

Laboratory diagnosis of influenza

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Influenza Viruses

- Orthomyxoviruses can be identified as influenza A, B and C viruses
- Influenza A infects a variety of different host species

There are 15 subtypes of H and 9N. H1N1, H2N2 and H3N2 have been recognized to cause human infection for decades

H1N1 - 1918 - 1957

1977 -

H2N2 - 1957 - 1968

H3N2 - 1968 -

- H1N2 - 2002 --

- Single stranded RNA virus with a core of 8 segments. Each segment corresponds to a gene

Influenza Viruses

- The envelop haemagglutinin (HA) attaches to host-cell receptors and stimulates the production of neutralizing antibody
- The surface spike neuraminidase (NA) permits transport of virus through mucin on cells of respiratory tract
- The nucleoprotein (NP) contains type specific antigens that distinguish between influenza type A, B and C
- PB1 and PB2 are polymerases involved in mRNA synthesis and PA in virion RNA synthesis
- M gene codes for membrane or matrix protein
- NS genes code for two proteins in infected cells involve in protein and RNA synthesis

Source of specimens

- Clinical specimens from hospitals for virology studies.
- Swabs from sentinel surveillance sites
- Swabs from investigation of institutional outbreaks
- Confirmatory service for private laboratories with positive screening tests.

Specimen collection

- Take specimen early during illness. Best result in the first 3 days of illness
- Throat / nose swabs
 - Nasopharyngeal aspirate
 - Tracheal aspirate
 - Bronchio-alveolar lavage
 - Sputum
- Transport to lab. as soon as possible. Place at 4°C if delay is inevitable (Isolation rate unchanged for up to 4 days at 4°C)
- Place at -70°C if not cultured within 4 days

Laboratory Methods

- Cell culture
- Immunofluorescence test
- Haemagglutination inhibition test
- Polymerase chain reaction
- Nucleotide sequencing

Rapid diagnosis of influenza A virus

- ❖ Directigen Flu A (Becton Dickinson)
- ❖ Now® Flu A (Binax)
- ❖ Immunofluorescence test
- ❖ Reverse transcription-PCR (In-house)

Directigen Flu A (Becton Dickinson)

- An enzyme immunoassay (EIA test) membrane test for influenza A viral antigen (nucleoprotein)
- Results are available in 15 minutes



Result of Directigen Flu A



Comparison of Directigen to Culture

<u>Specimen type</u>	<u>Sensitivity</u>	<u>Specificity</u>
NPA	92%	88
PS	67%	92%
NPS	88%	97%

Now® Flu A (Binax)

- An immunochromatography (ICT) technology
- Requires only one step
- Results are available in 15 minutes

Result of Now® Flu A (Binax)



Correlation between culture and immunofluorescence

<u>and</u>	<u>IF screen +ve</u>	<u>IF screen -ve</u>	<u>IF screen +ve</u>	<u>Both IF</u>
<u>ve</u>	<u>Culture +ve</u>	<u>Culture +ve</u>	<u>Culture -ve</u>	<u>Culture -</u>
RSV	54	5	15	
Influenza A	115	13	2	
Influenza B	94	29	0	
Parainfluenza 1	4	0	1	
Parainfluenza 2	3	0	0	
Parainfluenza 3	70	8	10	
Adenovirus	99	58	2	
TOTAL	439	113	30	<u>1566</u>

some specimens had more than a single virus detected. There the total number of viruses detected was greater than the number of specimens examined.

Reverse transcription-PCR

❖ For sub-typing and detection

❖ Principle

- Sub-type (H1 or H3) specific primer
- Target on viral hemagglutinin gene
- Viral RNA is extracted and transcribed into cDNA
- cDNA is then used as a target for polymerase chain reaction
- Each reaction is analysed by agarose gel electrophoresis and ethidium bromide staining

RT-PCR result (H3)



Cell culture

Inoculate into MDCK cells to isolate influenza virus

Identify by IF and HI tests

Specimens are also inoculated into LLC-MK2, HEp-2c, RD to isolate other respiratory viruses

Serology

Based upon a significant rise (generally 4 fold) in antibody titre to a given viral antigen over the course of the patients' illness.

-Complexment fixation test (CF)

type specific

-Haemagglutination inhibition test(HI)

subtype specific

-Neutralization test

measure protection

most sensitive and specific

Typing of Viruses

- All the influenza virus isolates would be typed with antisera from WHO.
- Atypical isolates would be sent to WHO Collaborating Reference Laboratories (e.g. US, UK etc) for further typing.

WHO Influenza Surveillance

World Health Organization

United States

United Kingdom

Australia

Japan

WHO Influenza Collaborating Reference Laboratories



110 WHO National Influenza Centres

Antigenic analyses of influenza A H3N2 viruses

Viruses	Isolation Date		Haemagglutination inhibition titre*								
			Post infection ferret sera								
			A/Pan 2007/99	A/NY 55/01	A/Egypt 130/02	A/Fuj 411/02	A/Fin 170/03	A/Send 4952/02	A/Wy 3/03	A/UK 1861/03	A/Ku 102/03
A/Panama/2007/99			2560	640	1280	80	80	160	320	160	160
A/New York/55/01			2560	2560	5120	160	160	160	640	160	320
A/Egypt/130/02			640	640	2560	80	80	80	320	80	160
A/Fujian/411/02			80	<	<	320	160	320	640	160	640
A/Finland/170/03			160	80	160	320	320	640	640	320	640
A/Sendai/4952/02			320	160	320	160	320	640	640	320	1280
A/Wyoming/3/03			1280	320	640	2560	2560	2560	5120	1280	5120
A/UK/1861/03			320	80	160	80	80	160	1280	640	640
A/Kanagawa/102/03			320	80	160	640	640	1280	2560	320	2560
A/Hong Kong/136/03	29.1.03	MDCK2 U	80	80	80	160	80	160	640	160	320
A/Hong Kong/137/03	29.1.03	MDCK2 U	80	80	80	160	160	80	640	320	640
A/Hong Kong/905/03	29.3.03	MDCK2 U	80	80	80	160	160	80	320	160	320
A/Hong Kong/1205/03	28.6.03	MDCK2 U	320	160	160	320	320	640	1280	640	1280
A/Hong Kong/1227/03	5.7.03	MDCK2 U	320	160	160	320	320	640	1280	640	640
A/Hong Kong/1244/03	16.7.03	MDCK2 U	80	<	40	80	80	160	320	80	320
A/Hong Kong/1254/03	19.7.03	MDCK2 U	80	80	80	160	160	640	1280	160	1280
A/Hong Kong/1256/03	15.7.03	MDCK2 U	320	160	160	80	80	160	640	80	640
A/Hong Kong/1261/03	22.7.03	MDCK2 U	320	80	80	80	80	80	640	640	320
A/Hong Kong/1262/03	22.7.03	MDCK2 U	80	80	80	160	160	80	640	160	640
A/Hong Kong/1264/03	22.7.03	MDCK2 U	80	80	80	160	160	640	1280	320	640
A/Hong Kong/1270/03	25.7.03	MDCK2 U	80	160	80	160	160	160	640	320	640
A/Hong Kong/1272/03	25.7.03	MDCK2 U	160	80	160	320	320	320	1280	320	640

Antigenic analyses of influenza B viruses

Viruses	Isolation date		Haemagglutination inhibition titre ¹					
			B/Shan ² 7/97	Post infection ferret sera				
				B/Shan 7/97	B/HK 335/01	B/Te 80/02	B/Bris 32/02	B/Sich 379/99
B/Shandong/7/97			2560	320	320	80	160	<
B/Hong Kong/335/01			1280	320	320	80	160	<
B/Tehran/80/02			1280	320	320	320	160	<
B/Brisbane/32/02			1280	320	320	80	160	<
B/Sichuan/379/99			<	<	<	<	<	80
B/Hong Kong/466/03	13.3.03	MDCK2 U	40	<	<	<	<	80
B/Hong Kong/553/03	31.3.03	MDCK2 U	5120	320	40	40	40	<

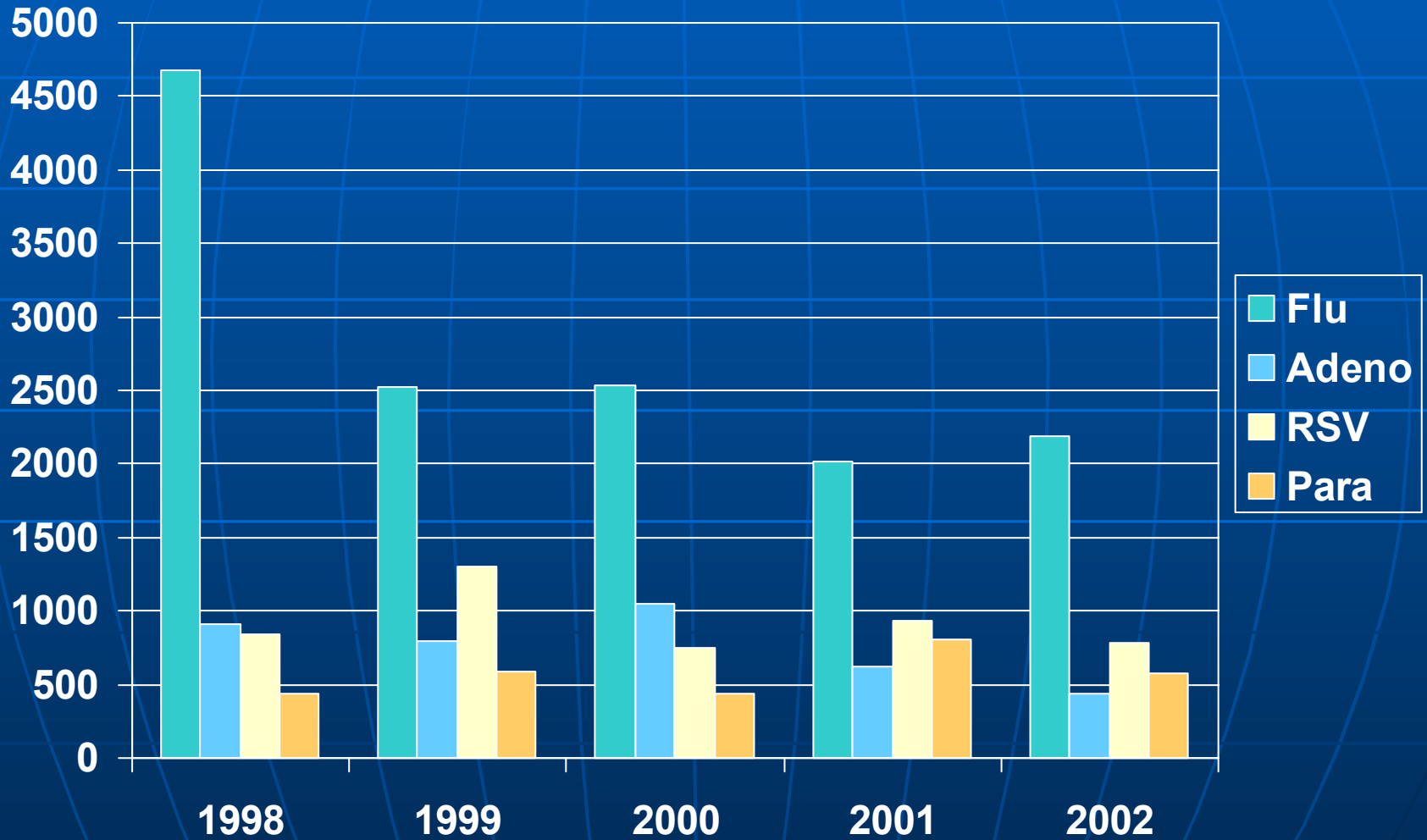
¹ < 40

² hyperimmune sheep serum

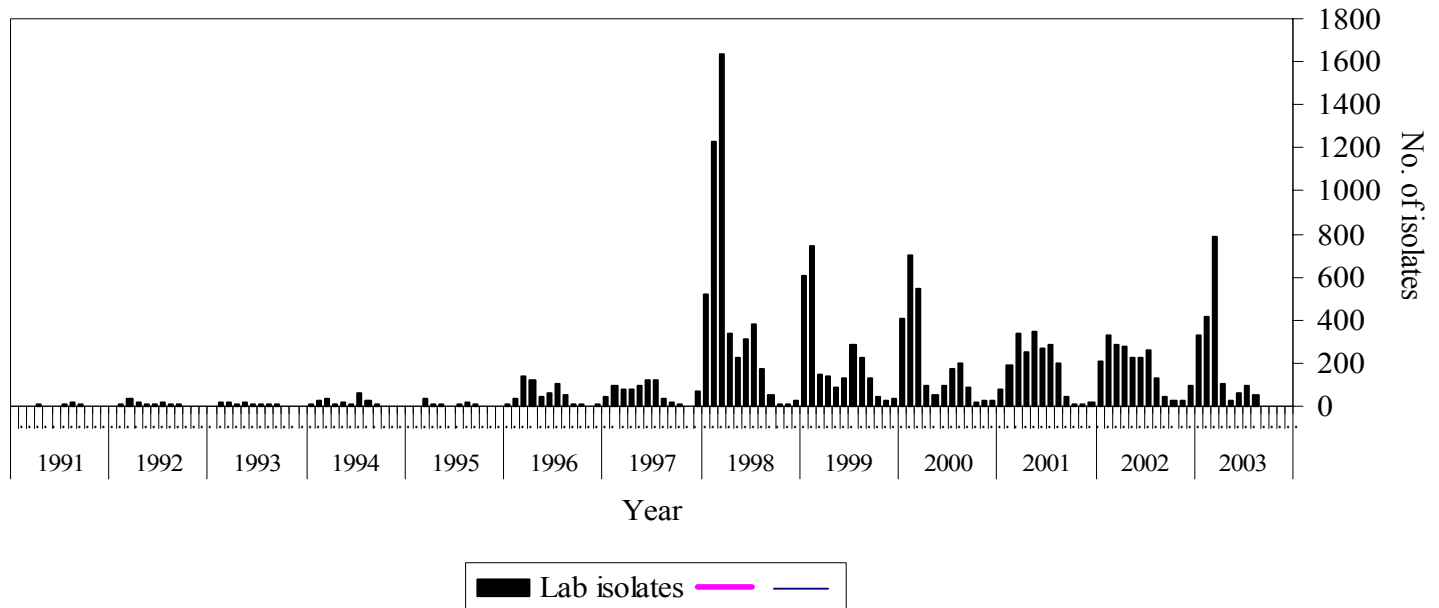
Specimens by Age Groups (1998-2002)

<u>Age Group positive</u>	<u>Specimens number</u>	<u>No. (%)</u>
0 - 4	53944	19259 (35.7)
5 - 14	13108	5118 (39.0)
15 - 24	2516	820 (32.6)
25 - 29	7115	1908 (26.8)
≥60	4184	1092 (26.1)

Respiratory Viruses by Year (1998-2002)



Lab influenza virus isolates 1991 - 2003 August



Recent Infections by Animal and Avian Influenza A Viruses

- 1997 Influenza A H5N1 virus (chick)
- 1999 Influenza A H9N2 virus (quail)
- 1999 Influenza A H3N2 virus (swine)
- 2001 Influenza A H1N1 virus (swine)
- 2003 Influenza A H5N1 virus (chick)

Confirmed Cases of H5N1 Infection in 1997

- 18 cases in total
- M : F = 8 : 10
- 11 children under 14, 7 adults
- 3 from Hong Kong Island
 - 6 from Kowloon
 - 3 from New Territories East
 - 6 from New Territories West
- History of possible exposure to poultry in 7
- 6 died 2 children <14, 4 adults

Virology

- All eight DNA gene segments of each of these viruses are of avian origin
- The human and chick viruses showed a high degree of homology
- The avian influenza virus H5N1 crossed the avian-human species barrier without prior adaptation in another mammalian species

Confirmed cases of H5N1 in 2003

- Father 33 and son 9
- Returned from Fujian after staying for 10-14 days
- Daughter 8 yrs. died of pneumonia in Fujian
- Father died on 17.2.2003
- Son recovered uneventfully

Confirmed cases of H5N1

NPA H5N1 virus isolated in MDCK cells
PCR - H5 specific

- A/HK/212/03 similar to A/HK/213/03
- No human influenza gene segments present
- All the internal genes are different from A/HK/156/97
- Antigenically different from A/HK/156/97

Avian-to-Human Transmission of Influenza H9N2 Virus

- H9N2 viruses were isolated from two children with mild influenza like symptoms
- H9N2 viruses have been isolated from different species of birds and pigs
- H9N2 viruses are widespread and have been found in Germany, Iran, Pakistan, Saudi Arabia

Swine-to-Human Transmission of Influenza Virus

- H3N2 virus isolated from 10 month-old baby with mild influenza symptoms
- Antigenically distinct from circulating human virus
- Related to early human and swine H3N2 virus infection
- Closely related to swine H3N2 virus prevalent in the Europe since the 80's
- Resistant to anti-influenza drugs amantadine and rimantadine

Swine-to-Human Transmission of Influenza Virus

- H1N1 virus isolated from 3 month-old baby with mild influenza symptoms
- Antigenically distinct from circulating human virus
- Related to early swine H1N1 virus infection
- Antigenically related to swine H1N1 virus A/New Jersey/8/76
- None of the other family members were positive for the virus

Conclusion

Laboratory diagnosis important for

- Treatment
- Infection control
- Investigation and management of outbreak
- Systematic collection and analysis of data provide coherent information on trends of infection
- Facilitate rapid isolation of new strains of influenza virus
- Detailed characterization of pathogens provide information for vaccine formulation