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IDAHO DEPARTMENT OF
HEALTH & WELFARE

2009 Influenza A(H1N1) Virus and Enhanced Surveillance

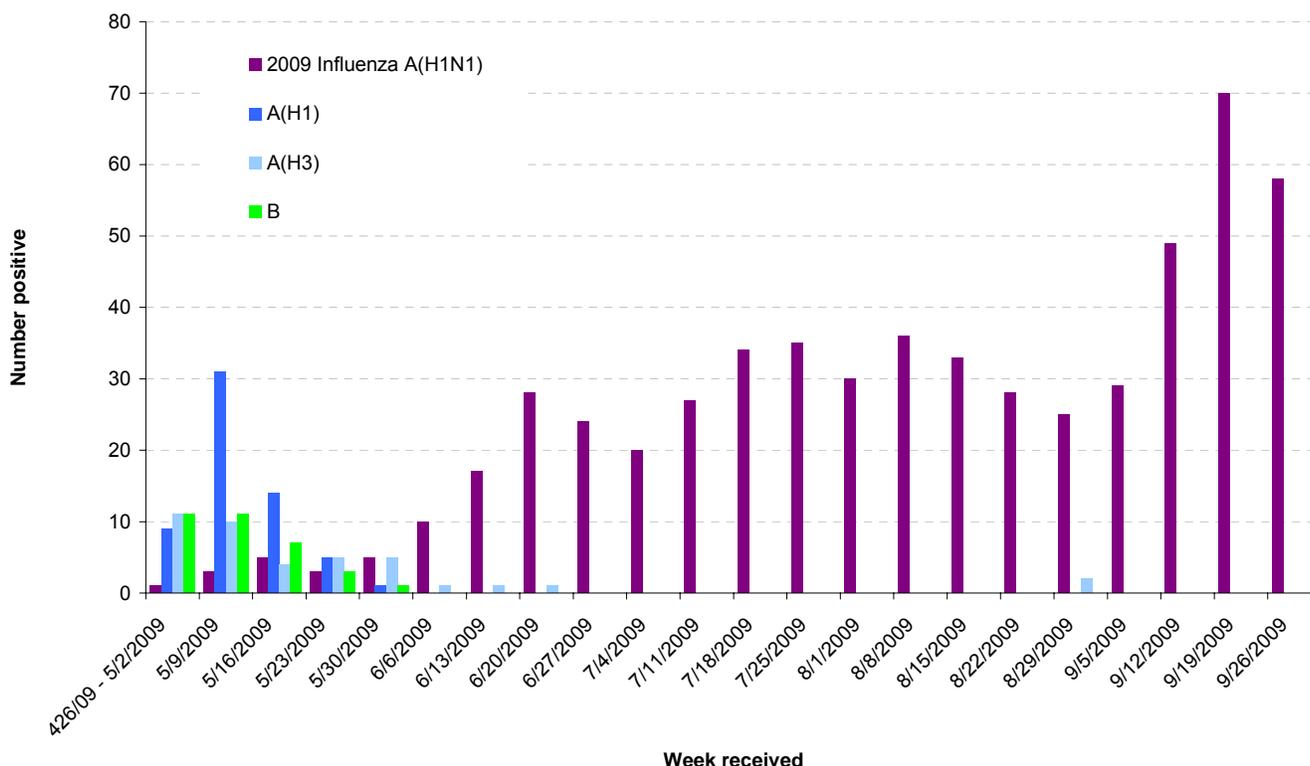
Colleen Greenwalt and Christopher Ball, Ph.D.

Influenza Activity

CDC estimates that between April and June 2009, more than 1 million cases of 2009 Influenza A(H1N1) occurred in the United States. Idaho Bureau of Laboratories (IBL) testing data (shown below) indicates that 2009 Influenza A(H1N1) was detected in late April and quickly became the most abundant strain circulating in Idaho. With the exception of 2 AH3 viruses detected mid-August, the 2009 Influenza A(H1N1) virus was the only strain

detected in Idaho from June 21 to October 2, 2009. IBL surveillance data suggests that 2009 Influenza A(H1N1) will co-circulate with other influenza strains throughout the 2009-2010 influenza season. It is conceivable that up to five subtypes could co-circulate: seasonal Influenza A(H1) and A(H3), 2009 Influenza A(H1N1), Influenza B(Yamagata), and Influenza B(Victoria).

**Influenza positive PCR results by week received--
Idaho Bureau of Laboratories, 4/26/09 - 9/26/09**



Testing Options

Rapid Influenza Diagnostic tests (RIDTs)

RIDTs detect influenza A or B antigens in 30 minutes or less. As reported in the August 7th, 2009 MMWR (http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5830a2.htm?s_cid=mm5830a2_e), overall sensitivities for the most frequently used RIDTs range from 40-69%. On August 10th, CDC issued "Interim Guidance for the Detection of Novel Influenza A Virus Using Rapid Influenza Diagnostic Tests."

http://www.cdc.gov/h1n1flu/guidance/rapid_testing.htm.

It is important to note that none of the FDA approved RIDTs can distinguish between influenza A virus subtypes (e.g. seasonal influenza A (H3N2) or seasonal influenza A (H1N1) strains), and RIDTs cannot provide any information about antiviral drug susceptibility.

Public Health Laboratories

At a minimum, the role of the public health laboratory (PHL) is to: detect emerging strains; assist with influenza surveillance; provide limited diagnostic services in the absence of commercial alternatives; and to facilitate the transfer of new testing technology to the private sector. In the current pandemic, the IBL has performed these PHL functions. Our surveillance efforts have established that the 2009 Influenza A(H1N1) strain is circulating throughout Idaho. As a result, the public health need to detect every case has diminished. Now that there are commercial diagnostic testing alternatives for patient care, the IBL is refocusing testing priorities to a more traditional surveillance role.

Private Sector Laboratories

The FDA has granted Emergency Use Authorization (EUA) to Focus Laboratories (now part of Quest Diagnostics) for their 2009 Influenza A(H1N1) rRT-PCR assay. **Diagnostic testing that does not meet public health surveillance priority needs should be routed to private sector labs in order to help protect PHL testing capacity.** Patient and Physician Fact Sheets about the Focus test can be found at: <http://www.questdiagnostics.com/2009H1N1/>.

Idaho Bureau of Laboratories Surveillance Testing Program

The IBL performs the FDA approved (or EUA authorized) real time RT-PCR IVD panels to detect Influenza A and B viruses, as well as the Influenza A sub-types [AH1,AH3, AH5, and 2009 A(H1N1)]. In addition, the IBL performs viral culture to obtain isolates needed for antigenic characterization and antiviral susceptibility studies. IBL is a member of the WHO surveillance network and forwards material and data to the CDC Influenza Branch.

Whom Will We Test?

Healthcare providers were informed in a HAN message (sent 10-07-09) that the IBL will only accept diagnostic test specimens from:

- A person hospitalized with suspected influenza-like illness, or
- A person with a fever AND either a cough or a sore throat AND who is:
 - a health care worker from a hospital setting, or
 - pregnant, regardless of hospitalization status, or
 - part of a possible outbreak in a facility or other special setting, as part of a public health district investigation.

During the 2009-10 influenza season, which started September 1st, 2009, LRN sentinel laboratories may be asked to submit a few representative respiratory specimens throughout influenza season, per established viral monitoring and surveillance testing relationships. These submissions will be used by IBL to perform viral monitoring of various influenza viral strains that may circulate during the winter months.

We anticipate that the 2009 Influenza A(H1N1) surveillance approach will evolve over the course of the influenza season as we learn more about the behavior of this virus. Future guidance documents will be distributed should these testing parameters change.

Specimen Types and Proper Collection Technique

Specimens acceptable for testing include the following: nasopharyngeal swabs, nasal aspirate or swab, or a combined nasopharyngeal/oropharyngeal swab. Only polyester or Dacron swabs with an aluminum or plastic shaft can be used. Swabs must be immediately placed into 1-3 mL of viral transport medium, kept cool (4°C) on ice packs, and transported to lab within a recommended 72 hour timeframe. Do not use the same specimen swab collected for shipment to IBL to perform a rapid influenza test. Any manipulation of the specimen will impact rRT-PCR testing. Specimens must be accompanied by a complete Influenza Submission Form available at <http://www.statelab.idaho.gov>

Surveillance specimen collection kits, complete with swabs, viral transport media, and IBL submission forms are available, free of charge, by contacting IBL at 208-334-2235 x 228.

Newer β -lactamases in Gram Negative Pathogens: Sorting it Out

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Extended-spectrum beta-lactamases (ESBLs) were first reported in the United States in 1983 and plasmid-mediated AmpC beta-lactamases over twenty years ago. At the Idaho Bureau of Laboratories (IBL), AmpC resistance is commonly detected in certain *Salmonella* serotypes and carbapenemases have arrived in Idaho! Today these enzymes are undermining the efficacy of third-generation cephalosporins against gram-negative bacteria.

The clinical laboratory plays an integral role in infection control. Identification of ESBL, plasmid-mediated AmpC, and carbapenemase resistance can be extremely challenging. Infection control is impossible without detection capabilities for these resistance mechanisms. Common differences among the newer beta-lactamases are listed in Table 1.

Table 1. General Differences: ESBL, AmpC, & Carbapenemase resistance

	ESBL	AmpC	Carbapenemases	
			KPC enzymes	Metallo-beta-lactamases
Affected by Beta-lactamase inhibitors ¹ ?	YES	NO ²	YES	NO
Inhibited by boronic acid?		YES		
Hydrolysis of:				
1 st to 3 rd generation cephalosporins	YES	YES	YES	YES
Cephameycins ³	NO (Sensitive)	YES (Resistant)	YES (Resistant)	YES (Resistant)
Cefepime	SOME	SOME	YES	YES
Carbapenems ⁴	NO	Slightly	YES	YES
Monobactams ⁵	YES	YES	YES	NO
Sensitivity to chelators? (e.g. EDTA)	NO	Slightly	NO	YES
Confirmatory tests	Double disk test, CLSI, commercial tests	AmpC Disk Test, AmpC inhibitors (e.g. boronic acid) Multiplex PCR ⁶	Modified Hodge Test (MHT), Tris/EDTA (TE) Disk Test, Indirect Test	Modified Hodge Test (MHT), Tris/EDTA (TE) Disk Test, Etest

¹ β -lactamase inhibitors: clavulanate, cloxacillin, sulbactam, tazobactam

² Clavulanate is an inducer of AmpC, *obscuring detection of ESBLs*

³ Cefmetazole, cefotetan, ceftiofur

⁴ Imipenem, meropenem, ertapenem

⁵ Aztreonam

⁶ Only for plasmid-mediated AmpC

Dilemmas and common problems encountered by clinical labs in the recognition of newer beta-lactamases are:

ESBLs

1. There are no CLSI guidelines for ESBL detection in organisms other than *Klebsiella* spp., *E. coli*, and *Proteus mirabilis*. If ESBLs have arrived in your patient population, it is dangerous to assume that ESBLs will only occur in *Klebsiella*, *E. coli* and *Proteus*!
2. Ceftazidime MICs are usually low for newer ESBLs (CTX-M β -lactamases) making ESBL screening with ceftazidime unreliable.
 - Cefepime is a more dependable screen for organisms harboring CTX-M β -lactamases.
 - Although common in Europe, Asia and South America, they have been reported in several states, including Idaho.
3. Because much variation exists in the phenotypic expression of different ESBLs, detection of these resistance mechanisms range from simple to exceedingly difficult.

AmpC

1. There are no CLSI-recommendations for detection of AmpC resistance.
2. AmpC genes can be chromosomal (*Enterobacter*, *Serratia*, *C. freundii*, *Providencia*, *M. morgani*, *H. alvei*, *P. aeruginosa*, *Aeromonas*, *E. coli*) or plasmid-mediated (*Klebsiella*, *E. coli*, *Salmonella*, *P. mirabilis*).
3. Decreased susceptibility to cefoxitin (FX) is a reliable screening test for AmpC resistance.
 - Most commonly occur as cefoxitin-intermediate or resistant isolates that are ESBL screen-positive but have negative ESBL confirmatory tests.
 - Reduced FX susceptibility can also be due to altered membrane permeability.
 - It is important to interpret AmpC disk test results in conjunction with carbapenem susceptibility results. Other enzymes such as carbapenemases also hydrolyze cefoxitin!
4. AmpC enzymes are NOT inhibited by available beta-lactamase inhibitors (clavulanate, and sulbactam) as are ESBLs.
5. Clavulanate is an inducer of AmpC, *obscuring detection of ESBLs*.
6. *Multi-drug resistance to unrelated drug classes* is associated with plasmid-mediated AmpC.
7. Associated changes in membrane permeability can also produce resistance to carbapenems.

Methods to prevent AmpC interference with ESBL detection:

1. Use cefepime as the indicator drug. Cefepime is not affected as much by AmpCs as are other drugs used for ESBL screening; or,
2. Use beta-lactamase inhibitors cloxacillin, sulbactam, or tazobactam instead of clavulanate.

Carbapenemase: KPC enzymes

1. Automated systems can be unreliable in detecting reduced carbapenem susceptibility.
 - Some *Enterobacteriaceae* are falsely susceptible to carbapenems with imipenem MICs of 4-8 $\mu\text{g/ml}$ (susceptible or intermediate by current CLSI breakpoints).
 - *Ertapenem and doripenem reduced susceptibility are more reliable indicators*.
 - Positive carbapenemase screen: ertapenem "I" or "R" or imipenem MIC $\geq 1 \mu\text{g/ml}$ (except *P. mirabilis*)

2. KPC-resistance genes have been reported in *K. pneumoniae*, *C. freundii*, *Enterobacter* spp., and *S. marcescens*.
3. ESBL confirmatory tests are positive because they hydrolyze cephalosporins and are inhibited by clavulanate, yet they are not ESBL-producers.
4. Modified Hodge Test (MHT) positive is an Infection Control EMERGENCY!
 - Although carbapenemases are therapeutic choices for ESBL-producers, *treatment failures are well-documented* in cases where organisms harbor these enzymes.
 - A false positive MHT can be due to high-level AmpC production.

Carbapenemase: Metallo- β -lactamases

1. Indicated by high carbapenem MICs.
2. Most commonly found in *Stenotrophomonas*, *Pseudomonas*, and *Acinetobacter*.
3. Phenotypic detection is based on sensitivity to EDTA.
4. Present significant therapeutic challenges.

The following guidelines can be useful indices of suspicion:

Cefoxitin resistance → suspect AmpC
 → interpret in conjunction with carbapenem susceptibility results

Reduced susceptibility to carbapenems (especially if ESBL confirmatory test positive)
 → suspect KPC

A number of significant benefits materialize from testing for newer beta-lactamases. Patient outcomes are improved, the escalation of resistance is reduced, resource consumption is optimized, and existing antibiotic formularies are protected.

Recent studies indicate low but increasing prevalence of plasmid-mediated AmpC β -lactamases, about where we were ten years ago with ESBLs, yet there are no CLSI-recommended guidelines for detection of this resistance mechanism. Most importantly, the natural spread of newer beta-lactamases is facilitated by the transfer of mobile elements into other microorganisms. How can we prevent these types of resistance from spreading if labs are not detecting them?

For questions regarding antimicrobial resistance detection, please call the Microbiology lab: **208.334.2235 ext. 257.**

References

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Lab Confirmation for VOC Exposures

Ian A. Elder, Ph.D.

The Idaho Bureau of Laboratories (IBL) has finalized implementation of a Centers for Disease Control and Prevention (CDC) method that can be used to confirm exposure to volatile organic compounds (VOCs). The VOCs measured by this method are benzene, carbon tetrachloride, chloroform, 1,2-dichloroethane, ethylbenzene, styrene, tetrachloroethene, toluene, and xylenes. Health-related information for these toxicants can be found at ATSDR's Toxic Substances Portal:

<http://www.atsdr.cdc.gov/substances/index.asp>

In the Laboratory Response Network (LRN) procedure, VOCs in blood are analyzed by solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC/MS). Method implementation at IBL included participation in a CDC validation study, completion of a standard operating procedure, integration into the laboratory information management system, and acquisition of "qualified status" through participation in LRN proficiency tests.

The analysis is available through standard Idaho LRN activation procedures (i.e. referral through a district health department). The optimal amount of specimen is at least 5 mL of blood with a minimum requirement of 1.5 mL. Specimens must be collected in vacutainers containing heparin (green top) or sodium fluoride (grey top) anticoagulant. If isopropanol is used to

disinfect the venipuncture site, prevent contamination of the sample by swabbing the venipuncture site with a gauze bandage and allow the site to dry for 5 to 10 seconds prior to collection. Headspace in the vacutainers should be minimized when possible.

Samples should be placed in a refrigerator or cold shipping container within 30 minutes of sample collection and transported to IBL as quickly as possible. Samples should be shipped at 4-10 °C. The test requisition form can be found in the Clinical Chemistry section of the IBL website:

<http://www.statelab.idaho.gov>



Blood Samples in Autosampler Tray