



# Laboratory Investigation of Pandemic Influenza A (H1N1) 2009

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# When laboratory diagnosis is needed?

- Diagnosis of new cases in a community
- The patients are severely ill.
- The patients are in the high risk groups: pregnancy, obese, having underlying diseases.
- For epidemiological study: investigate immune status of the population, asymptomatic infection

# Lab diagnosis of respiratory viruses

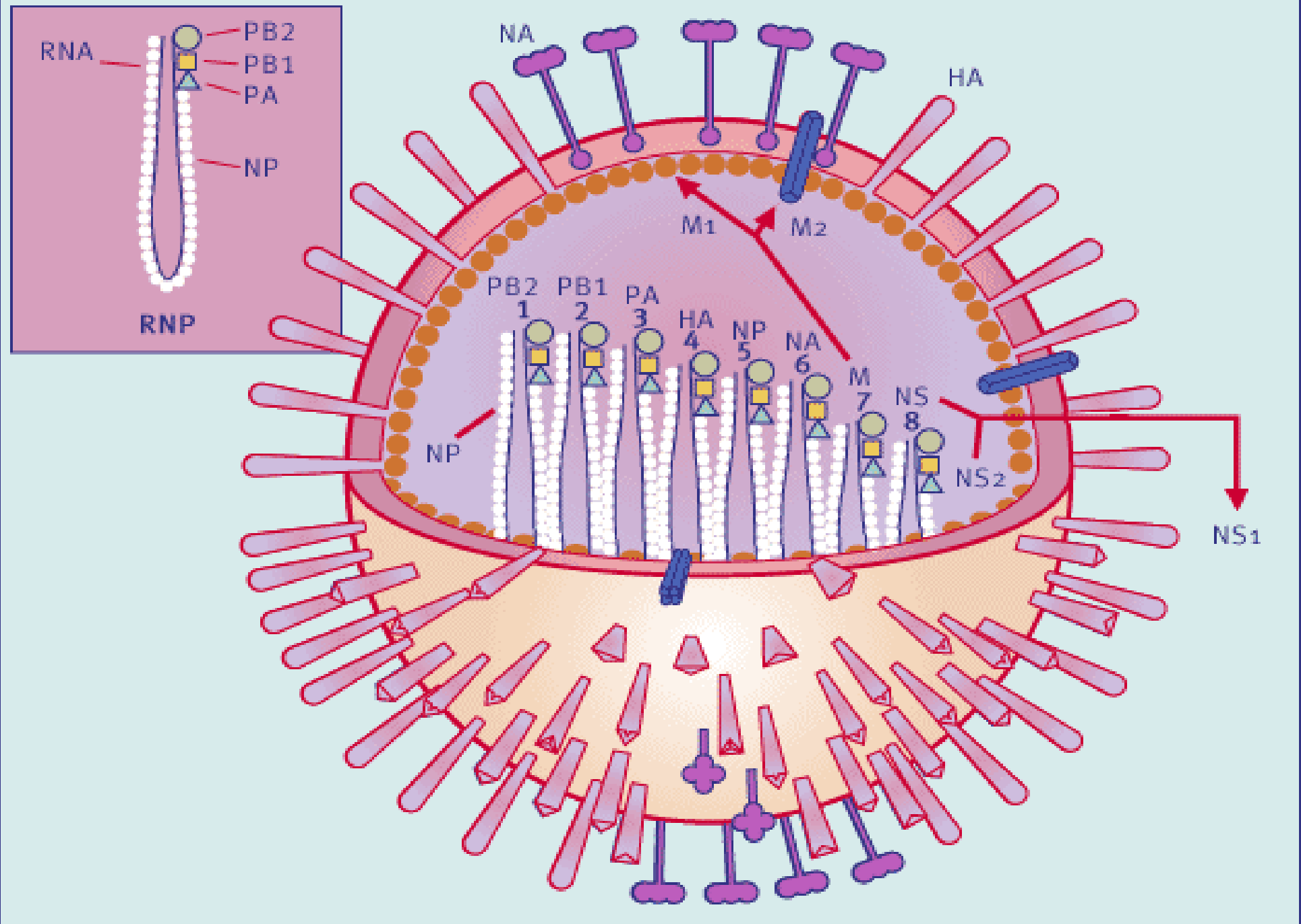
## **Rapid diagnosis**

- Antigen detection by IFA, immunochromatography
- Genome detection

## **Virus isolation**

## **Antibody detection**

# Influenza A virus



# Clinical samples and lab investigation

NP wash/aspirate, sputum, throat swab, endotraheal swab/aspirate, tissues

- Antigen detection
- Virus isolation
- Molecular diagnosis

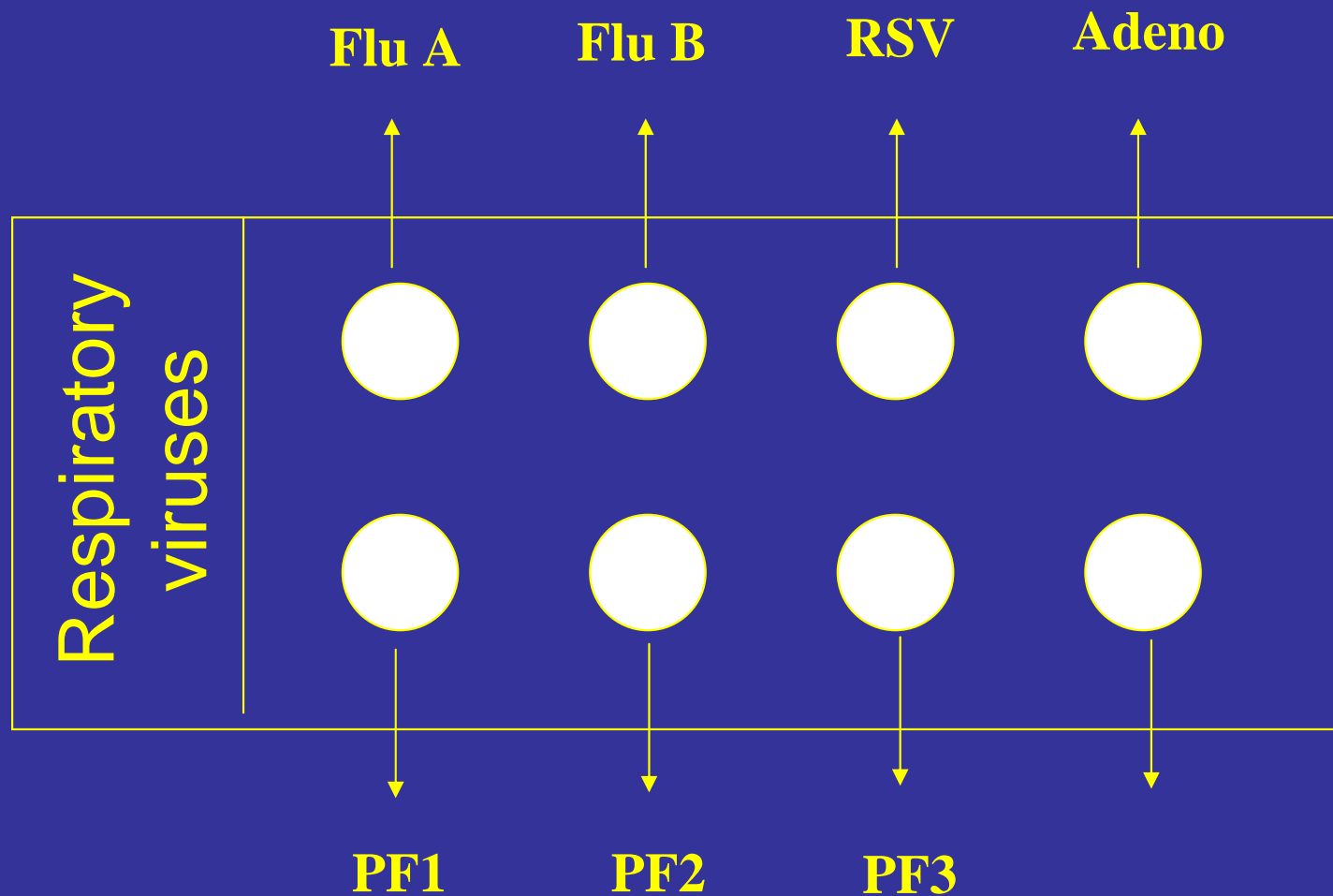
Paired blood for antibody detection

Working with lived virus should be performed in BSL2 enhanced lab.

**Antigen detection:**  
**Diagnosis at level of type A or B**

# Rapid Influenza Antigen Tests

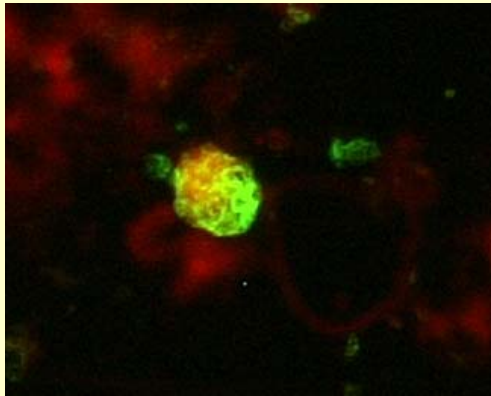
<b>Test</b>	<b>Sensitivity, % (95% CI)</b>	<b>Specificity, % (95% CI)</b>	<b>PPV, %</b>	<b>NPV, %</b>
BD Directigen EZ Flu A+B	46.7 (34.6-59.1)	100 (86.2-100)	100	89.6
BinaxNOW Influenza A&B	38.3 (27.1-51.0)	100 (86.2-100)	100	88.2
QuickVue Influenza A+B	53.3 (40.9-65.4)	100 (86.2-100)	100	90.8



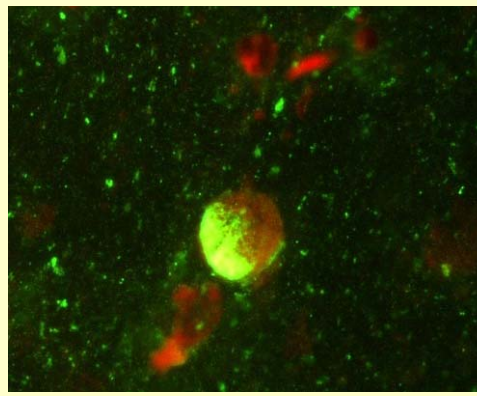
Indirect immunofluorescence test for respiratory viruses



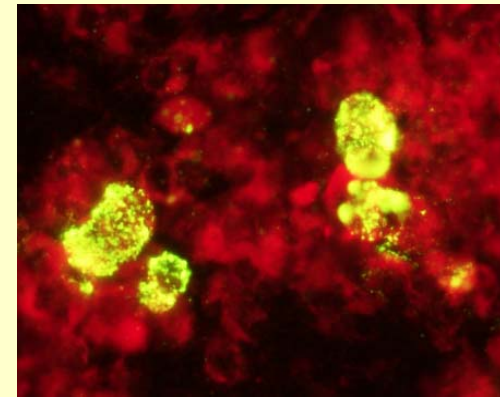
Immunofluorescence staining of virus infected cells in NPA. Positive cells exhibit an apple green color under fluorescence microscope.



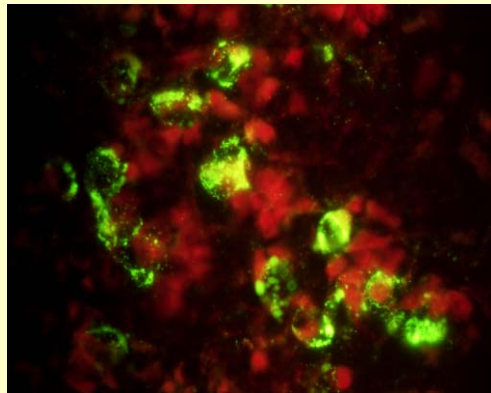
Flu A



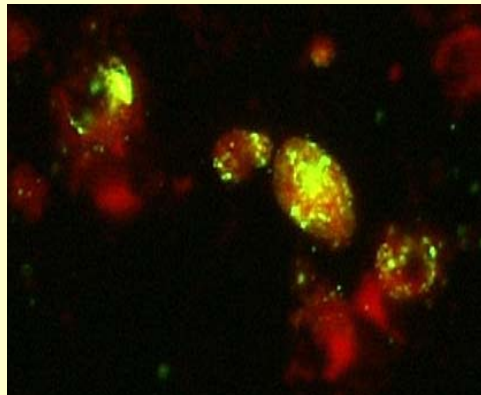
Flu B



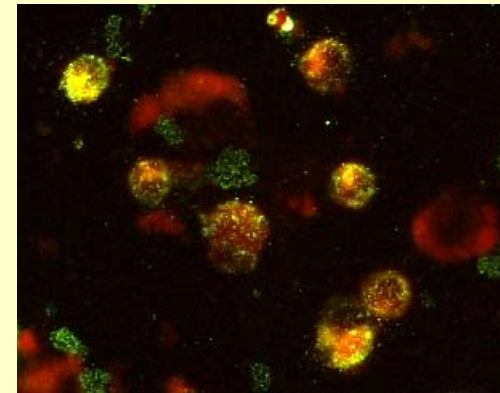
PIV1



PIV3



RSV



Adenovirus

**Diagnosis at level of types, subtypes  
and strain by molecular techniques**

# Molecular diagnosis at level of types and subtypes

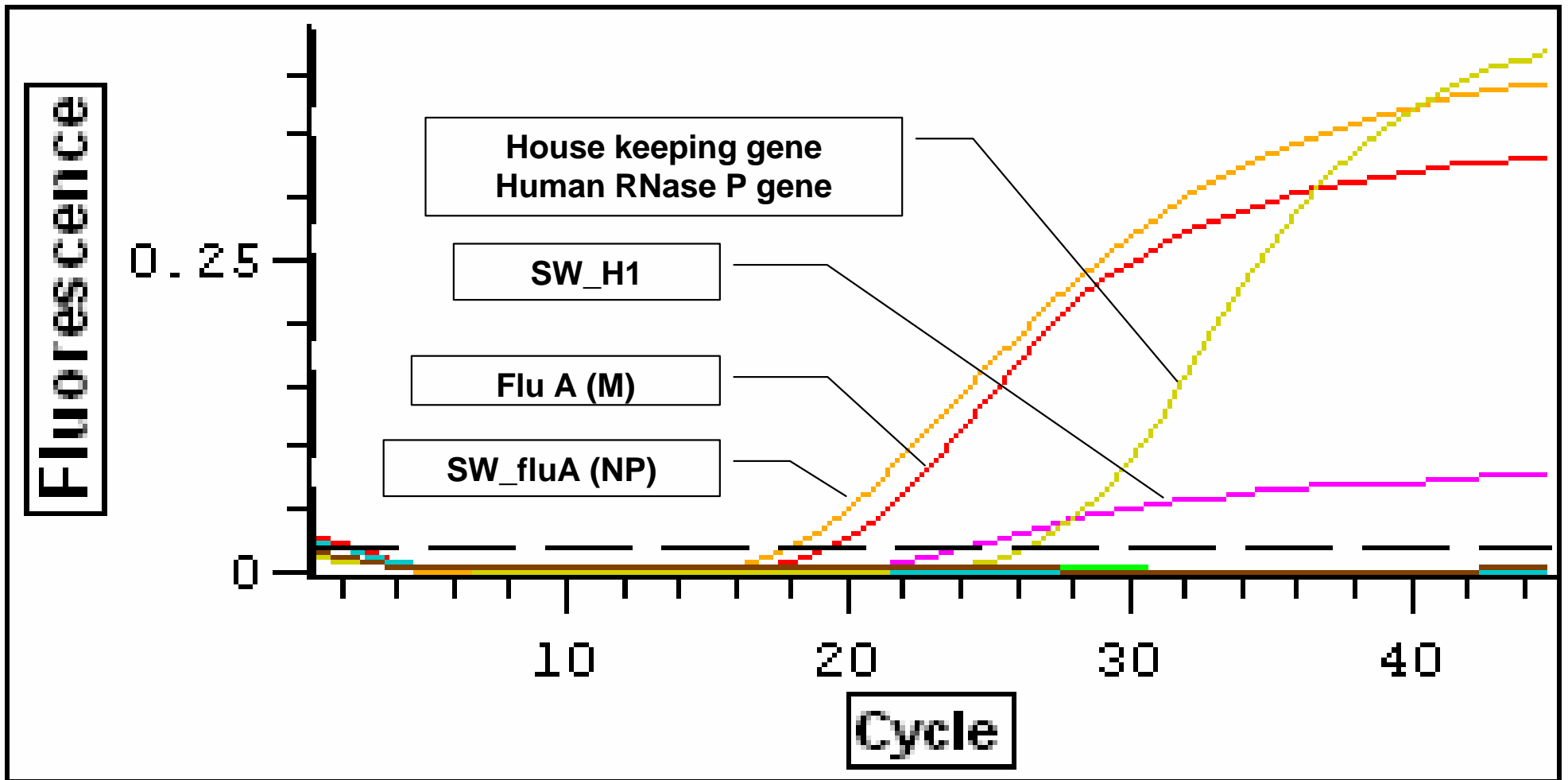
- RT-PCR, nucleotide sequencing
- M or NP gene is used to design universal primers for all flu A
- H and N genes for subtyping, strain differentiation
- House keeping gene to check for quantity of RNA in clinical samples

**Human (seasonal) influenza viruses:  
H1N1, H3N2, fluB**

**Avian influenza H5N1, H7N7 viruses**

**Pandemic influenza (H1N1) 2009 virus**





## CDC protocol for diagnosis of pandemic influenza (H1N1) 2009

# Virus shedding

- How high and how long?
- Comparison between 2009 pandemic flu and seasonal flu

- Viral shedding period = 4.9 d (range 1-11 days)
- Amount of viruses shed in term of M copy numbers/ml of VTM =  $10^{5-9}$

Virus isolation in MDCK cells or embryonated eggs should be performed in BSL 2 enhanced laboratory.



# Purposes for detection of antibody to pandemic influenza 2009 virus

- Disease diagnosis
- Vaccine evaluation
- Seroepidemiology



# Serological tests

Paired blood 1-4 weeks interval

- **Hemagglutination inhibition (HI) assay**
  - for diagnosis of human flu
  - for human vaccine evaluation (HI titers  $\geq 40$  is assumed to be the protective level.)
- Microneutralisation assay is recommended for avian H5N1 and pandemic influenza H1N1 viruses

A photograph of a plant with green, trifoliate leaves and a central spike of pink flowers. The flowers are in various stages of bloom, with some fully open and others as buds. The background is a soft-focus green.

## Acknowledgements

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