


Laboratory Testing Plans and Recommendations for the Upcoming Influenza Season

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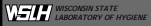

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Objectives

At the end of the session, participants will be able to:

- Identify three laboratory methods to test patient specimens for influenza.
- Describe considerations in the use and interpretation of rapid tests for influenza.
- Summarize plans for laboratory-based surveillance of influenza in Wisconsin during the 2009-10 season.

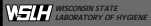

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Brief Recap of Last Season

Antigen Detection (Rapid) Testing Data Reported to WSLH, 2003/4 – 2008/9 Seasons

Season	Number Positive				Number Tested	% Positive
	Influenza A	Influenza B	Influenza A & B	Influenza Unknown		
2003/2004	4623	26	0	1915	19321	34%
2004/2005	9693	1508	6	815	50543	24%
2005/2006	2614	886	18	135	25647	14%
2006/2007	4100	254	43	312	28450	17%
2007/2008	9526	4448	85	435	62563	23%
2008/2009	2139	1990	33	94	40776	10%

Not a particularly exciting year, until...

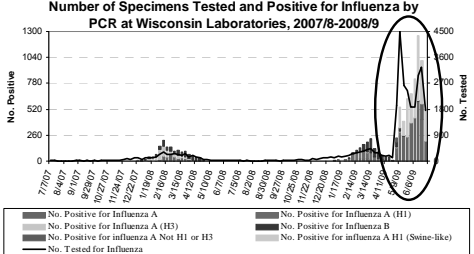



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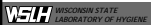

Brief Recap of Last Season

The "season" became more exciting in May

Number of Specimens Tested and Positive for Influenza by PCR at Wisconsin Laboratories, 2007/8-2008/9



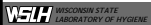

Novel H1N1 PCR testing was performed at WSLH, Milwaukee Health Department Laboratory, Midwest Respiratory Virus Program, Marshfield Clinic Research Foundation

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Brief Recap of Last Season

- April 15, 2009: CDC testing confirms 1st human cases of novel H1N1 virus.
- April 17, 2009: CDC testing confirms 2nd human case of novel H1N1 virus.
- April 23, 2009: 7 total cases - 5 cases of novel H1N1 confirmed in California; 2 cases in Texas.
- April 26, 2009: U.S. declares public health emergency.
- April 30, 2009: Per CDC - 91 total cases of novel H1N1 influenza in: Arizona, California, Indiana, Kansas, Massachusetts, Michigan, Nevada, New York, Ohio, Texas.
- May 3, 2009: Per CDC – 226 total cases in 30 states, including Wisconsin.
- June 11, 2009: W.H.O. raised the worldwide pandemic alert level to Phase 6. •••

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Brief Recap: novel H1N1 Influenza Detected throughout the State

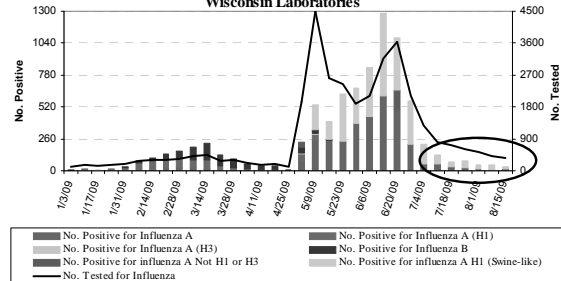


In Wisconsin:
Confirmed Cases: 4,583
Probable Cases: 1,814
Total Cases: 6,397

Source: <http://pandemic.wisconsin.gov>

Brief Recap: Influenza Detections Continued through the Summer

Number of Specimens Tested and Positive for Influenza by PCR at Wisconsin Laboratories



Expectations for 2009-2010 Season

- Probable (?) circulating viruses
 - Seasonal Influenza viruses (in the vaccines)
 - Influenza A (H1N1) - A/Brisbane/59/2007 (H1N1)
 - Influenza A (H3N2) - A/Brisbane/10/2007 (H3N2)
 - Influenza B - B/Brisbane/60/2008 (B/Victoria lineage)
 - Novel H1N1 Influenza A virus
 - Influenza A/California/07/2009 (H1N1)
- Concerns:
 - Novel H1N1 Influenza A virus could mutate into a form that would cause more severe illness.
 - Antiviral susceptibility patterns could change.

Expectations for 2009-2010 Season

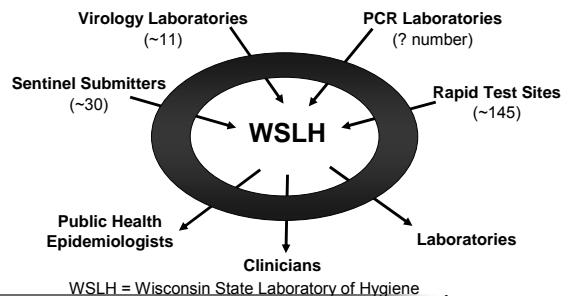
- Purpose of fee-exempt testing during Spring 2009 response was case identification
- Purpose of fee-exempt testing during 2009-2010 season is surveillance.
 - WSLH will discontinue fee-for-service testing

Laboratory-Based Influenza Surveillance in Wisconsin

Expectations for 2009-2010 Season

- Objectives of influenza surveillance
 - Determine **when** influenza viruses are circulating*
 - Determine **where** influenza viruses are circulating*
 - Track geographic spread of influenza
 - Track influenza-related illness
 - Measure the impact of influenza on serious illness and mortality
 - Detect changes in influenza viruses*
 - Infection by novel viruses*
 - Optimize use of vaccines and antivirals*
- * = dependent on laboratory contributions

Components of Wisconsin's Laboratory-Based Influenza Surveillance



Laboratory-Based Influenza Surveillance During 2009-10 Season

- Sentinel Submitters: ~ 30 enrolled healthcare providers provide limited number of specimens to WSLH.
- Virology (culture) Laboratories: ~ 11 laboratories provide weekly reports and selected viral isolates.
- PCR Laboratories: ? Laboratories provide weekly reports and selected positives.

Laboratory-Based Influenza Surveillance During 2009-10 Season

- Rapid Test (antigen detection) Sites: Sites provide weekly reports and selected samples to WSLH.
 - 20 enrolled "Structured" submission sites to send first 5-10 per week (regardless of rapid test result)
 - These sites will be specifically recruited and enrolled.
 - Other rapid test sites to send first positives, until notified to discontinue.
 - Notices will be provided in Virus Surveillance Reports, WCLN Laboratory Messages and posted on the WSLH website.
 - Requests for specimens and notices to discontinue submissions will be based on regional activity.

Laboratory-Based Influenza Surveillance During 2009-10 Season

- Fee-exempt testing criteria for novel influenza A (H1N1):
 - Pregnancy
 - Child 2 years of age or less
 - Healthcare Worker
 - Resident or Staff of Residential or Correctional Facility
 - Hospitalization
 - Death
 - Public Health Department Approval

Laboratory-Based Influenza Surveillance During 2009-10 Season

- Note: "*Positive rapid influenza A test result*" is no longer included as an automatic criterion for fee-exempt testing, but ...
- Fee-exempt confirmatory testing of rapid influenza A test results will be available (just as in previous years), but will be discontinued in each region based on activity.

Laboratory-Based Influenza Surveillance During 2009-10 Season

- Packet of information, including forms & instructions, will be provided to viral culture, PCR, & rapid test sites (as in previous years).
 - Will also post components to the WSLH website
- Web-based reporting is available for rapid testing sites; instructions will be in the packet.

Laboratory-Based Influenza Surveillance During 2009-10 Season

- New forms will be provided in packets and posted to WSLH website.
- “Fillable” requisition form for novel H1N1 surveillance will be posted on the WSLH website.
 - *Please complete submitter information before printing.*



Laboratory-Based Influenza Surveillance During 2009-10 Season

- Updates and reports will be provided via WCLN laboratory message, WSLH Virus Surveillance Report, and website.
- State and regional graphs for Culture, PCR, & antigen detection will be posted to the WSLH website – click on “Surveillance Reports” at <http://www.slh.wisc.edu/labupdates/>



Laboratory-Based Influenza Surveillance During 2009-10 Season

- Teleconferences and webinars will be held as needed.
- Annual influenza update teleconference will be provided in October.



Influenza Testing



Molecular Testing at WSLH and MHD

- CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel [rRT-PCR Flu Panel (IVD)]
 - FDA-cleared
 - Available to Public Health Labs
 - Universal influenza A detection
 - Subtypes
 - Seasonal A/H1
 - Seasonal A/H3
 - A/H5 (Asian lineage)
 - Universal influenza B detection



Molecular Testing at WSLH and MHD

- rRT-PCR novel A/H1 Flu Panel
 - FDA-EUA
 - Universal influenza A detection
 - Subtypes novel A/H1
 - Detects novel H1 matrix gene
 - Novel H1 hemagglutinin gene



Acceptable Specimen Types

- Upper respiratory tract
 - Nasal or naso-pharyngeal (NP) swabs
 - Throat swabs
 - NP aspirates or washes
- Lower respiratory tract
 - Tracheal aspirates
 - Bronchoalveolar lavages
- Store at 2-8°C ≤ 72 hours or freeze at ≤ -70°C.
 - Transport with cool-pack



Reporting

- Possible results for the rRT-PCR novel A/H1 Flu Panel
 - No influenza A Virus RNA detected
 - Influenza A H1 (Swine-like) Virus RNA detected
 - Influenza A Virus RNA detected; NOT H1 (Swine-like)
 - Influenza A Virus RNA detected; equivocal for H1 (Swine-like)
 - Influenza A Virus RNA detected; unable to subtype as H1 (Swine-like)

Surveillance Testing Algorithms at WSLH

- Early season (low prevalence)
 - Screen for influenza A and B
 - If flu A + → Subtype novel A/H1, seasonal A/H1, A/H3
- Surge algorithm if novel A/H1 predominates
 - Run novel A/H1 flu panel
 - Flu A +, novel H1 + = novel A/H1
 - Flu A +, novel H1 - → Subtype sA/H1, sA/H3
 - Test for flu B based on epidemiologic need if B circulating

Surveillance Testing Algorithms at WSLH



- Super Surge with novel A/H1 predominating
 - Test for flu A only
 - Report with comment that novel A/H1 is predominate influenza A virus circulating
 - Continue surveillance for non-novel A/H1 on a subset of specimens
 - Store specimens for testing post-outbreak

Surveillance Testing Algorithms at WSLH-- Other Respiratory Viruses

- Throughout the season test a subset of flu A negative specimens for other respiratory viruses
 - Use a RVP that detects 17 viral targets
 - Approximately 40% of influenza A negative specimens are positive for other respiratory viruses

Surveillance for anti-viral Resistance

- Screen for adamantane and oseltamivir (Tamiflu®) resistance
 - Pyrosequencing
 - Test for presence of mutations that confer resistance
 - M2 gene for adamantanes
 - NA gene for oseltamivir



Commercial Molecular Influenza Assay Options*

- Focus Laboratories---Influenza A H1N1 (2009)
 - FDA-EUA real-time PCR
 - Differentiates novel H1N1 from seasonal
 - ABI 7500
 - MagNA Pure LC extraction
- Prodesse---ProFlu-ST
 - FDA-EUA pending real-time PCR
 - Differentiates novel H1N1 from seasonal
 - SmartCycler
 - MagNA Pure LC or NucliSENS easyMAG

*WSLH does not endorse the use of any commercial products. This list is provided for informational purposes only.

Commercial Influenza Options

- Luminex xTAG RVP
 - Detects novel H1N1, but cannot identify the subtype
 - Influenza A +, seasonal H1 -, H3 –
 - Influenza A-unsubtypeable
 - Possible novel A H1
- Other products in various stages of development

Laboratory Developed Molecular Tests

- ASR reagents are available
- Validation
 - WSLH will provide a panel of specimens at a nominal cost to help with validation of LDTs or verification of FDA-EUA cleared tests



National Commercial Laboratories*

- Focus Laboratories
- Specialty Laboratories
- Mayo
 - Does not differentiate novel H1N1 from seasonal subtypes
- ARUP
 - Sends specimens to Focus
- LabCorp
 - Does not offer molecular influenza testing

*WSLH does not endorse any commercial laboratories. This list is provided for informational purposes only.

Rapid Influenza Diagnostic Test (RIDT's)

Laboratory Diagnosis of Influenza

Comparison of Test Methods for Influenza

Test Method	Time to Results	Comments
Serology	>2 wks	Retrospective, requires paired sera
Culture	1-10 days	Still gold standard(?), requires expertise, provides virus for studies
Molecular (RT-PCR)	2-4 hrs	Becoming gold standard(?), requires expertise & expensive equipment
Antigen Detection (IF)	2-4 hrs	Requires reading expertise & IF microscope
Antigen Detection (Rapid EIA-like)	15-30 min	Widely available, requires little expertise

Rapid Influenza Diagnostic Tests *What we knew before*

- Performance limitations
 - **Sensitivity**
 - Inherent limitations with device, specimen dependent (type, quality, timing, handling), age dependent
 - **Predictive Values (PVs)**
 - Dependence on prevalence
- Strategies to optimize rapid test results
- Biosafety concerns
 - **Why the concern?**
 - **Strategies to enhance biosafety & reduce potential exposure**



Rapid Influenza Tests: Addressing Biosafety Concerns

- Why the concern?
- Strategies to enhance biosafety & reduce potential exposure
 - Consider use of additional personal protective equipment (PPE) during test performance
 - Consider use of physical barriers for test performance (e.g., bench shields)
 - Use biosafety cabinet (BSC), if available
 - Sequester/isolate testing area
 - Collect & communicate patient clinical, epi, and travel history and risk factors to testing staff
 - Assess risk of on-site testing based on patient information



Rapid Influenza Diagnostic Tests *Re-visiting with Novel Influenza A H1N1*

Key references: Rapid tests

- *Interim guidance for the detection of novel influenza A virus using rapid influenza diagnostic tests, August 10, 2009.*
www.cdc.gov/h1n1flu/guidance/rapid_testing.htm
- *Evaluation of rapid influenza diagnostic tests for Detection of novel influenza A H1N1 virus – United States, 2009.*
MMWR, August 7, 2009 / 58(30):826-829
<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5830a2.htm>



Rapid Influenza Diagnostic Tests *Re-visiting with Novel Influenza A H1N1*

Key findings and recommendations (I):

- Limited data on performance of RIDT, nevertheless...
 - “...the sensitivity of RIDTs to detect novel influenza A(H1N1) is equal to or lower than the sensitivity to detect seasonal influenza viruses.”
 - A number of factors contribute to lower sensitivity
- A negative RIDT result does not rule out infection
 - **Significant consequences**



Rapid Influenza Diagnostic Tests *Re-visiting with Novel Influenza A H1N1*

Key findings and recommendations (II):

- Specificity of RIDTs is generally high, however...
 - **Remember, PVP depends on specificity and prevalence**
 - **Consider confirmatory testing during periods of low influenza activity**
- A positive RIDT result may be useful for treatment decisions when influenza circulating in the community
- Laboratories should add a statement about test limitations in the results report to help physician in patient management



Rapid Influenza Diagnostic Tests *Re-visiting with Novel Influenza A H1N1*

Key references: Biosafety

- *Interim biosafety guidance for all individuals handling clinical specimens or isolates containing 2009-H1N1 influenza A virus (novel H1N1), including vaccine strains.*
www.cdc.gov/h1n1flu/guidelines_labworkers.htm



Rapid Influenza Diagnostic Tests *Re-visiting with Novel Influenza A H1N1*

Key findings and recommendations:

- When performing RIDTs, use splash protection
 - Lab coat, gloves, eye protection, facemask
 - If there is aerosol risk, use BSC
- For more complex procedures, perform in Class II BSC in BSL-2 laboratory
 - BSL-3 no longer required for culture
- Recommendations given for lab wastes, disinfection and occupational health



Rapid Influenza Diagnostic Tests *Re-visiting with Novel Influenza A H1N1*

The WSLH view:

- Users must understand test limitations
- Clinical judgments should not be based on test result alone
 - Consider clinical presentation and epidemiological data
- We cannot recommend whether to use these tests nor can we recommend a particular test
- We are not able to provide specimens for validation testing
- We will limit confirmatory testing to defined surveillance purposes



Conclusion



H1N1 Response Survey Feedback

- To those who have not provided feedback about our response actions last spring – it's not too late. Send in the survey or complete it online at <http://wslh.outbreakfeedback.sgizmo.com>.
- To those who have provided feedback–
 - Thank you for your compliments.
 - Thank you for telling us what didn't work so well.
 - Thank you for your suggestions for improvements.



Feedback Responses: Suggestions in place or under development

- Courier Options: Developing alternative courier options and will announce when arrangements completed.
- Communications: Will try to communicate changes in advance and consolidate messages *whenever possible*.
 - Will maintain result interpretation information on website to help clarify result and comment terminology.
 - Will add "Q & A" section to the website.



Feedback Responses: Suggestions in place or under development

- Forms: Will update forms and post "fillable" test requisition forms on the website; suggest that you complete the submitter information before printing.
- Webinars: WSLH is assessing "webinar" format for scheduled audioconferences and updates, in addition to teleconference format; you would be able to access via telephone or via computer.



Contacts

- WSLH
 - Customer Service
608-262-6386 or 800-862-1013
 - Clinical Orders
608-265-2966 or 800-862-1088
 - 24/7 Emergency Answering Service
608-263-3280
 - PCR Validation Panels (Audrey Prieve)
608-265-9754 or ap@mail.slh.wisc.edu
 - WSLH Virus Surveillance Reports
Email **WCLN@mail.slh.wisc.edu**



Thank you for your support.

Please send questions and comments
to:

Email **WCLN@mail.slh.wisc.edu**

FAX **WCLN** at **608-265-9091**

