



# Challenges and Strategies for Detection of Influenza

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# Three Approaches to Detection

- Detection of live virus
- Detection of viral antigen
- Detection of viral nucleic acid

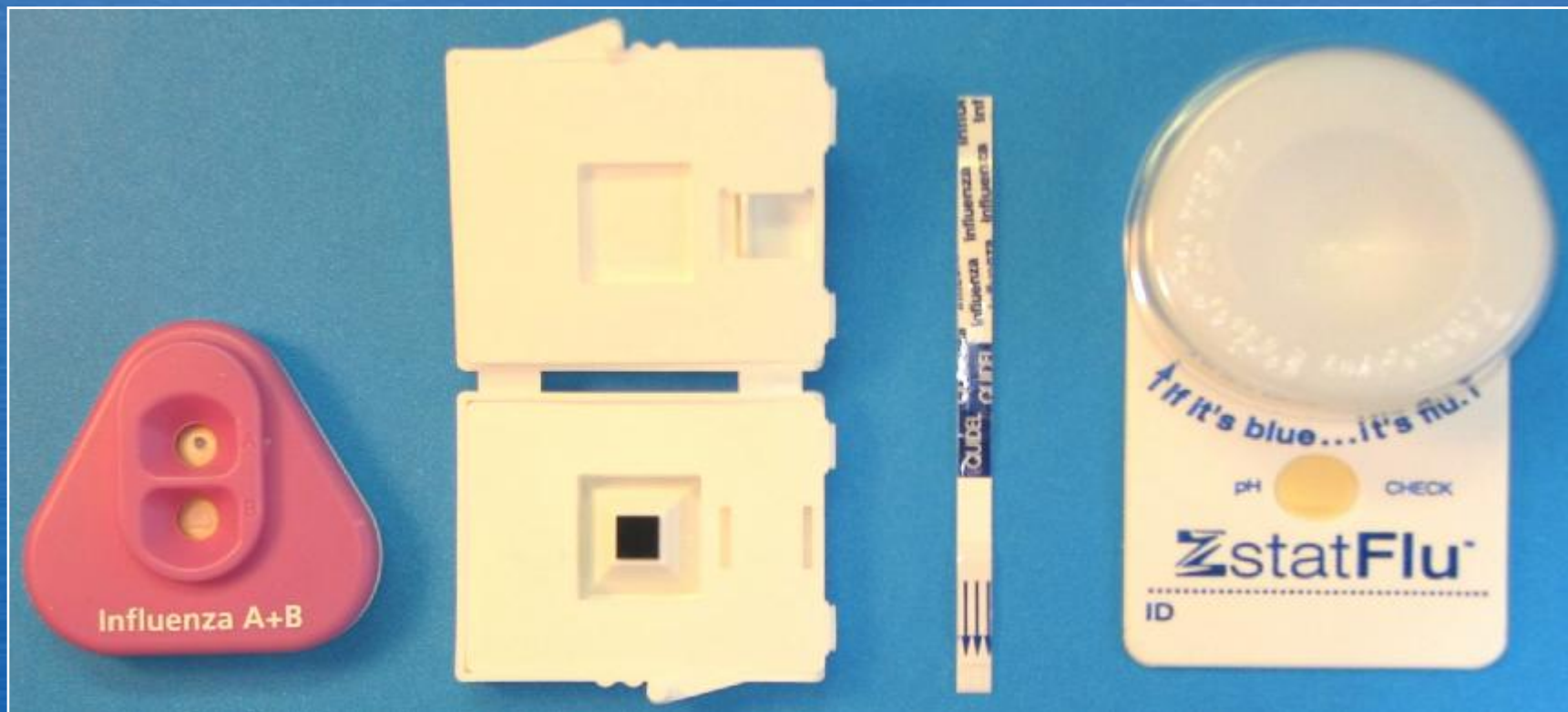


# Antigen (Protein) Based Methods

- Rapid Tests
- Hemagglutination (HA)
- Hemagglutination Inhibition (HAI)
- Immunofluorescence (IFA)
- ELISA



# Rapid Tests: Usual Suspects



Directigen  
Fu A + B

FLU OIA

QuickVue

ZstatFlu



# Rapid Tests: Flu A & B





# Rapid Tests

## Advantages:

- Rapid – results in <30 minutes
- Simple
- On-site or point of care
- Clinical material or grown virus

***Useful for clinical settings and outbreak investigations***



# Rapid Tests

## Disadvantages

- Less sensitive than viral culture or RT-PCR – *false negatives*
- Some kits cannot type (A/B)
- Cannot subtype (H3, H1, H5?)
- Cost (\$12 - \$25)

***Unsuitable for most surveillance and reference labs***



# Rapid Tests – Capabilities

- **Detects only Influenza A viruses**
  - Directigen Flu A
- **Detects and distinguishes between A & B**
  - Directigen Flu A + B; FLU OIA A/B
  - XPECT FLU A/B; NOW FLU A/B
  - Capilia Flu A B; Influa A B Quick
- **Detects but does not distinguish between A & B**
  - QuickVue Influenza Test
  - FLU OIA
  - ZstatFlu



# HA and HAI

## Advantages

- General subtyping to detailed antigenic characterization
- “Gold standard” for vaccine selection

## Disadvantages

- Pure virus culture of high titers necessary
- Immune sera necessary
- Time consuming and labor intensive

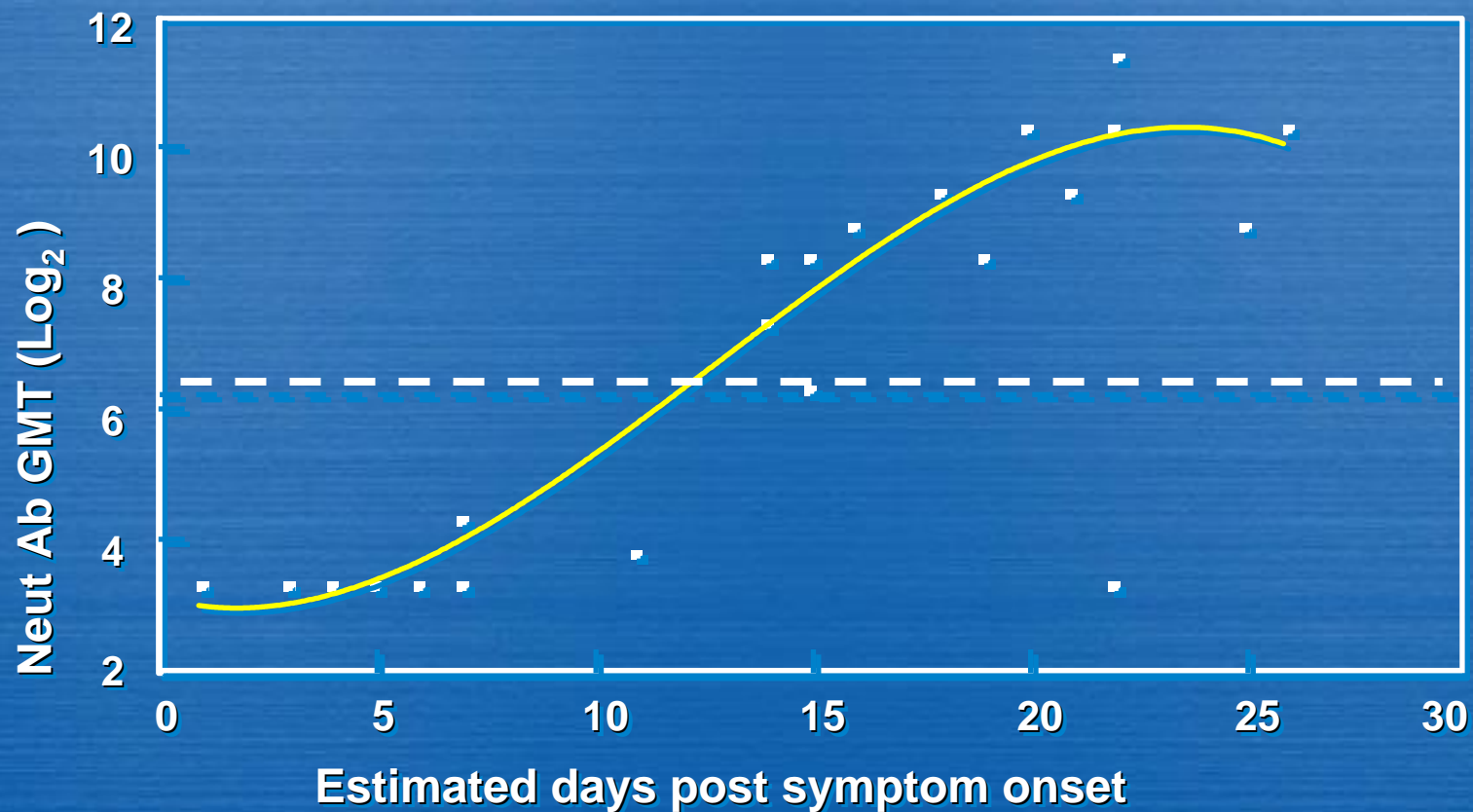


# Serology

- § Acute and convalescent sera are useful but not necessary for suspect avian influenza cases.
- § HI is often not effective for detecting H5 neutralizing Ab in sera from human cases.
- § Neutralization test is more effective for detection of antibodies to avian viruses
  - ÿ Technically difficult, requires live virus, BSL3+ containment, confirmation by Western blot desirable
- § ELISA often demonstrates high level of nonspecificity



## Kinetics of Antibody Response in H5N1-infected Humans Determined by Micro-Neutralization Assay





# Polymerase Chain Reaction (PCR)

- Conventional
- Real-time (SYBR<sup>®</sup> green, dual-labeled probes, molecular beacons, etc.)
- RT-PCR-ELISA
- Luminex/Bioplex bead array
- Microarray (“gene chips”)



# PCR Detection of Influenza Viruses

## Advantages:

- Rapid – *15 minutes to 24 hours*
- Sensitive\* – *1 to 10 viral particles*
- Specific\*
- Low Biocontainment – *BSL-2*
- High Throughput – *can be automated*

\* *Only as good as the probes & primers!*



# PCR Detection of Influenza Viruses

## Disadvantages:

- Technically difficult
- Risk of cross contamination or carry-over contamination (false-positive results)
- Differences between protocols (primers, chemistries, etc)
- Cost (per assay & start-up costs)



# PCR Systems – A Few Examples!



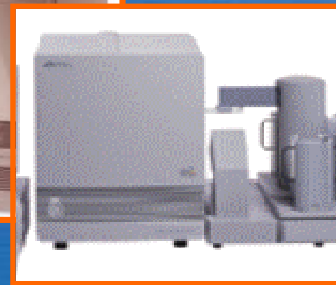
Roche  
Lightcycler



Stratagene Mx3000p/3005p



ABI 7500



ABI PRISM 7900



BioRad iCycler



Cepheid Smartcycler I & II



Corbett Rotor-Gene



Eppendorf Realplex 2/4



MJ MiniOpticon





# "Real-Time" RT-PCR

## **Simplified protocol**

- Lower risk of technician error
- Lower risk of contamination

## **Universal assay format**

- Comparable results with different platforms, chemistries, etc.

## **No processing of amplified DNA**

- Low risk of "carry-over" contamination



# “Real-Time” RT-PCR for Influenza

- Virus detection – type A/B
- Subtyping – H1, H3, H5, etc.
- Specimen quality control
- Estimate virus load



# Caveats – regardless of method

- Influenza viruses are constantly evolving
- Reagents may require periodic modifications to maintain sensitivity and specificity
- All test results must be interpreted in the context of clinical and epidemiologic background!
- Confirm results whenever possible *before* announcing findings!



# Role of Laboratory Surveillance

- Many respiratory outbreaks show “flu-like” symptoms
- H5 influenza + SARS suspect case definition similar
- Effective laboratory testing for influenza necessary to rapidly “rule-in” or “rule-out” influenza

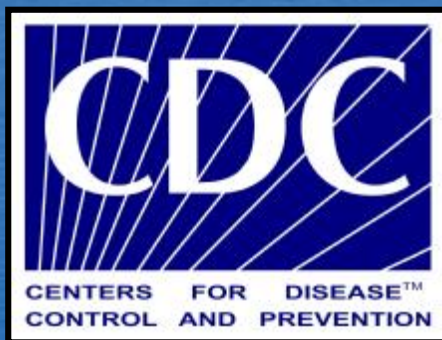


# Conclusions

- A number of testing strategies can be used to meet the epidemiological situation and laboratory capabilities.
- Planning is crucial to develop laboratory capability, capacity, and competency to report effective results in timely manner.



# Acknowledgments



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# HI REACTIONS OF H5 INFLUENZA SPECIMENS

## STRAIN DESIGNATION

## REFERENCE ANTISERA

### REFERENCE ANTIGENS

1. A/TERN/S. AFRICA/61
2. A/HONG KONG/156/97
3. A/GOOSE/HK/437-4/99
4. A/HONG KONG/213/2003

### TEST ANTIGENS

5. A/VIETNAM/1194/2004
6. A/VIETNAM/1203/2004
7. A/VIETNAM/1204/2004

GOAT

FER

CHIC

FER

TN/SA

HK/156

GS/HK

HK/213

1280

320

640

20

640

320

1280

80

320

320

1280

80

1280

320

1280

320

10

5

80

5

10

5

160

5

10

5

80

5

Sera from 2003 H5 kit

3/2/2007



## Comparison of HI and Neutralizing Antibody Titers for Detection of Anti-avian H5 Antibody in H5N1 Cases

Case no.	HK/156/97 (H5N1)		Dk/Sing/97 (H5N3)	
	HI	Neut	HI	Neut
4	<20	160	<20	160
8	<20	80	<20	40
16	<20	320	<20	160
6	80	1,280	80	1,280
7	80	1,280	80	640



# Differences Among Human H5N1 Virus HA Proteins





# WHO Reagent Kit for Identification of Influenza (H5) Virus

## HAI reagents

- 5ml influenza A (H5) control antigen (inactivated)
- 1ml sheep H5 specific serum (lyophilized)
- RDE

## IFA reagents

- 0.5ml influenza A (H5) specific monoclonal antibody pool