Avian Influenza: Laboratory Issues

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The Influenza A Virus

- 16 Serologically distinct HAs
  - (H1-H16)
- 9 Serologically distinct NAs
  - (N1-N9)
- Nomenclature
  - A/Chicken/HK/5/98 (H5N1)

Slide courtesy of Dr. David Wentworth
“Classic” methods for influenza diagnosis

- Antigen detection using kit-based test on patient specimens - 20 mins
- DFA - 2 to 4 hours
- Culture - 3 to 14 days
- Conventional PCR to sub-type e.g. H3N2 - 1 to 2 days
- Strain determination by sequence e.g. A/Fujian - 2 to 7 days
- Need for rapid molecular method

Real-time PCR
Detection of viral antigen

- Point-of-care FDA-approved rapid tests
- Immunofluorescence-based detection assays
- Will detect H5 avian influenza (CDC)

Sensitivity is a major issue!
Cell culture

• For respiratory viruses we use primary rhesus monkey kidney cells and two cell lines derived from human lung tissue (A549 and HEL)

• Performed as tube culture
Viruses causing respiratory infection

**Influenza**, respiratory syncytial virus, parainfluenza virus, adenovirus, enterovirus, rhinovirus, human metapneumoviruses and several human coronaviruses
How is virus growth evaluated?

- Tube cultures monitored under microscope for 14 days
- Time consuming and requires high skill level
- After virus growth detected, need to perform additional assays to confirm identification
PCR - A little goes a long way

Exponential amplification

(template DNA) 1st cycle 2nd cycle 3rd cycle 4th cycle

wanted gene

$2^2 = 4$ copies

$2^3 = 8$ copies

$2^4 = 16$ copies

$2^5 = 32$ copies

$2^{36} = 68$ billion copies

(Andy Vierstraete 1999)
Conventional PCR assay for subtyping influenza virus

- Based on amplification of HA target gene using specific primers
- Amplicon can then be used for sequence analysis for strain identification
- Requires gel analysis
- TAT about 2 days
Wadsworth Center in-house conventional PCR for H5

A/Turkey/Ontario/7732/66(H5N9)

51F1

H5-869R

HA-1144

H5-1660R

H5-1735R

St. George June 2005
Taqman technology for real-time PCR

Very sensitive, very specific
Turn around time about 6-8 hours from sample receipt
Current Influenza A Taqman Assay

From $10^{-2}$ to $10^{-8}$

97% Efficiency
Y-intercept 35.4
$R^2 .995$

Slide courtesy of Dr. Amy Dean
Influenza A Virus - real time assay

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- Nomenclature
  - A/NY/5/04 (H3N2)
  - A/Chicken/HK/122/04 (H5N1)
CDC Real-time HA assays, APHL website

H1
1779

H3
1764

H5
1772

H7
1792

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Wadsworth Center Strategy for avian influenza

Parallel assays
1. Wadsworth Center Influenza A/B real-time assay (M1 & NS1 targets)
2. CDC subtype-specific real-time assays, H1, H3, H5 & H7 (HA target)
3. Wadsworth Center conventional PCR sub-typing assay (H1, H3, H5)
4. Culture when H5N1 ruled-out
Laboratory safety issues for H5N1 viruses

- Agricultural as well as human pathogen
- Requires BSL-3 Ag (BSL-3 with enhancements)
- Molecular manipulations may be performed at BSL-2 with appropriate precautions
- Culture must only be performed at BSL-3 Ag (BSL-3 with enhancements)
Biological Safety Level-3/Ag

- Personal protective equipment (PPE) - Full Tyvek body suit, head covering, shoe covers, double gloves
- Hepa-filtered respirator
- All work in biological safety cabinet with Hepa-filtered exhaust
- Restricted-entry laboratory under negative air pressure
- Shower-out capability
- Decontamination of all laboratory waste and effluent
CDC Criteria for testing

Hospitalized patients with

- Radiographically confirmed pneumonia, ARDS or other severe respiratory illness

AND

- History of travel within 10 days of onset of symptoms to country with documented H5N1 avian influenza in poultry and/or humans

http://www.cdc.gov/flu/avian/professional/han020405.htm
CDC Criteria for testing (cont)

Consider testing for hospitalized or ambulatory patients with

• Documented temperature of >100.4°F

AND

• One or more of following: cough, sore throat, shortness of breath

AND

• History of contact with poultry or a known or suspected human case of influenza A (H5N1) in an H5N1-affected country within 10 days of symptom onset.
What samples do we need?

- NP aspirate or wash, NP swab, pooled OP/nasal swab
- Swabs should be collected with dacron or rayon swabs with plastic shaft and placed in viral transport medium
- Serum (acute and convalescent)
- Ship as soon as possible after collection
- Keep COLD (4°C) using frozen ice packs
- If cannot ship for >2 days, freeze at -70°C and ship on dry ice
Communication
Acknowledgements

Reference lab staff:
Kim Rush-Wilson
Meghan Fuschino
Theresa Church
Greg Farrell
Matt Kleabonias
Jenny Kinne
Bill Spargo
Sara Griesemer

Kirsten St. George
Amy Dean
Dave Wentworth