oxidase-positive, nonmotile, yellow-pigmented nonfermenter that does not grow on MacConkey. Treatment of chryseobacterial infections is difficult because they are inherently resistant to many antimicrobial agents commonly used to treat infections caused by gram-negative bacteria. Fortunately, they are often susceptible to agents generally used for treating infections caused by gram-positive bacteria[4]. *Rothia* belongs to the family *Micrococcaeae,* although it is included with coryneform bacteria because some species are rod-like. *Rothia* has been associated with endocarditis, bacteremia, and respiratory tract infections. The *Bacillus cereus* group includes *B. cereus, B. anthracis, B. thuringensis,* and *B. mycoides*. The 16S rRNA gene sequence analysis cannot distinguish these organisms beyond the group level. It is important to

(continued on next page)

Figure 1. ABI310 Chromatogram. The colored peaks correlate with specific bases: blue=C, red=T, black=G and green=A.



16S Gene Sequence Analysis Aids Infectious Disease Diagnoses

(continued)

rule out *B. anthracis* for the reason that approximately 20% of untreated cases of cutaneous anthrax result in death either because the infection becomes systemic or because of respiratory distress caused by edema in the cervical and upper thoracic regions. The arm wound isolate was beta-hemolytic, enabling the submitting lab to rule out *B. anthracis*.

16S rDNA gene sequence results are dependent on the quality of the sample. Mixed cultures will result in a chromatogram that is not interpretable and no identification will be determined. IBL's Molecular lab employs CLSI document MM-18A, *Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing, Approved Guideline*. 2008, for guidance on the ability of the 16S rDNA gene sequence to differentiate organisms to the genus and species level.

[1] Claridge III, J.E. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious disease. *Clin. Microbiol. Rev.* 17:840-862.
[2] Ruoff, K.L. 2002. Miscellaneous Catalase-Negative, Gram-Positive Cocci: Emerging Opportunists. *J. Clin. Microbiol.* 40(4):1129-1133.
[3] Mohammed, M.J. and F.C. Tenover. 2000. Evaluation of the PASCO **Strep** Plus Broth Micro-dilution Antimicrobial Susceptibility Panels for Testing ***Strep**tococcus pneumoniae* and Other **Strep**tococcal Species. *J. Clin. Microbiol.* 38(5):1713-1716.
[4] Murray P., et al. Manual of Clinical Microbiology, 9th Edition, American Society of Microbiology, Washington D.C., 2007.

Do your shippers need recertification?

Register online at www.nltn.org/302-10.htm

National Laboratory Training Network

Packaging and Shipping Division 6.2 Materials

An Interactive Online Training Course

This course is intended for those learners seeking recertification in packing and shipping of infectious substances (Division 6.2 materials). This course does not apply to individuals seeking initial certification.

Meet IBL's New Personnel

Kathy Cunningham joined IBL on November 29th as Data Coordinator. She has a B.B.A. in Business Management from Boise State University and was previously employed by MPC Computers as an IT Business Analyst. Kathy will be working with the Lab Information Management System (LIMS) at IBL.

Kathy lives in Greenleaf with her husband, Shannon, and the "kids": Gersh (Rottweiler/ Dobie mix), Corkie (Chihuahua), and Suzie (cat). Kathy and Shannon enjoy fishing, camping, and playing on their boat and four-wheelers.

Welcome, Kathy!

Tim Warden grew up in Payette, and has always lived within 50 miles from home, with the exception of 7 months in Denver and about 5 days in Tempe. He and his wife, Karen, have two married daughters and a fifteen year old grandson. They enjoy hanging around the Oregon coast. Tim loves to backpack in the Seven Devils or Sawtooths when he has the time.

Tim worked as an equipment technician and has done some underground utility construction in the past. He last worked at Micron in the facilities control room. When Micron had the big workforce reduction, he was able to go to school full time for a year and finish his AAS in electronics technology.

We welcome Tim to our facility maintenance crew!

INFLUENZA WORD FIND PUZZLE

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

ACTIVITY
ANTIGENIC SHIFT
ANTIVIRAL DRUGS
ANTIVIRAL RESISTANCE
CDC
FLUVIEW
GENETIC SHIFT
GETREADYFORFLU.ORG
INFECTION CONTROL
INFLUENZA
NOVEL STRAINS
PANDEMIC
PREVENTION
RESPIRATORY
RT-PCR
SEASONAL
SUBTYPE
SURVEILLANCE
VACCINE
WHO

(answers on last page)

Seasons Greetings from IBL's Microbiology Section!

Left to right:
Raemi Nolevanko
Steve Gregoire
Dr. Christopher Ball
Amanda Bruesch
Kari Getz
Rachel Beukelman
Colleen Greenwalt
Vivian Lockary
Vonnita Barton
Walt DeLong
Sadika Kobic
(Rachel Ketterling not pictured)

SOLUTION TO INFLUENZA WORD FIND PUZZLE

(Over,Down,Direction)

ACTIVITY(15,10,SW)
ANTIGENIC SHIFT(12,3,SE)
ANTIVIRAL DRUGS(5,14,S)
ANTIVIRAL RESISTANCE(4,23,N)
CDC(25,29,E)
FLUVIEW(27,22,W)
GENETIC SHIFT(20,1,SW)
GETREADYFORFLU.ORG(1,17,NE)
INFECTION CONTROL(6,13,E)
INFLUENZA(20,3,E)
NOVEL STRAINS(28,10,W)
PANDEMIC(17,19,SE)
PREVENTION(2,15,NE)
RESPIRATORY(16,27,N)
RT-PCR(24,27,NE)
SEASONAL(11,19,S)
SUBTYPE(30,24,NW)
SURVEILLANCE(29,13,NW)
VACCINE(30,13,N)
WHO(1,1,SE)

Special thanks to our contributors to the Clinical Forum – in addition to their daily responsibilities, they also graciously agreed to write for us!

Clinical Forum Newsletter Committee:

Dr. Christopher Ball
Colleen Greenwalt
Steve Gregoire
Vivian Lockary

UPCOMING TELECONFERENCES

New Breakpoints & new AST recommendations
Presented by TNT
January 5, 2011, 10:00 MT

Case Histories and Surveillance for MDRO
Presented by TNT
January 18, 2011, 12:30 MT

Interesting cases in Bacteriology
Presented by TNT
February 2, 2011, 11:00 MT

Antimicrobial Susceptibility Testing Update
Presented by APHL/CLSI
February 2, 2011, 11:00 MT

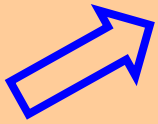
The MSDS:
Not just for Safety Professionals Anymore
Presented by APHL
February 8, 2011, 11:00 MT

An Overview of Molecular Methods for Pathogen Diagnosis
Presented by APHL
February 15, 2011, 11:00 MT

Molecular Diagnostic Testing:
Updated Specimen Handling Guidance
Presented by APHL/CLSI
March 17, 2011, 11:00 MT

To receive email notification of upcoming teleconferences, contact Dave Eisentrager at:

Eisentra@dhw.idaho.gov



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Happy Holidays from our staff to yours!