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

![Influenza positive PCR results by week received](image-url)
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:

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 (4ºC)
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

Vivian Lockary, MPH, MT(ASCP)

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

<table>
<thead>
<tr>
<th>Hydrolysis of:</th>
<th>ESBL</th>
<th>AmpC</th>
<th>Carbapenemases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Affected by Beta-lactamase inhibitors?</strong></td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Inhibited by boronic acid?</strong></td>
<td>YES</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>1st to 3rd generation cephalosporins</strong></td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Cefamycins</strong>&lt;sup&gt;3&lt;/sup&gt;</td>
<td>NO (Sensitive)</td>
<td>YES (Resistant)</td>
<td>YES (Resistant)</td>
</tr>
<tr>
<td><strong>Cefepime</strong></td>
<td>SOME</td>
<td>SOME</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Carbapenems</strong>&lt;sup&gt;4&lt;/sup&gt;</td>
<td>NO</td>
<td>Slightly</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Monobactams</strong>&lt;sup&gt;5&lt;/sup&gt;</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Sensitivity to chelators? (e.g. EDTA)</strong></td>
<td>NO</td>
<td>Slightly</td>
<td>NO</td>
</tr>
<tr>
<td><strong>Confirmatory tests</strong></td>
<td>Double disk test, CLSI, commercial tests</td>
<td>AmpC Disk Test, AmpC inhibitors (e.g. boronic acid)</td>
<td>Modified Hodge Test (MHT), Tris/EDTA (TE) Disk Test, Indirect Test</td>
</tr>
</tbody>
</table>

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<sup>1</sup> β-lactamase inhibitors: clavulanate, cloxacillin, sulbactam, tazobactam

<sup>2</sup> Clavulanate is an inducer of AmpC, obscuring detection of ESBLs

<sup>3</sup> Cefmatazole, cefotetan, cefoxitin

<sup>4</sup> Imipenem, meropenem, ertapenem

<sup>5</sup> Aztreonam

<sup>6</sup> 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. morganii, 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 cloxaxillin, 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 µg/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 ≥ 1 µg/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

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