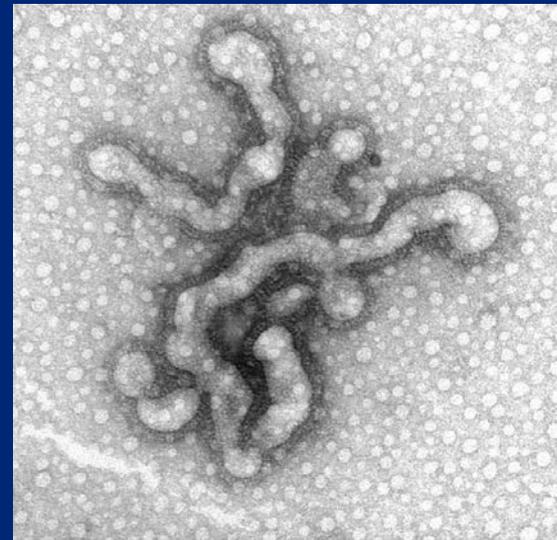


Influenza Surveillance: Texas DSHS Laboratory

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Overview of Testing in the DSHS Lab

- Test Methods used for Influenza Surveillance
- Summary of 2011-2012
- Updates for next year
- Important points for submitters

Rapid Influenza Diagnostic Tests

- Sensitivity vs gold standards

- More Information

[http://www.cdc.gov/flu/professionals/
diagnosis/clinician_guidance_ridt.htm](http://www.cdc.gov/flu/professionals/diagnosis/clinician_guidance_ridt.htm)

Test Methods used for Influenza Surveillance

Diagnostic Methods

- Culture
 - Immunofluorescence
 - Hemagglutination/Inhibition
- PCR/Molecular based
 - Real time RT-PCR
 - Multiplex assays
 - ❖ Pyrosequencing (Not diagnostic)

Culture

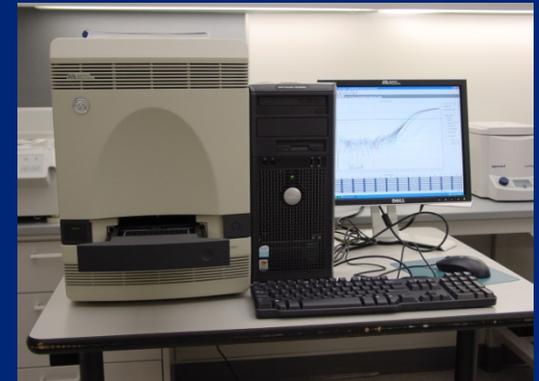
- Traditional method
 - The virus is infectious!
 - Isolate for further studies
 - Antigenic characterization: Strain id
 - Anti-viral resistance testing
 - Vaccines
 - Important for surveillance
 - Longer TAT: 3-15 days

Culture Confirmation

- Observation - Minimum 10 days
- Immunofluorescence
 - Ag + FI-Ab
 - 2.5 hours
 - A and B, subtypes
- Hemagglutination/Inhibition (Limited)
 - A and B, subtypes: H1, H3, 2009 H1N1
 - Strain lineage (B), Yamagata-like or Victoria-like
 - CDC, further characterization

Real time RT-PCR

- CDC assay: Seasonal, Pandemic H1N1
- Primer/Probe sets: A, B, H1, H3, pdmA, pdmH1
- Detect unsubtypeables, H5, variants (H3v)
- Monitor amplification in real time
- Sensitivity vs culture
- No Isolate for further studies
- Faster TAT



Multiplex Respiratory Virus Panel

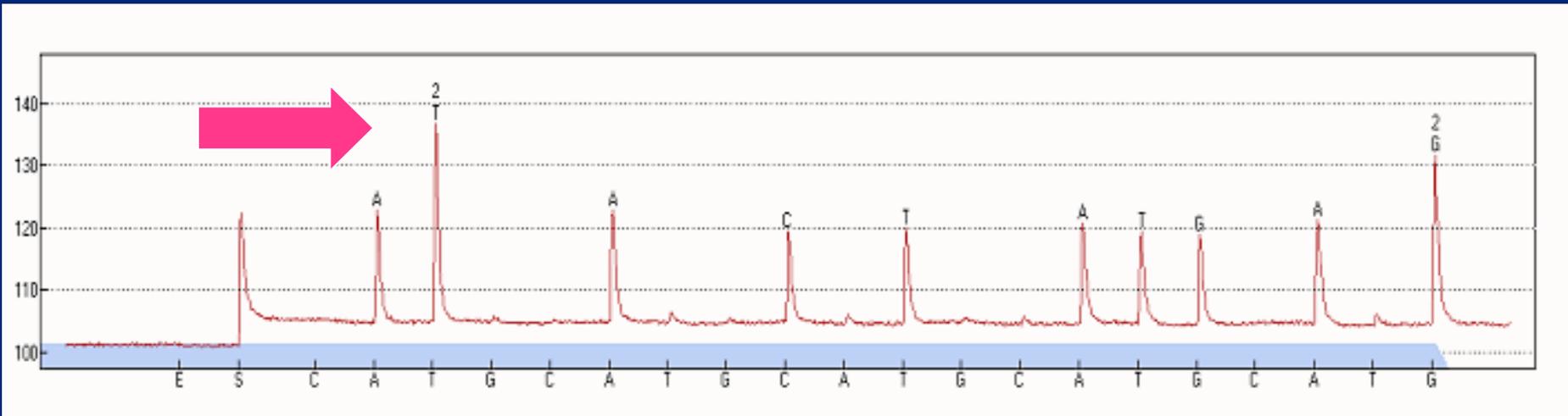
- 12 targets:
 - RSV, Rhino, Flu A/H1/H3/B, Adeno, Para 1,2,3, Metapneumoviruses
- Nasopharyngeal swabs in VTM
- *Labor intensive*, additional workspace
- Expensive,
- Luminex xTAG RVP



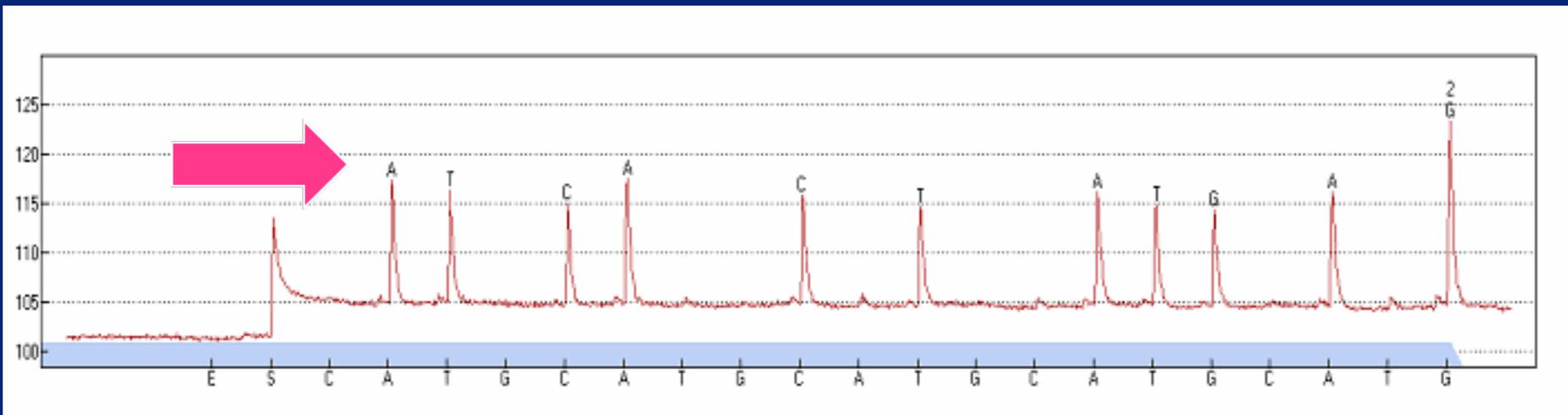
Pyrosequencing Antiviral Resistance

- Sensitive, clinical specimens
- Known mutation that confers resistance to oseltamivir
- 2009 Influenza H1N1
- High throughput, 85-95 specimens
- 104 tested, 4 positive for the mutation
- Surveillance only, not for diagnostic use @ DSHS lab





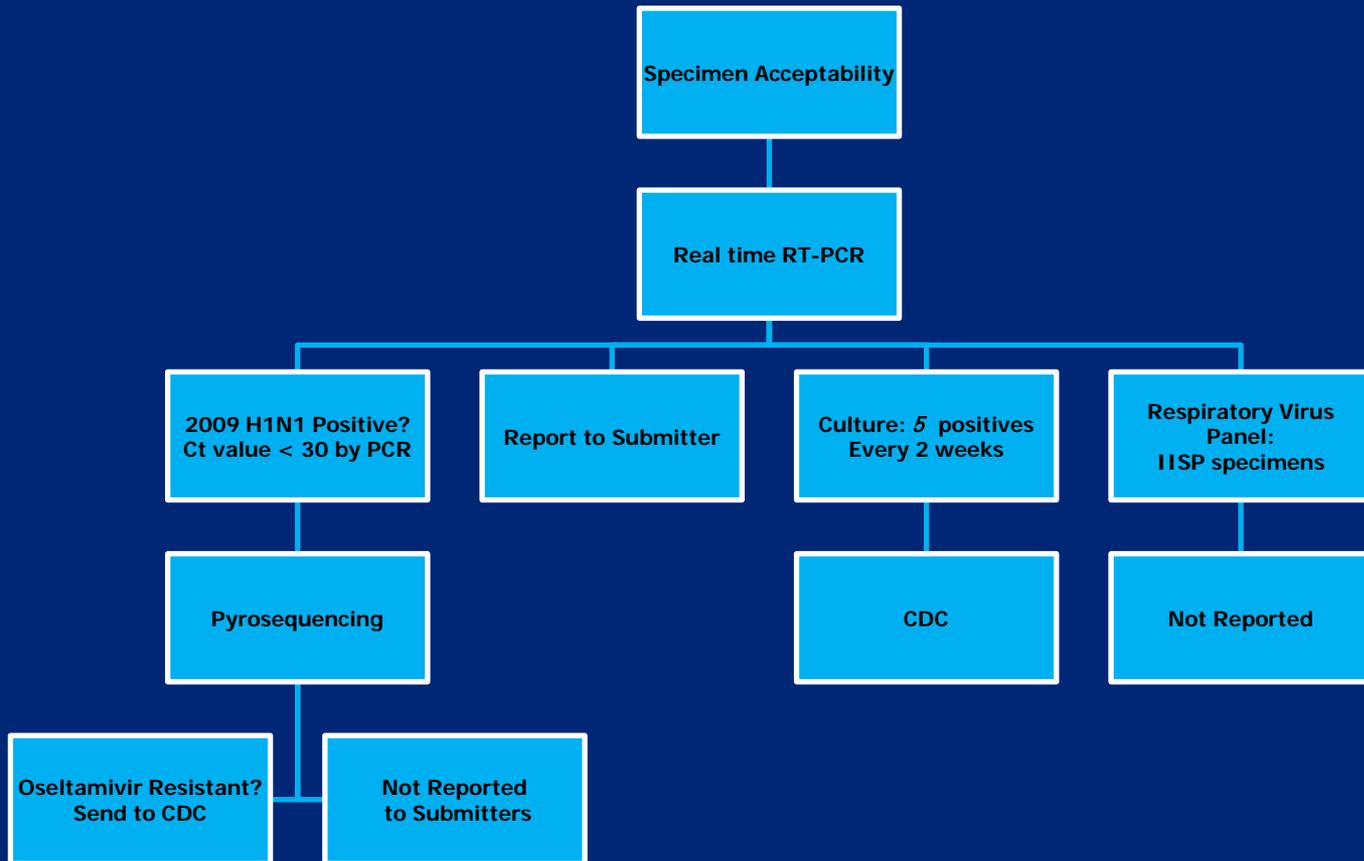
Pandemic H1N1pdm-Resistant-H275Y



Pandemic H1N1pdm-Wildtype

2011-2012 Summary

2011-2012 Influenza Season

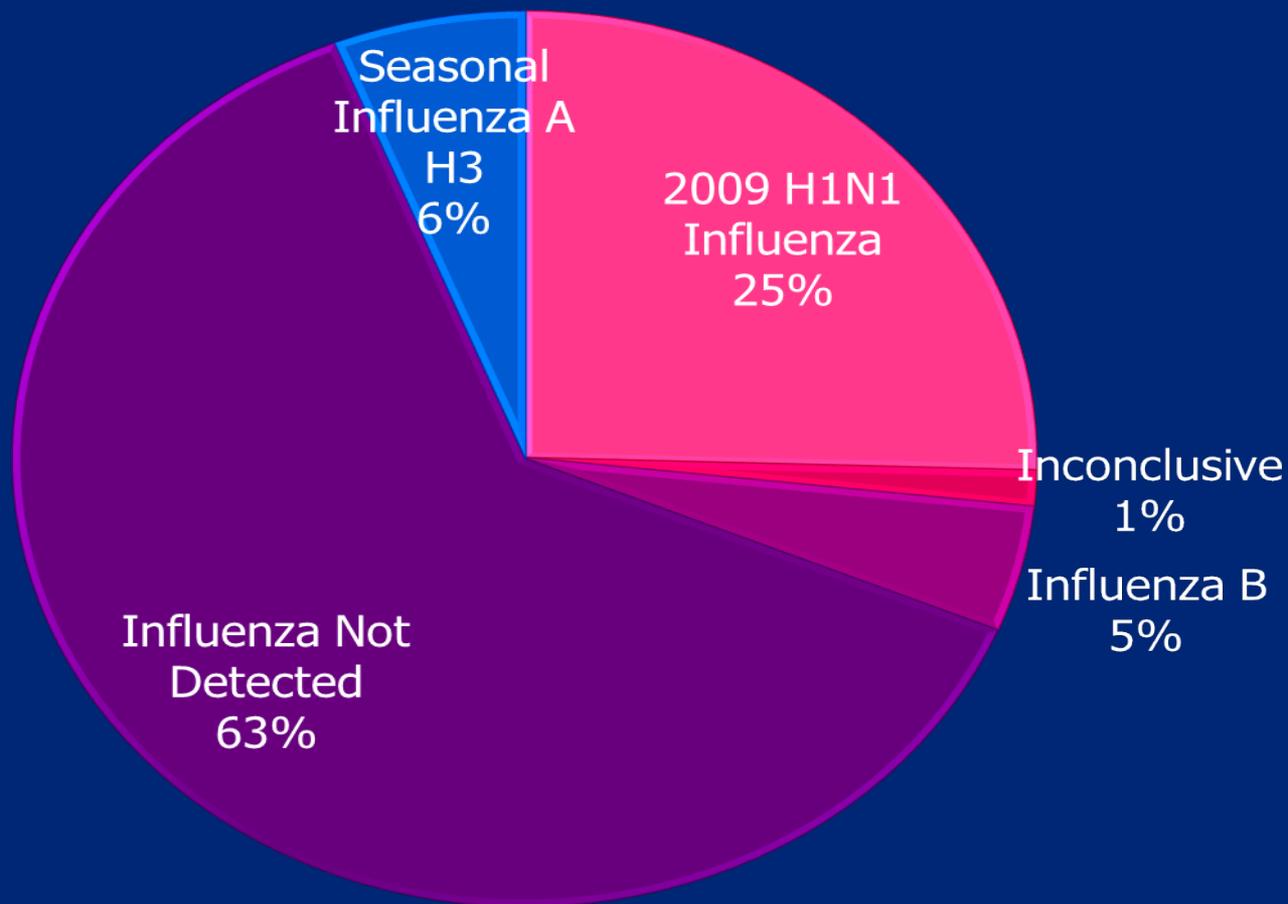


2011-2012 Summary: Real time RT-PCR

2009 H1N1 INFLUENZA	117
INCONCLUSIVE	6
INFLUENZA B POSITIVE	17
INFLUENZA NOT DETECTED	289
SEASONAL INFLUENZA A H3	28
TOTAL TESTED	457

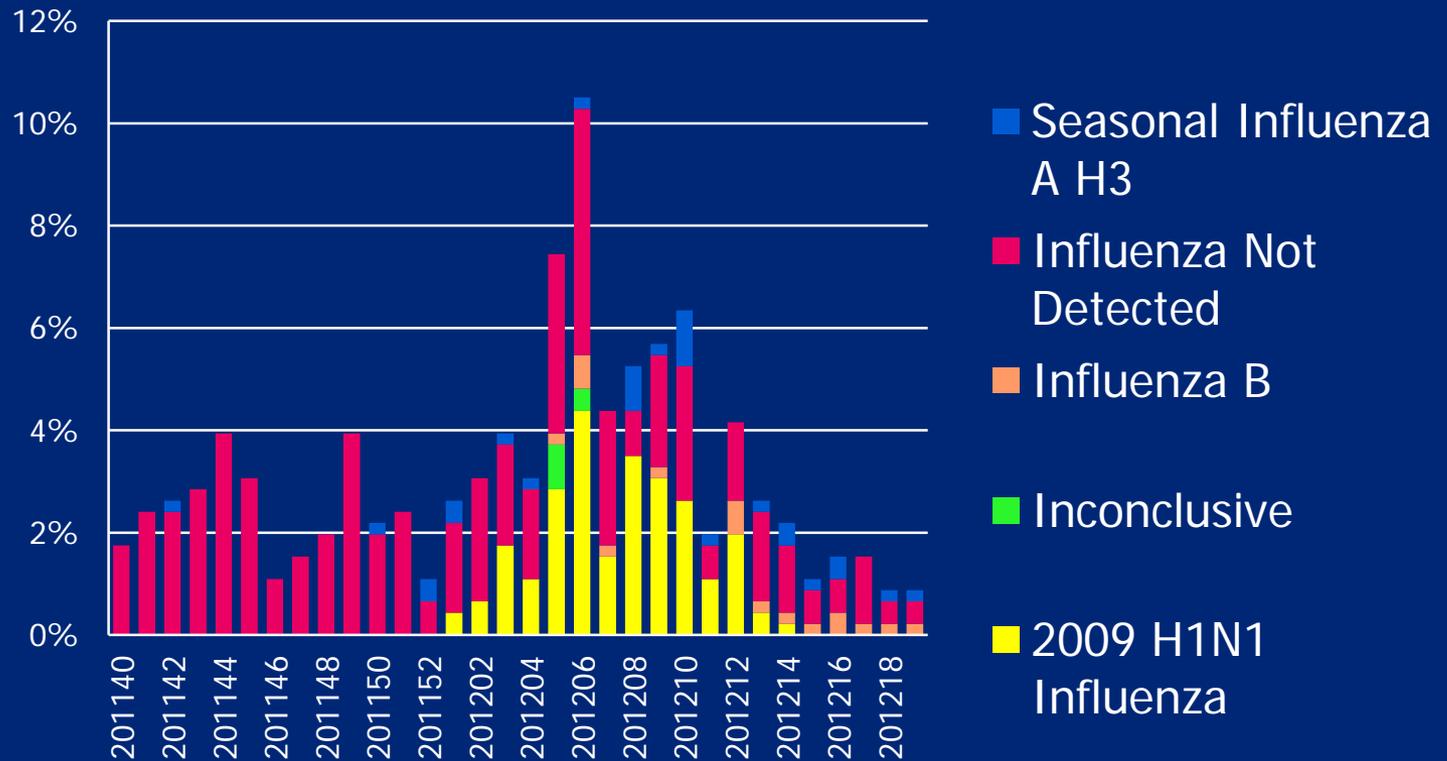
2011-2012: Results

% of Total Tested



2011-2012

MMWR week 40 - week 20



2011-2012 Recap

- Tested 457 specimens, real-time RT-PCR
- 4 specimens containing mutation conferring resistance to oseltamivir
- IISP study, RVP

CDC Surveillance: What do we send?

- Always
 - Anything unusual
 - Vaccinated cases: *if we know*
 - Unsubtypeable specimens
- 2011-2012
 - Up to 5 isolates and original clinical materials every 2 weeks: Surveillance (AgC/*AVR)

CDC Submissions

- Antigenic Characterization.....Drift
- Vaccine Studies: Will it grow in eggs?
- Genetic Sequencing.....Drift
- Antiviral Resistance
 - Pyrosequencing
 - NA Inhibition assay

CDC Submissions

A/California/07/2009-Like (H1N1)pdm09	16
A/Perth/16/2009-Like (H3N2) GP	8
B/Brisbane/60/2008-Like (Victoria)	4
B/Wisconsin/01/2010-like (Yamagata)	6
Influenza A(H1N1)pdm09 by PCR	1
Pending	3
*Antiviral resistance only	1
Total	39

*4 sent for Antiviral Resistance

How do we choose the samples?

- Recent collection dates
- Geographic spread
- Leftover sample available?
- Did it grow in culture?

Updates for next year?

Additional Acceptable Specimens: CDC Assay Real Time RT-PCR

- Upper Respiratory Specimens
 - Nasal swabs
 - Throat swabs
 - Nasal aspirates
 - Nasal Washes
- Lower Respiratory Specimens
 - Bronchoalveolar lavage
 - Bronchial wash
 - Tracheal aspirate

Specimen Acceptability Criteria

- Recommended: Nasopharyngeal swabs
- Received within 72 hours cold
- If received >72 hours, freeze and ship on dry ice

Viral Transport Media

- DSHS media
 - Quality control in house

- Commercial media.....IISP
 - Follow manufacturer's recommendations

Viral Transport Media

- Viral transport medium
- Hanks balanced salt solution
- Tryptose-phosphate broth
- Sucrose-phosphate broth
- Cell culture medium
- Veal infusion broth

- Supplement with a protein or stabilizer, bovine serum albumin or gelatin at 0.5 to 1%.

- Antibiotics

Important points for Submitters

- If possible, ship specimens same day as collection, COLD on ice packs
- If specimens are not shipped the same day as collection, freeze the specimen and ship on dry ice.
- Use synthetic swabs
- Swabs should ALWAYS be in VTM

QUESTIONS?

Acknowledgements

- **Viral Isolation Team**
 - Crystal Van Cleave
 - Jennifer Gonzales
 - Charles Parmely
 - Amanda Lucas
- **Reporting Group**
- **Container Prep**
- **CDC Influenza Division**
- **Specimen Acquisition Branch**
- **LRN Laboratories**