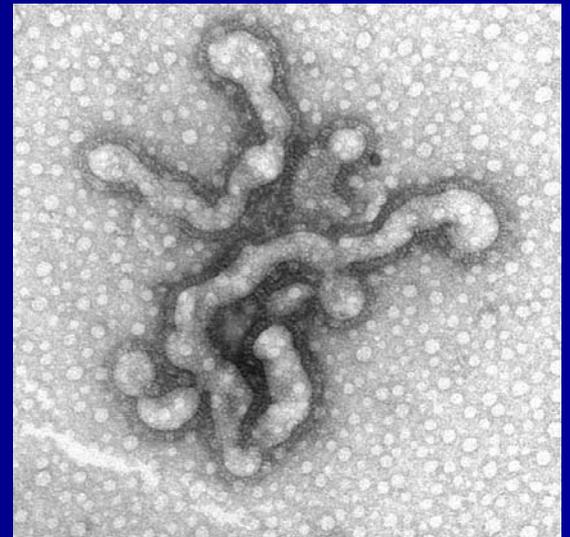


Influenza Surveillance: Texas DSHS Laboratory

Martha Thompson, MPH
Viral Isolation Team Leader
Medical Virology Group
Laboratory Services Section
Texas Department of State Health Services



Agenda

- Influenza diagnostic methods
- Role in Influenza surveillance
- *Plan* for Fall of 2010
- Important points for submitters 😊

Rapid Influenza Diagnostic Tests

- Simple, rapid, and on site
- Sensitivity is still an issue
- Positive: $\sqrt{\sqrt{\sqrt{\quad}}}$
- Negative: ???
- Prevalence
 - False Positives
 - False Negatives

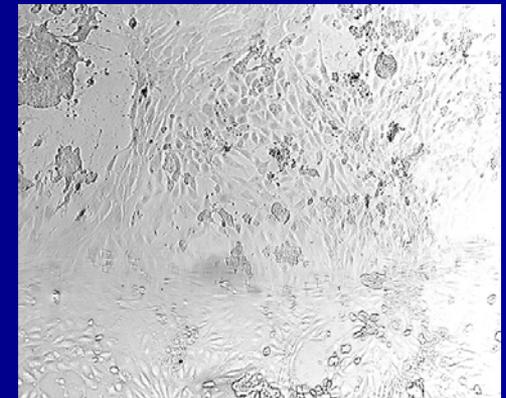
Diagnostic Methods

- Culture
 - Immunofluorescence
 - Hemagglutination/Inhibition
- PCR/Molecular based
 - Real time RT-PCR
 - Multiplex assays

Traditional Methods

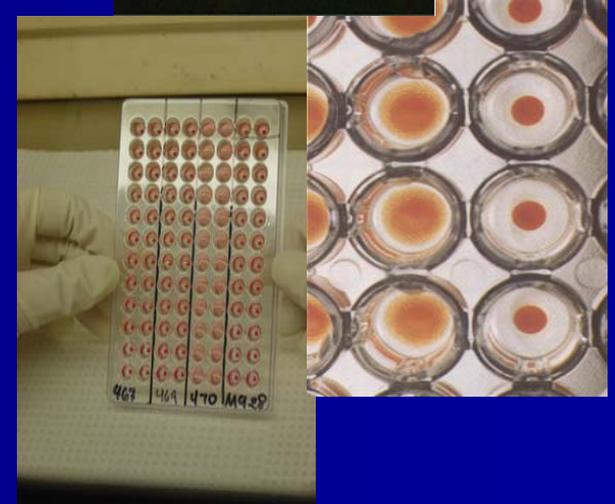
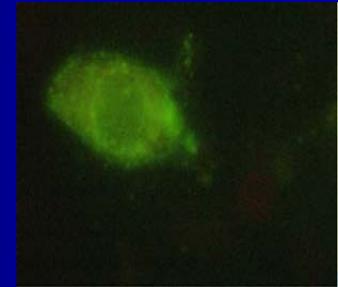
- Culture

- 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

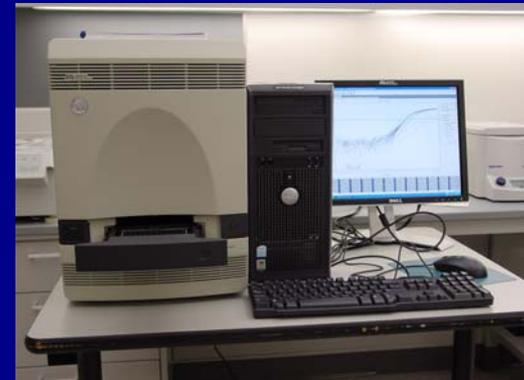
- Observation
 - Minimum 10 days
- Immunofluorescence
 - Ag + FI-Ab
 - 2.5 hours, bench time
 - A and B, subtypes
- Hemagglutination/Inhibition
 - A and B, subtypes: H1, H3, 2009 H1N1
 - Strain lineage (B), Yamagata and Victoria
 - CDC, further characterization



---WHO KIT---

Real time RT-PCR

- CDC assay: Seasonal and 2009 H1N1
- Monitor amplification in real time
- Sensitivity vs culture
- No Isolate for further studies
- Faster TAT
- Bench time for 19 – 36 specimens
 - 5-7 hours
- Reporting time
 - 1-4 days
 - Method of reports
 - Repeat



Workflow: Real time RT-PCR

- Specimen Receiving
- Data Entry
- Testing
 - Extraction
 - Reagent Prep
 - Specimen Addition
 - 7500 set up/Addition of plate
 - Analysis
- Enter Result
- Results Reported



Equivocals and Inconclusives

- Equivocal: 2 of 3 targets met
- Inconclusive:
 - Internal control failure of specimen
 - Low copy number, not all targets met

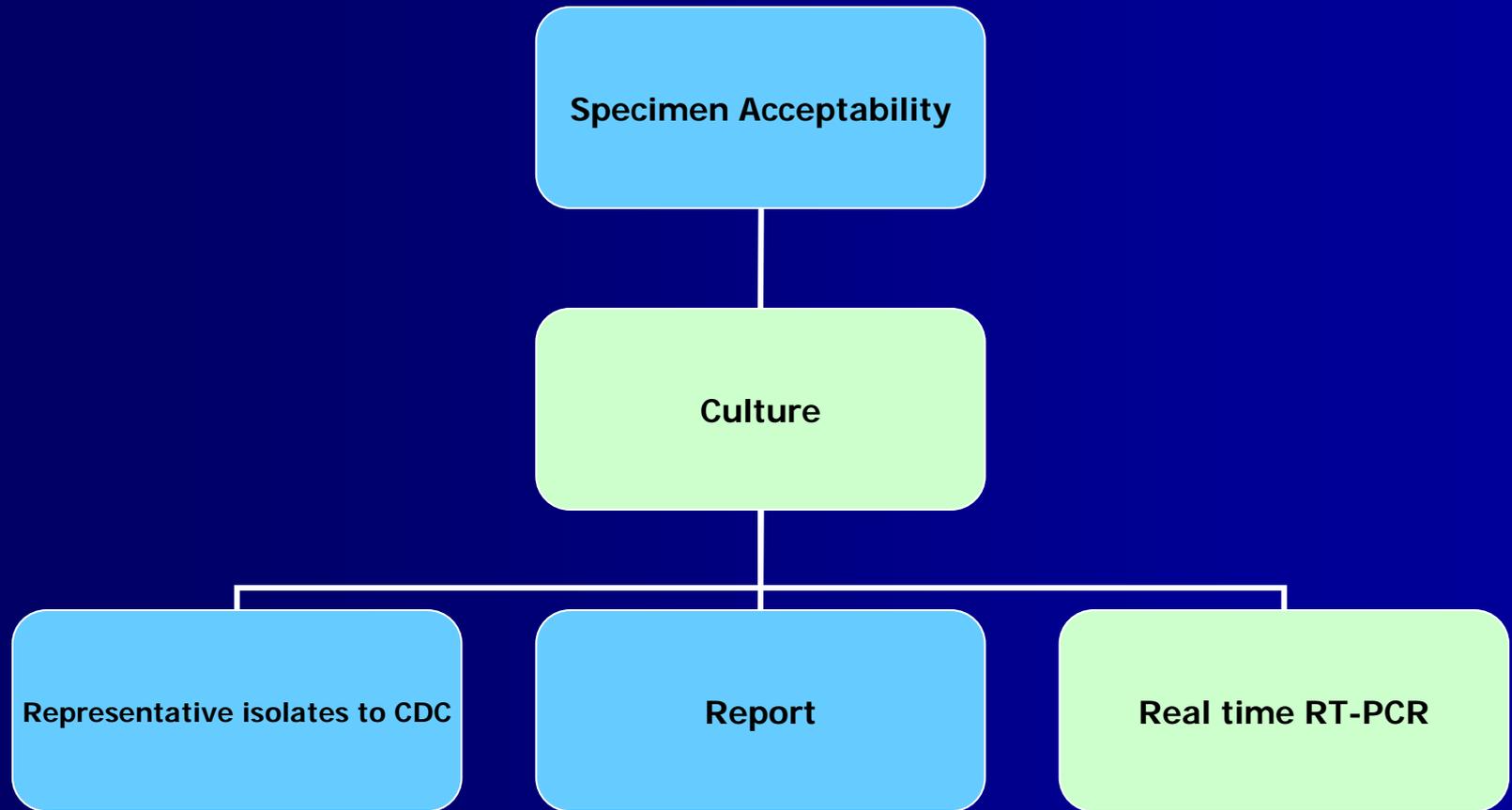
Luminex xTAG RVP

- Multiplex assay
- Nasopharyngeal swabs in VTM
- 12 Respiratory targets:
 - RSV, Rhino, Flu A/H1/H3/B, Adeno, Para 1,2,3, Metapneumoviruses
- Labor intensive, additional workspace
- Expensive
- Additional screening assay during April/Summer 2009 pandemic response

Anti-Viral Resistance

- Pyrosequencing, sensitive, clinical specimens
- Perform separate PCR reaction
- Known mutation that confers resistance to oseltamivir
- 2009 Influenza H1N1
- High throughput, ~ 90 specimens

Prior to April 2009



Previous Seasons 1999-2010

Flu Season*	Total specimens	Total positive for flu	%pos	A	B	Source
1999-2000	701	291	41.51%	287	4	2006 Epi Annual Report
2000-2001	1081	481	44.50%	234	247	2006 Epi Annual Report
2001-2002	1013	462	45.61%	410	52	2006 Epi Annual Report
2002-2003	1199	633	52.79%	227	406	2006 Epi Annual Report
2003-2004	1097	563	51.32%	562	1	2006 Epi Annual Report
2004-2005	1422	606	42.62%	498	108	2006 Epi Annual Report
2005-2006	872	382	43.81%	324	58	2006 Epi Annual Report
2006-2007	801	409	51.06%	716	85	unfinished annual summary
2007-2008	1570	765	48.73%	546	219	Annual summary, web
2008-2009	1434	779	54.32%	509	270	z_flusurveillance_060710
2009-2010	350	161	46.00%	159	2	0910DSHSspecimens

Courtesy: Lesley Brannan, TX DSHS IDCU

April 24, 2009 - October 1, 2009

Total Tested	10089
2009 Influenza A H1N1	2496
Seasonal Influenza A H1	36
Seasonal Influenza A H3	125
Influenza B Positive	39
Equivocal	3
Inconclusive	52
Negative	7324

April/Summer 2009

Specimen Acceptability

```
graph TD; A[Specimen Acceptability] --> B[Real time RT-PCR]; B --> C[Report];
```

Real time RT-PCR

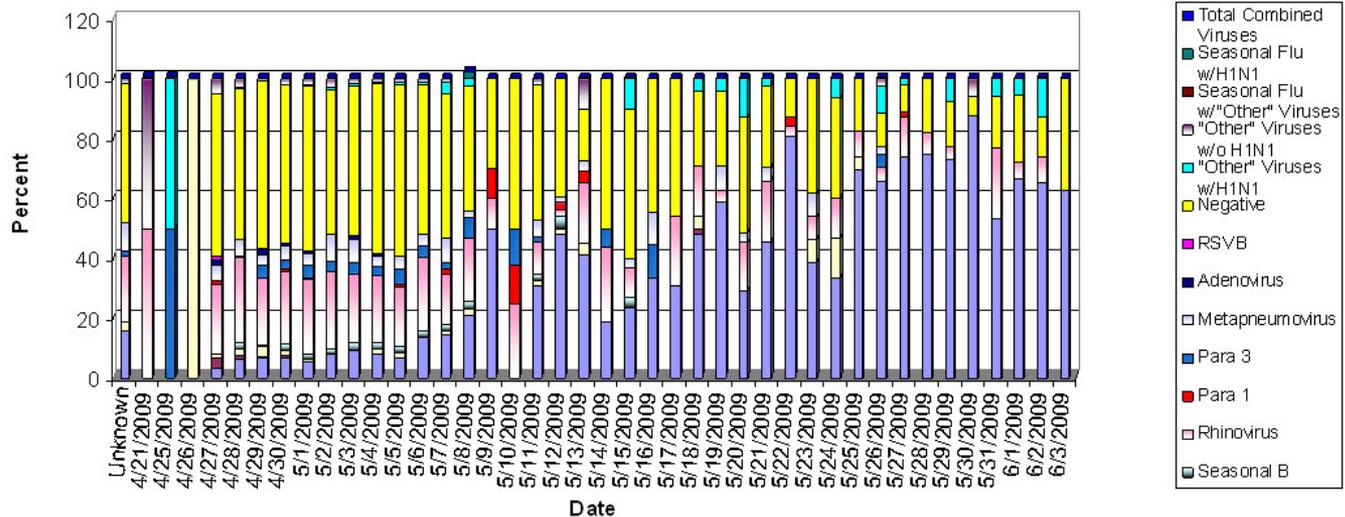
Report

Laboratory Challenge: Novel virus = New test

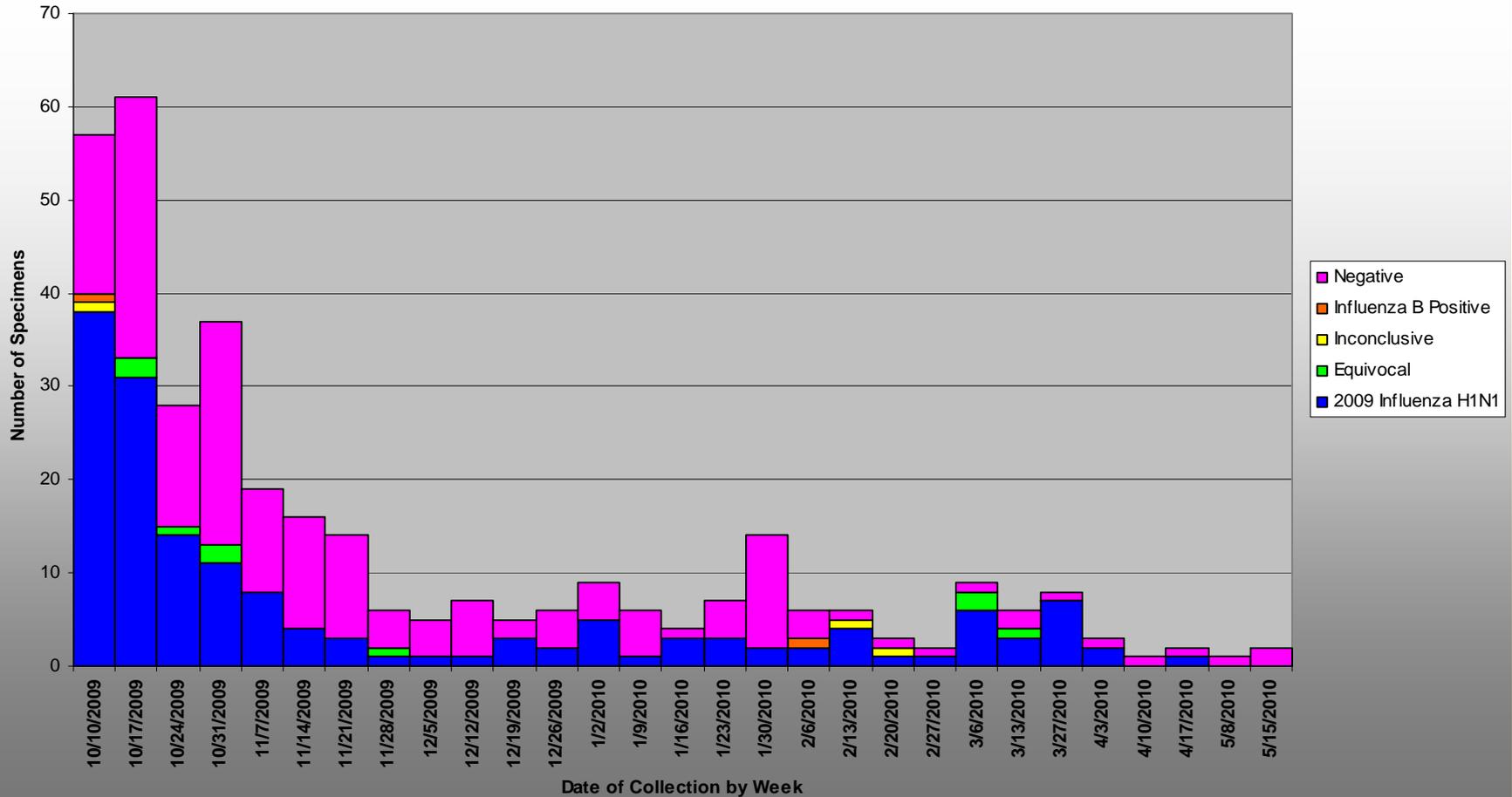
- Specimen Volume
- Triage
- Staffing
- Training
- Equipment/Supplies/Reagents

Summary of RVP analysis during April/May/June 2009

Viruses Detected - Luminex Testing



2009-2010: DSHS Lab



2009 – 2010 Summary

2009 Influenza H1N1	158
Equivocal	9
Inconclusive	3
Influenza B Positive	2
Negative	178
Total Tested	350

CDC Surveillance: What do we send?

- Always
 - Anything unusual
 - Vaccinated cases: if we know***
 - Unsubtypeable specimens
- 2009 – 2010
 - 5 isolates and original clinical materials every 2 weeks: Surveillance (AgC/AVR)
 - 5 clinical samples weekly: AV Resistance
 - Positive 2009 H1N1

How do we choose the samples?

- Most recent collection dates
- Geographic spread as much as possible
- Is there leftover sample available?
- Did it grow in culture?

2009-2010 CDC Surveillance Submissions

Surveillance Isolates submitted	56
A/BRISBANE/59/2007-LIKE(H1N1)LOW	1
A/CALIFORNIA/07/2009-LIKE (H1N1)v	44
B/BRISBANE/60/2008-LIKE	1
INFLUENZA PANDEMIC A(H1N1)v BY PCR	6
Pending	4

*83 specimens submitted for Antiviral resistance testing

Important Points

Specimen Acceptability Criteria

- Pending updated CDC assay
- Seasonal vs H1N1
- Nasopharyngeal swabs
- Received at 72 hours cold
- >72 hours, freeze and ship on dry ice

When in doubt....

- 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

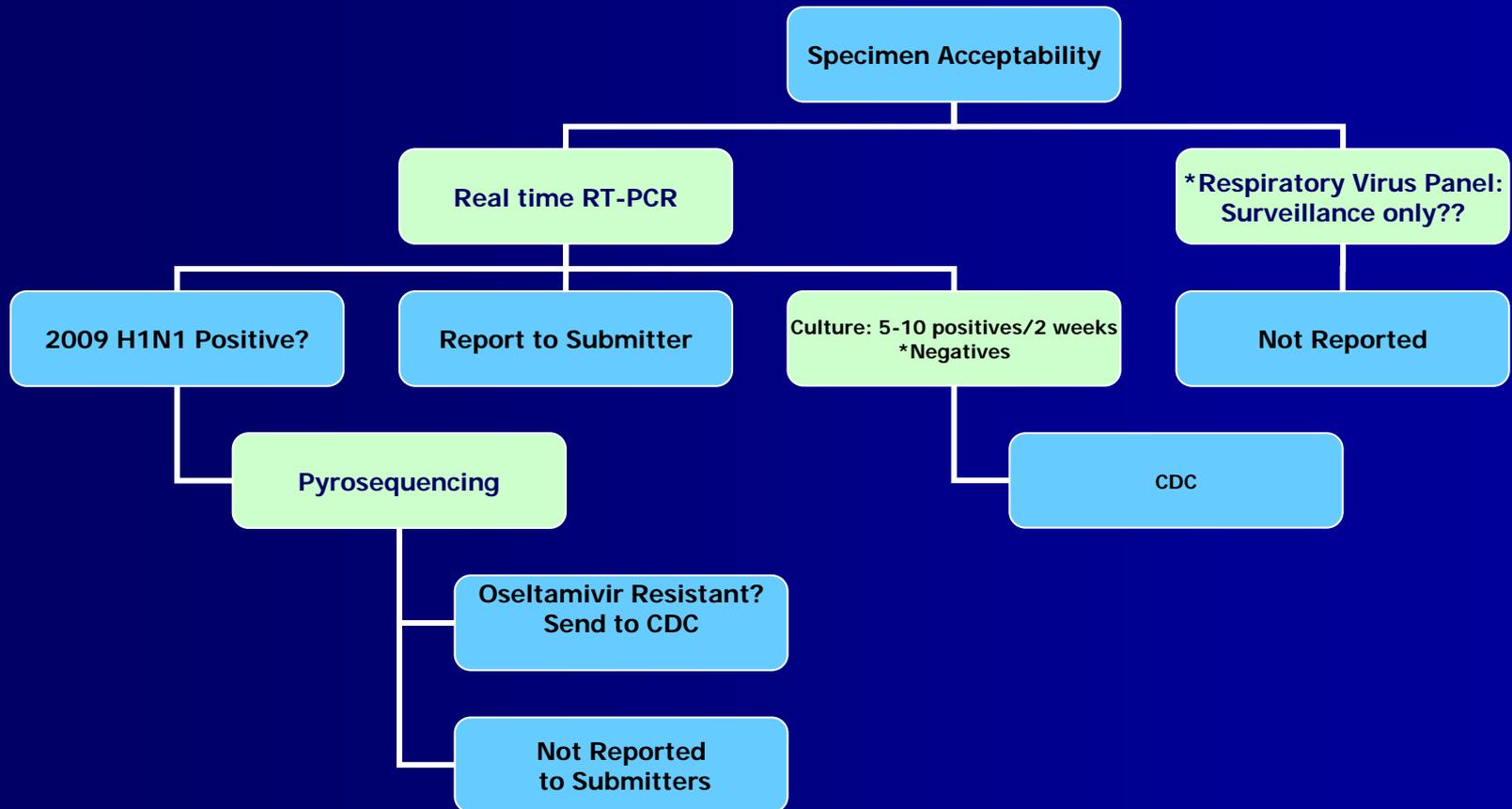
Viral Transport Media

- 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

Viral Transport Media

- DSHS media
 - Quality control in house
- Commercial media
 - Follow manufacturer's recommendations

Fall 2010 Plan for Influenza



*Subject to change

Acknowledgements

- DSHS Laboratory: ALL 400+ STAFF
- Viral Isolation team
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 - Walter Douglass
 - Salvador Arreola
- Laboratory Management
 - Susan U. Neill, Ph.D
 - Eldridge Hutcheson, Ph.D
- Reporting Group
 - Sandra Navarro
 - Tiffunee Odoms
- Microbiological Sciences Branch
 - Consumer Microbiology Group
 - Clinical Bacteriology Group
 - Serology Group
 - Molecular Group
 - Media Prep Team
 - Mycobacteriology/Mycology Group
- Container Prep
- Texas LRN Labs
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