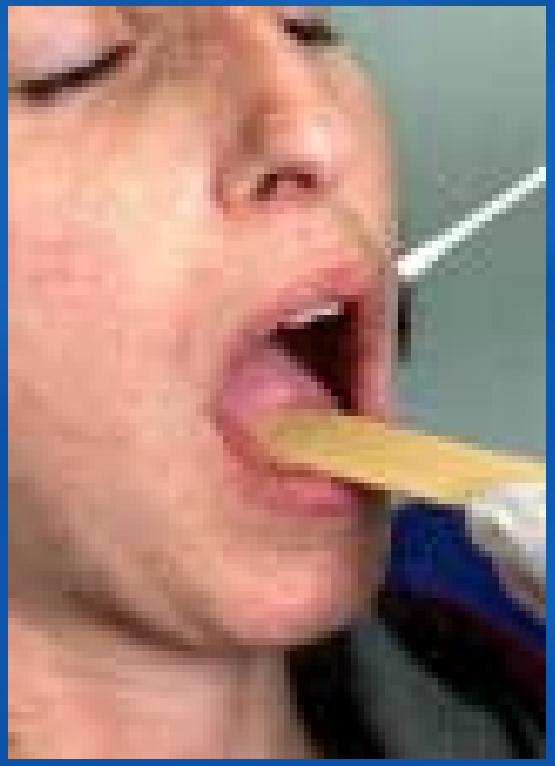
Laboratory Diagnosis of Influenza



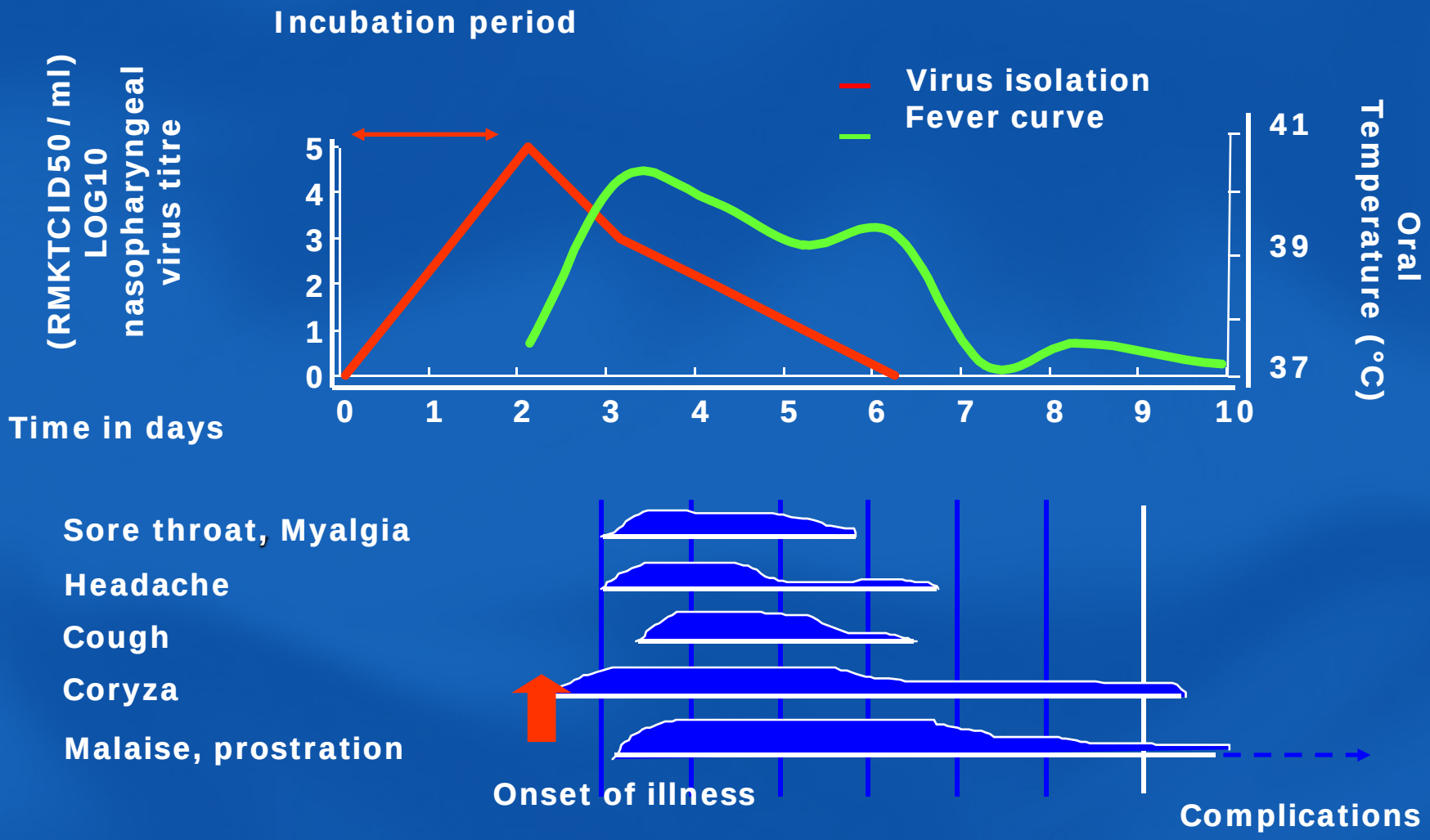
Specimens for Viral Identification

Identifying viruses in clinical specimens is a specimen-driven process

Clinicians need to be aware of:

1. Types of viruses that can be isolated from different specimen types
2. Pathogenesis of the disease
3. The limitations of the lab tests performed

Classical Influenza



Kilbourne E. D., Influenza, New York, plenum 1987:156-218

Influenza Viral Shedding

Influenza A & B viruses are primarily shed in the upper respiratory tract of humans

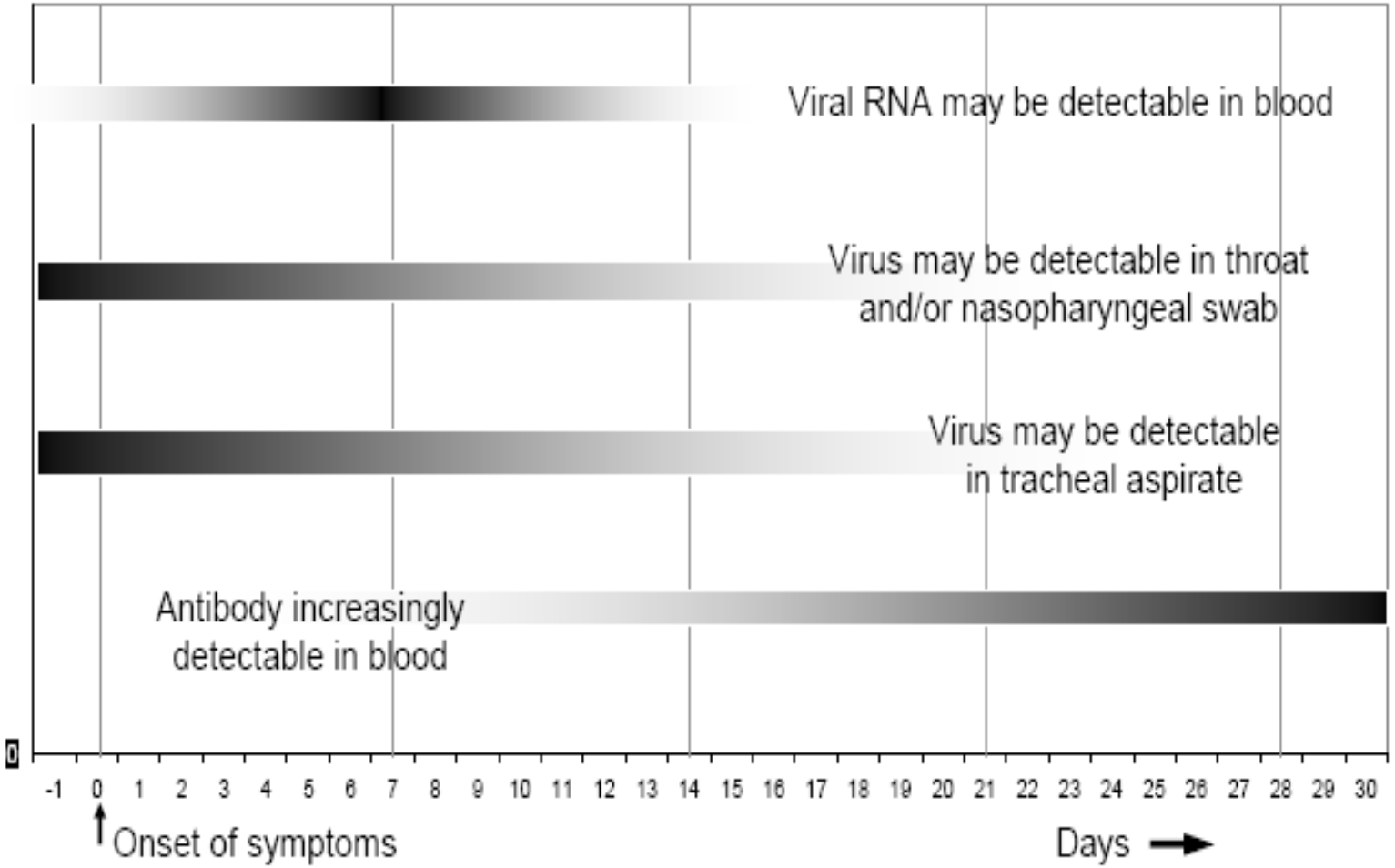
- Viral shedding occurs the day before illness onset
- Peak viral shedding on Day 1 of illness
- Duration
  - Adults may shed viruses for 4-6 days
  - Young children may shed for longer periods
  - Immunocompromised can shed for months

Clinical Specimen Sources

Plan for specimen collection before you leave for the field from

- Suspected cases
  - Symptoms consistent with influenza
- Contacts of cases
  - Including people living or working with suspected cases

Virus Excretion, Viral RNA in Blood and Antibody Response in H5N1 Infection in Humans



What Specimens to Collect from Suspect Cases

1. Preferred samples

- From upper respiratory tract
  (take both types of specimen to allow detection of A(H5N1) and other influenza viruses):

  - Posterior-pharyngeal (throat) swabs are currently the highest yielding upper respiratory tract specimens for detecting avian influenza (H5N1) (unlike human influenza)

  - Nasal swabs with nasal secretions (from the anterior turbinate area) or nasopharyngeal aspirates or swabs are appropriate specimens for detecting human influenza A and B and therefore useful if the influenza is not due to avian influenza (H5N1)

What Specimens to Collect from Suspect Cases

1. Preferred samples

Lower respiratory tract:

- If the patient is intubated, take a tracheal aspirate

  or

- collect a sample during bronchoalveolar lavage

Secondary specimens

(These are not essential but can be useful if materials are available)

- Plasma in EDTA (for detection of viral RNA)
- Rectal swab —especially if the patient has diarrhea
- Spinal fluid if meningitis is suspected and a spinal tap is to be performed for diagnostic/therapeutic purposes
- Blood:
  - Serum (acute and convalescent, if possible)

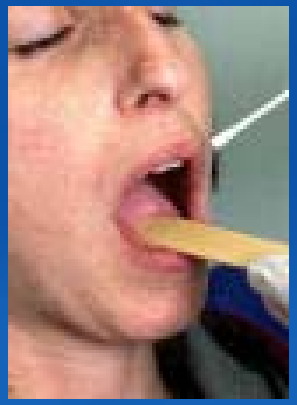
Laboratory Tests

Detection and Characterisation of Influenza

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

Routine Influenza Diagnostics & Analysis

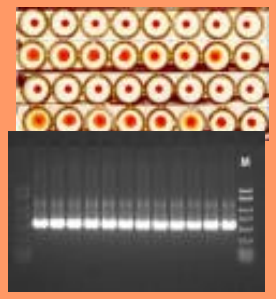
Patient clinical details: Influenza like illness, temperature, cough malaise



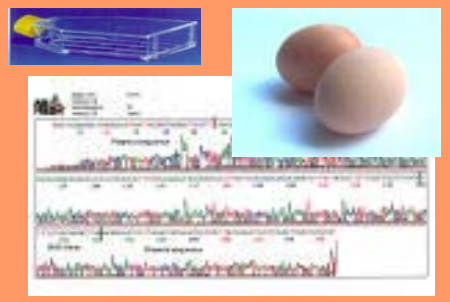
Patient sample: throat swab gargle/wash, aspirate (nasopharyngeal /bronchoalveolar lavage)

Rapid test*
Directigen Flu A+B
Binax Now A/B
Capilia Flu A,B

Lab assay
Direct IFA
RT-PCR
HI assay



Further analysis
Virus Culture
Extensive HI
Sequence
HA & NA



Diagnosis of Influenza

- **Viral culture**
  - "Gold" standard
- **Antigen detection kits**
  - “Point of care” rapid assay
  - Hemagglutination (HA) test
  - Hemagglutination Inhibition (HI) test
  - Immunofluorescence Assay (IFA)
- **Molecular detection tests**
  - PCR Assay
  - Real-Time PCR

Laboratory Diagnosis for Avian Influenza

Tests for respiratory samples:

- PCR-based techniques
- Virus isolation
- Immunofluorescence
- Rapid antigen detection

Blood used for:

- Measurement of specific antibodies (most common for influenza, sera is used)
- Viral isolation (whole blood if viremia is a consideration)
- PCR-based techniques (sera)

Laboratory Tests for Avian Influenza A (H5N1)

- **PCR based techniques - recommended**
  - Sensitivity depends on the particular test, the influenza strain, and the type of specimen used
- **Virus isolation**
  - Technically difficult
  - Requires a BSL-3 laboratory with enhancements

Laboratory Diagnostic Methods for Avian Influenza

- **Most commonly applied methods:**
  - PCR
  - Possibly cell culture
- **Recommendation:**
  - PCR, if there is equipment, BSL-2 lab with BSL-3 precautions
  - If positive, send specimen for cell isolation in culture at BSL-3 lab

Guidelines for Potential Avian Influenza Specimens

- **Use BSL-2 laboratory with BSL-3 practices for:**
  - Diluting specimens
  - Nucleic acid extractions
  - Diagnostic testing that does not involve culturing
- **Use BSL-3 laboratory with BSL-3 practices for:**
  - Culturing avian influenza virus

WHO H5N1 Laboratory Network

All viruses from human cases of avian influenza should be shipped to a WHO reference laboratory for:

- Vaccine development
- Antiviral susceptibility testing
- Other activities of public health significance

Who should be tested for H5N1 influenza??

- Hospitalised and/or fatal cases *AND*
- Has documented fever ≥38°C *AND*
- Has pneumonia (CXR), ARDS, or other severe respiratory illness, with no alternate diagnosis *AND*
- Meets criteria A *or* B *or* C below, within 10 days of symptom onset

A: History of travel to a country with H5N1 cases in birds or humans
AND has at least one of the following

1. Direct contact (touching) with sick or dead poultry
2. Direct contact with surfaces contaminated with poultry feces
3. Consumed raw or incompletely cooked poultry or poultry products
4. Direct contact with sick or dead wild birds suspected or confirmed to have H5N1
5. Close contact (within 1 meter) of a person hospitalized or dead due to a severe unexplained respiratory illness

B: Close contact of an ill patient who was confirmed or suspected to have H5N1

C: Worked with live H5N1 influenza virus in a laboratory

Consider Testing
(case by case basis)

- Someone with mild or atypical disease who meets criteria A, B or C

OR

- Someone with severe or fatal respiratory disease whose epidemiological information is uncertain, unavailable or otherwise suspicious, for example:
  - Returned traveler from H5N1affected country with unknown exposure(s)
  - Person who has contact with sick or well-appearing poultry

Managing and Analysing Laboratory Data

How to Present Results

Report:

- Time and place of the outbreak
- Prevalence of infection
- Clinical information about cases
- Epidemic curve

Share results with local health officials and the WHO Global Influenza Program

All test results must be interpreted in the context of clinical and epidemiologic background

Confirm whenever possible

Conclusions

- Influenza viruses are evolving — whether testing using antigenic or genetic detection strategies, test reagents may require periodic updates or modifications to maintain test sensitivity/specificity

Summary

- Most countries will be able to perform some basic influenza identification tests on a clinical specimen, but more specific testing may need to be done at a higher level national or regional laboratory

- When you report on the progress of an outbreak investigation, share investigation results with local health officials