Strengthening Laboratory Capacities: Sharing Insights and Approaches to Managing Bird Flu Outbreaks and panel discussion

Studying Avian Influenza: The Role of Serology and Virus Isolation

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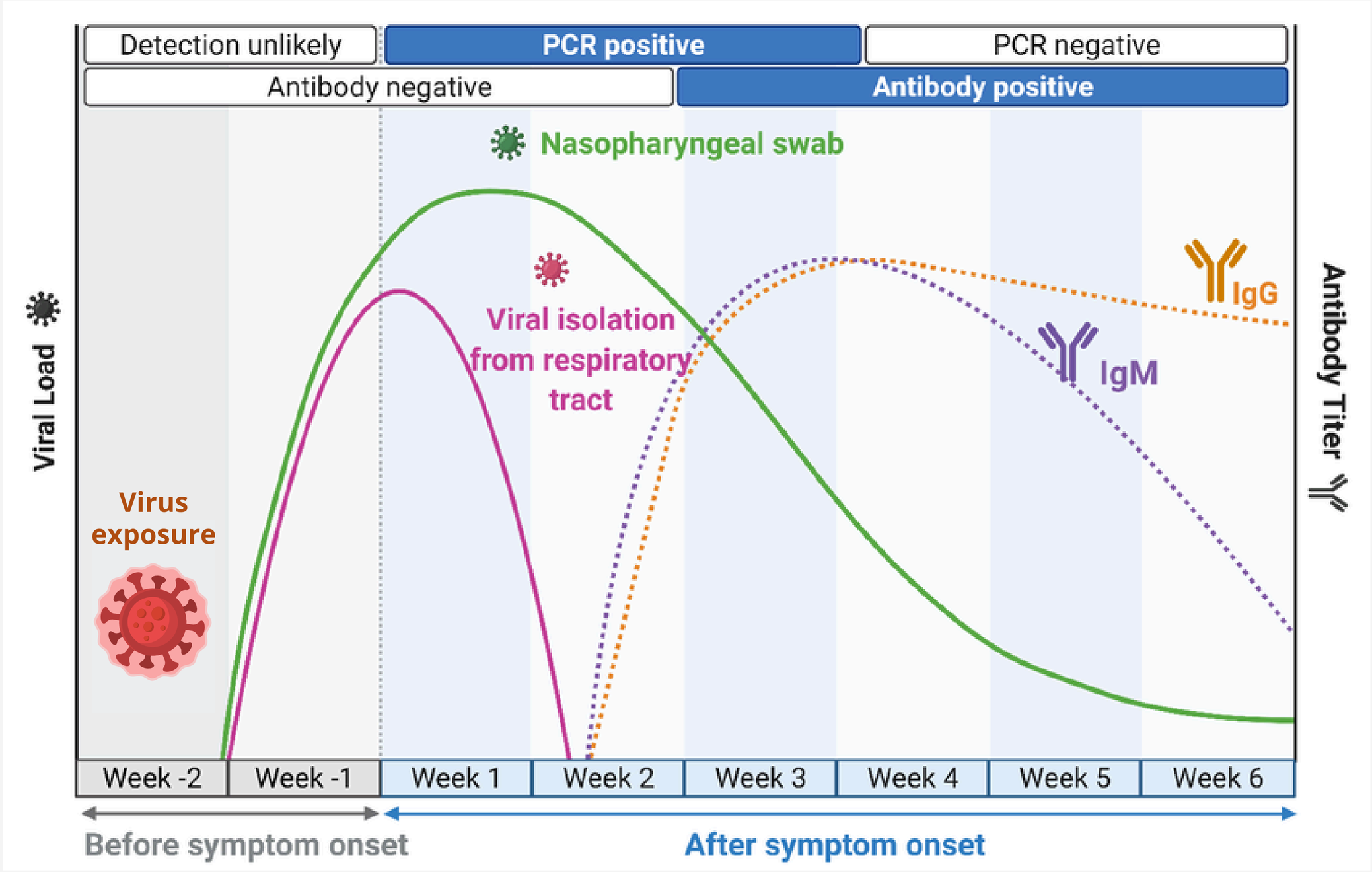
การประชุมวิชาการเชิงปฏิบัติการ
"Pandemic Preparedness: Bridging Lessons from Influenza to COVID-19 and Beyond"

สมาคมไวรัสวิทยา (ประเทศไทย)
21 กรกฎาคม 2568



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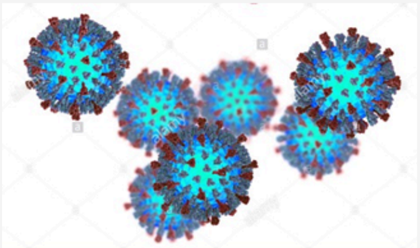
Laboratory investigations of viral diseases

Accessibility

Detection of virus/viral products

Detection of immune response



Virus isolation

Genome detection

Antigen detection

Serology IgM

Serology IgG

Confidence

Roles of Serology

1 Diagnostic

- Detect specific antibodies in sera, indicating exposure and immune response.
- Rising antibody levels between acute and convalescent serum samples (4-fold rising).
- Diagnosis in cases of suspected past infections
- Assists in diagnosing infections when virus is no longer detectable

2 Subtype identification

- Hemagglutoination inhibition test is particularly useful for identifying the H subtype of an unknown AI virus isolate.

Roles of Serology

3 Epidemiology study

- Determine the prevalence of infections in a population
- Estimate attack rate or case-fatality rate, which are necessary to assess how much community transmission has occurred and its burden
- Provide public health surveillance and understanding disease dynamics

4 Complementary to Molecular Methods

- In some cases, serology can enhance the diagnostic yield of other methods like RT-PCR, especially for respiratory viruses where shedding can be intermittent.

5 Vaccine evaluation

- Indicate vaccine effectiveness
- Evaluate herd immunity

Limitation of serology

Window period

- Antibodies take time to appear after infection.

Cross-reactivity

- May react with antibodies to related viruses.

Cannot Differentiate Natural Infection vs. Vaccination

- Individuals who are vaccinated may develop antibodies similar to those from natural infection.

Delayed Antibody Response

- In some individuals (e.g., immunocompromised or elderly), the antibody response is weak or delayed.

Limitation of serology

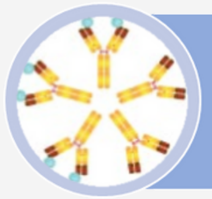
Need for Paired Sera

- To confirm recent infection, you typically need paired acute and convalescent serum samples (2–4 weeks apart)

Test Variability

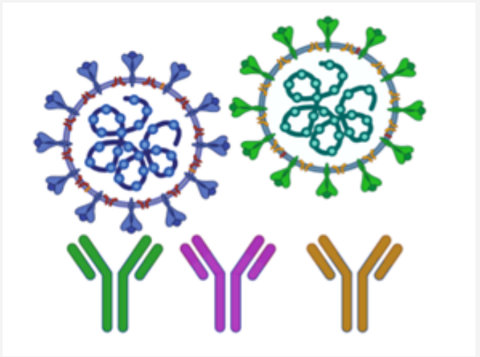
- Differences in serological tests (e.g., HI, MN, ELISA) can cause inconsistent results.

Serology tests



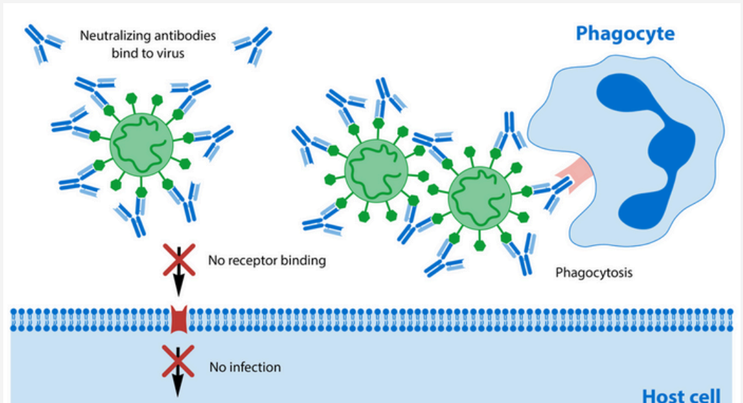
Binding antibody

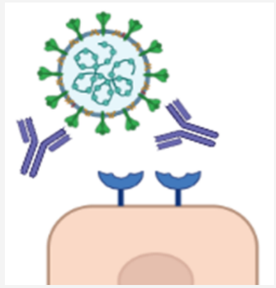
- Bind specifically to viral antigens but may not inhibit the viral infectivity.
- Not all binding antibodies are neutralizing
- Positive result may not be related to protection.



Neutralizing antibody

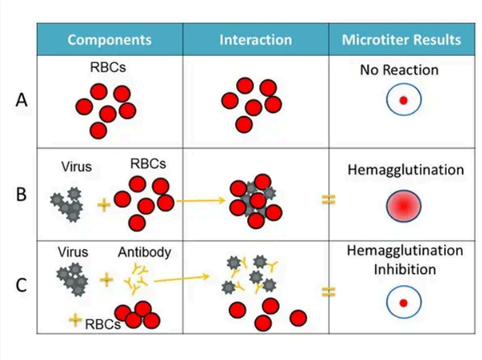
- Bind and neutralize the viral infectivity. Protective antibodies prevent the cells from viral infection.
- Neutralization in vitro is usually related to protection in vivo.



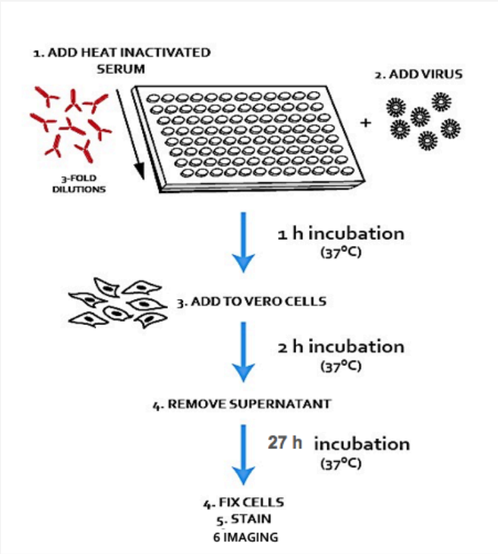


Serology tests

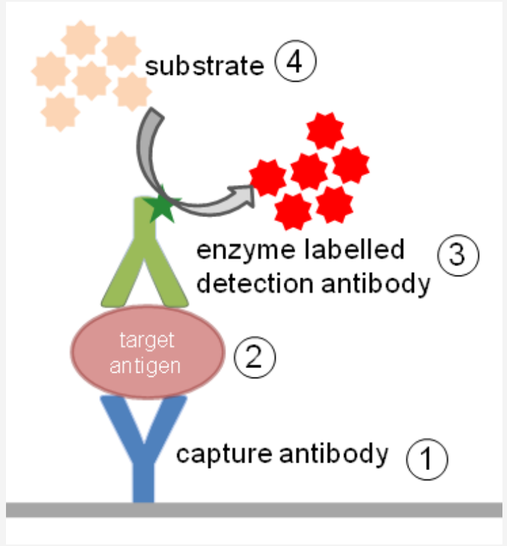
Hemagglutination inhibition assay



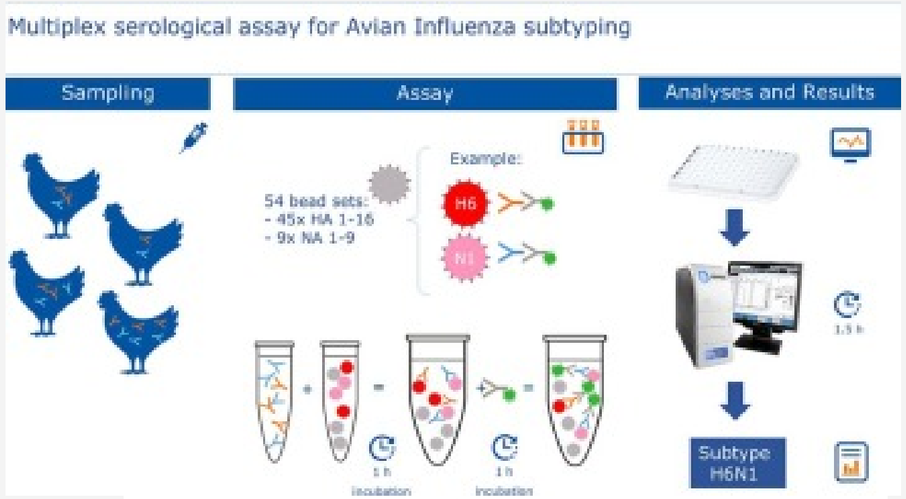
Microneutralization assay



ELISA



Multiplex serological assay



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The following testing was conducted in multiple replicates:

- Microneutralization (MN) assay: The main serology test is called a microneutralization (MN) test. This is a highly sensitive and specific test for identifying influenza virus-specific antibodies in blood sera. The MN assay was performed to measure for neutralizing antibodies against the RG H5 viruses.
- Hemagglutinin inhibition (HI) assay: The HI assay tests for antibodies to the hemagglutinin of the specific influenza virus using red blood cells (RBC). Multiple types of RBCs were assessed in the HI assays to detect antibodies in the sera.
- The Multiplex Antibody Detection Assay (MIADA): a test which looks for antibody binding to 28 different influenza antigen targets (28-plex), it also measures the total immunoglobulin (Ig), IgG, IgM and IgA antibody responses.
- Seasonal Influenza Serum Absorption: an additional step that removes antibodies against seasonal influenza viruses that might be cross-reactive prior to testing against A(H5N1) viruses was also performed. This step helps to rule out the possibility of cross-reactivity from prior exposure to seasonal influenza viruses (through infection or vaccination) which might generate a false positive H5N1 serology result.

Historical World Health Organization serological case definition using single serum

Single convalescent serum, collected at ≥ 21 days after symptom onset (or last exposure to infected animals/persons), with a **microneutralization antibody titer ≥ 1:40** to an A(H5) virus,

AND either

- **1)** a positive result using a different serological assay (e.g., **hemagglutination inhibition (HI) antibody titer≥40**) or
- **2)** an H5-specific positive result from another immunological assay such as ELISA or **multiplex binding antibody assay.**

Virus isolation

1 "Gold Standard" for Diagnosis

- Virus isolation involves culturing the virus from patient samples in cell lines or other systems
- A definitive proof of viral presence and infectivity

2 Disease Pathogenesis and Surveillance

- Provide crucial information about the virus's characteristics, pathogenicity, and useful for epidemiological investigations

Virus isolation

3 Genetic Characterization

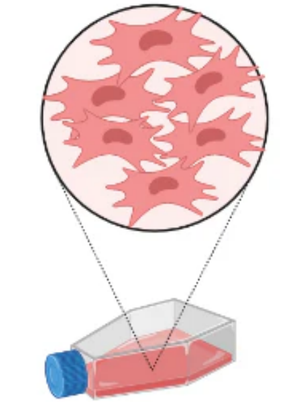
- Isolated viruses can be genetically characterized, allowing for phylogenetic analysis to track the evolution and spread of different strains and lineages.

4 Essential for Drug and Vaccine Efficacy Studies

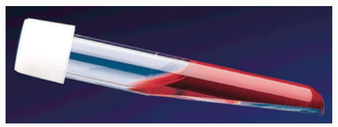
- Provide developing treatments or vaccines
- Live virus is essential to test antiviral activiry, a definitive proof of viral presence and infectivity
- Resistance mechanisms to existing drugs
- Vaccine evaluation

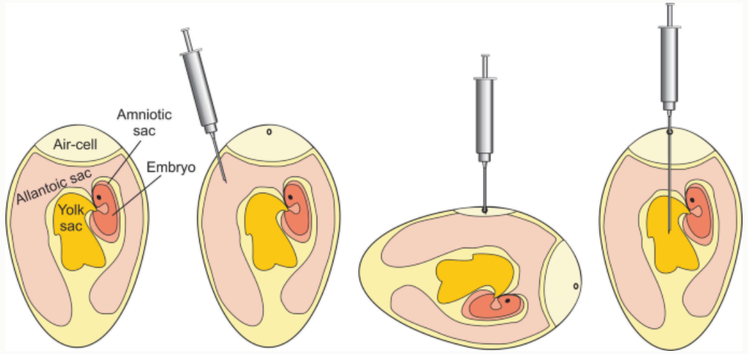
Virus isolation

Host system for avian influenza virus isolation



Adherent cell line

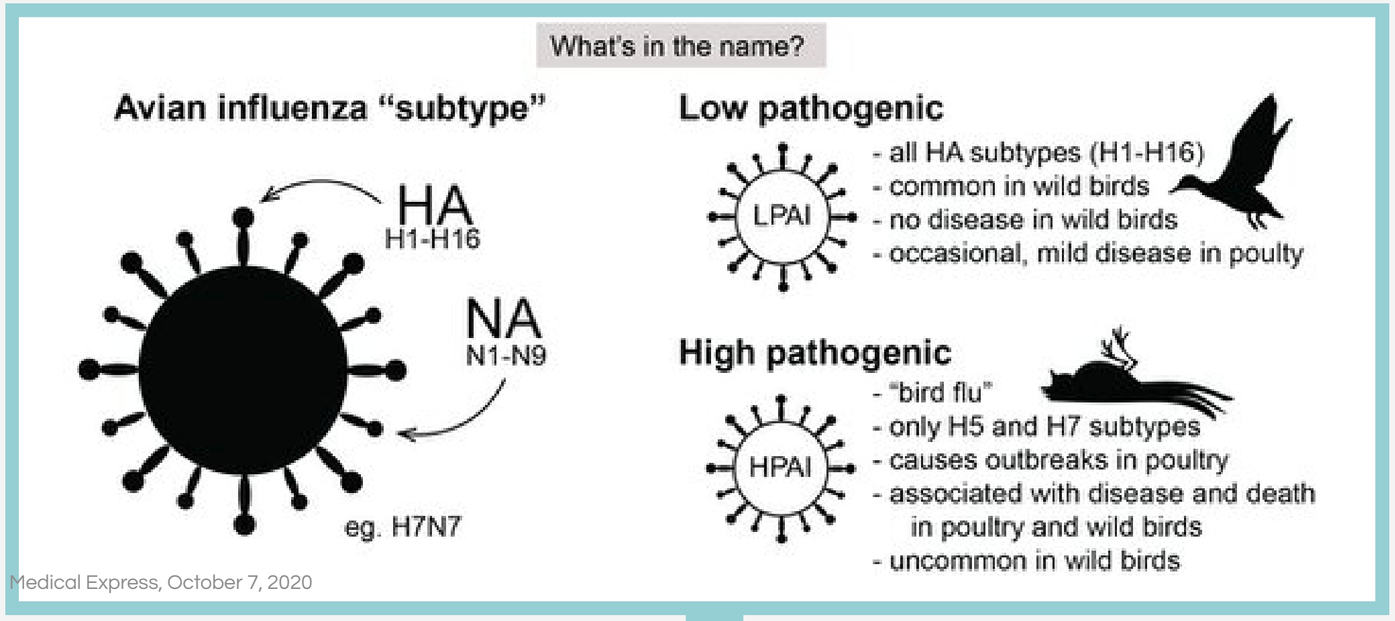




Embryonated chivken eggs

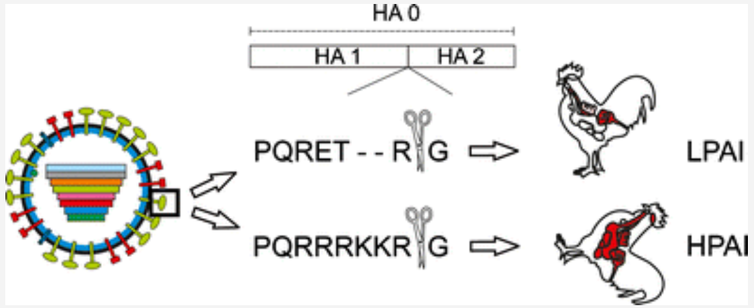
doi.org/10.1007/978-1-0716-0346-8_12

Highly or Low Pathogenic avian influenza virus ?



Medical Express, October 7, 2020

- Biosafety Level ?
- Virus isolation in cell culture: Add TPCK trypsin into culture medium ?



Virus recognition by Hemagglutination assay

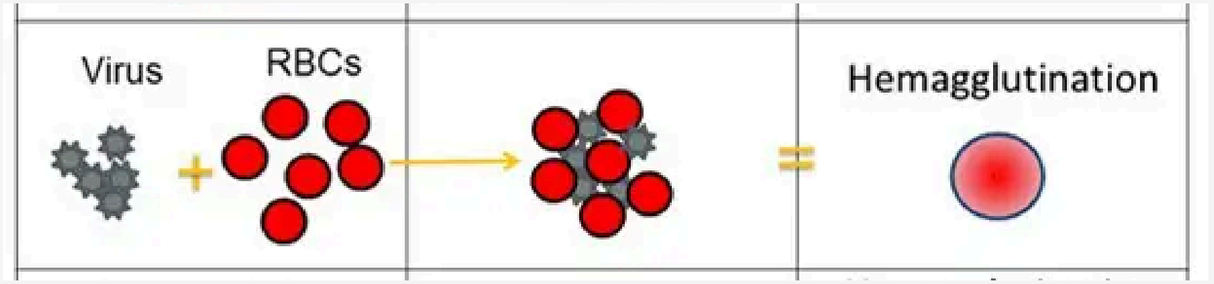


TABLE 1. HA titers of influenza A viruses as assayed with erythrocytes from different species

Influenza A virus and HA GMT	Passage history	Accession no.	HA titer by erythrocyte source[a]				
			Goose	Chicken	Guinea pig	Human	Horse
H1N1 human influenza virus							
A/New Caledonia/20/99-like virus (Siriraj 07/00)	MDCK7	EF568930	128	64	128	64	<2
H3N2 human influenza viruses							
A/Sydney/05/97-like virus (Siriraj 08/98)	MDCK4	EF568929	64	64	128	64	<2
A/Fujian/411/02-like virus (Siriraj 03/04)	MDCK6	EF568924	32	<2	64	64	<2
A/Fujian/411/02-like virus (Siriraj 01/03)	MDCK3	EF568925	32	<2	32	32	<2
A/Fujian/411/02-like virus (Siriraj 02/03)	MDCK2	EF568926	4	<2	32	32	<2
A/California/07/04-like virus (Siriraj 12/04)	MDCK4	EF568927	4	<2	16	32	<2
H5N1 influenza viruses							
A/Thailand/1(KAN-1)/04	LLC-MK2, MDCK8	AY555150	512	512	128	128	128
A/Thailand/2(SP-33)/04	MDCK6	AY555153	64	64	64	32	16
A/Thailand/3(SP-83)/04	MDCK5	AY577314	64	64	16	32	16
A/Thailand/5(KK-494)/04	MDCK4	AY627885	128	64	32	64	32
A/Thailand/676(NYK)/05	MDCK9	DQ360835	256	256	512	256	32
A/Great Barbet/Thailand/VSMU-2-CBI/2005	MDCK2	EF206697	128	128	64	64	32
A/Green Peafowl/Thailand/VSMU-3-CBI/2005	MDCK2	EF206700	256	128	64	64	64
A/Gray-Crowed Crane/Thailand/VSMU-4-CBI/2005	MDCK2	EF206696	512	256	128	128	128
A/Tree Sparrow/Thailand/VSMU-16-RBR/2005	MDCK4	EF178506	64	64	32	32	16
A/Golden Pheasant/Thailand/VSMU-21-SPB/2005	MDCK1	EF178517	128	64	32	16	32
A/Pigeon/Thailand/VSMU-25-BKK/2005	MDCK3	EF206698	64	64	32	16	16
A/Chicken/Thailand (Suphanburi)/137/05	MDCK4	EF568922	512	512	64	64	128
A/Tiger/Thailand/VSMU-11-SPB/2004	Egg 1	EF178531	512	512	256	256	256
A/Clouded Leopard/Thailand (Chonburi)/AI-1216A/2004	Egg 1	EF568923	512	512	256	256	<2
HA GMT of H5N1 viruses			190.21	156.03	74.25	67.25	33.62

Table 1
Hemagglutinating activity of replicating LPAIV.

Viruses	HA titers as determined by erythrocytes from				
	Goose	Guinea pig	Human	Chicken	Horse
A/aquatic bird/Hong Kong/DI25/2002 (H1N1)	256	256	128	128	64
A/wild duck/Shan Tou/992/2000 (H2N8)	256	256	128	128	64
A/duck/Shan Tou/1283/2001 (H3N8)	256	256	128	64	128
A/duck/Shan Tou/461/2000 (H4N9)	512	512	256	256	128
A/duck/Jiangxi/6151/03 (H5N3)	256	256	128	256	64
A/heron/Hong Kong/LC10/2002 (H6N8)	1024	1024	256	256	128
A/ostrich/Zimbabwe/222/1996 (H7N1)	512	512	256	256	128
A/mallard/Alberta/242/2003 (H8N4)	512	512	256	256	256
A/quail/Hong Kong/G1/1997 (H9N2)	1024	512	256	1024	<2
A/chicken/Hong Kong/G9/1997 (H9N2)	512	512	256	512	<2
A/duck/Hong Kong/Y280/1997 (H9N2)	1024	1024	512	512	<2
A/duck/Shan tou/1796/2001 (H10N8)	256	256	128	128	128
A/duck/Shan tou/1411/2000 (H11N2)	256	512	128	128	128
A/red-necked stint/Australia/5745/1981 (H12N9)	512	512	128	256	128
A/gull/MD/704/1977 (H13N6)	128	128	64	128	<2
A/mallard/Gurjev/263/1982 (H14N5)	1024	1024	256	512	256
A/duck/Australia/341/83(H15N8)	128	256	128	128	32
A/shore bird/DE/172/2006 (H16N3)	128	128	64	128	64

Limitation of virus isolation

Host adaptation

- When influenza viruses from human or avian are cultured in host system, amino acid mutations of the hemagglutinin may occur.
- Some AIV strains may not replicate efficiently in embryonated eggs or common cell lines like MDCK.

Time-Consuming

- Virus isolation in cells or eggs takes several days (typically 3–7 days) and several passages.

Required specialized facilities and Lab personnel

- BSL-3 for HPAI and well trained personne

Limitation of virus isolation

Sensitivity Depends on Sample Quality (Need viable virus)

- Poor sample collection or improper transport can result in failure to isolate virus.
- Virus is viable only for a limited time outside the host.

Laboratory-Generated Mutations

- Serial passages during isolation can introduce mutations, which may alter antigenicity or virulence

Biohazard Risk

- Strict containment and personal protection are necessary.

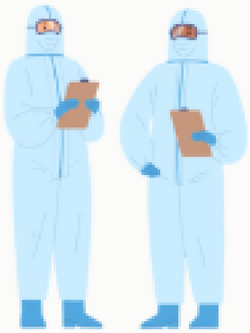
Laboratory preparedness for serology and virus isolation

Lab facilities

- BSL 2-enhanced
- BSL-3 Lab
- Equipment
- PPE
- Cell culture
- Embryonated chicken eggs
- Reagent

Protocol

- SOP
- Workflow
- Method
- Standard protocol: DMSC, CDC, WHO
- Control materials



Lab personnel

- Team
- Training
- Baseline medical condition, Baseline serum
- Vaccination and prophylaxis

My Research Story

JOURNAL OF CLINICAL MICROBIOLOGY, July 2007, p. 2284–2286 Vol. 45, No. 7
0095-1137/07/$08.00+0 doi:10.1128/JCM.00921-07

Erythrocyte Binding Preference of Avian Influenza H5N1 Viruses▽†

Suda Louisirirotchanakul,[1] Hatairat Lerdsamran,[1] Witthawat Wiriyarat,[2] Kantima Sangsiriwut,[1] Kridsda Chaichoune,[2] Phisanu Pooruk,[1] Taweesak Songserm,[3] Rungrueng Kitphati,[4] Pathom Sawanpanyalert,[4] Chulaluk Komoltri,[5] Prasert Auewarakul,[1] and Pilaipan Puthavathana[1]*

JOURNAL OF VIROLOGY, Sept. 2007, p. 9950–9955 Vol. 81, No. 18
0022-538X/07/$08.00+0 doi:10.1128/JVI.00468-07

An Avian Influenza H5N1 Virus That Binds to a Human-Type Receptor▽

Prasert Auewarakul,[1]* Ornpreya Suptawiwat,[1] Alita Kongchanagul,[1] Chak Sangma,[6] Yasuo Suzuki,[7] Kumnuan Ungchusak,[4] Suda Louisirirotchanakul,[1] Hatairat Lerdsamran,[1] Phisanu Pooruk,[1] Arunee Thitithanyanont,[2] Chakrarat Pittayawonganon,[4] Chao-Tan Guo,[7] Hiroaki Hiramatsu,[7] Wipawee Jampangern,[3] Supamit Chunsutthiwat,[5] and Pilaipan Puthavathana[1]

CLINICAL AND VACCINE IMMUNOLOGY, July 2009, p. 978–981 Vol. 16, No. 7
1556-6811/09/$08.00+0 doi:10.1128/CVI.00062-09

Kinetics and Longevity of Antibody Response to Influenza A H5N1 Virus Infection in Humans▽

Rungrueng Kitphati,[1] Phisanu Pooruk,[2] Hatairat Lerdsamran,[2] Siriwan Poosuwan,[1] Suda Louisirirotchanakul,[2] Prasert Auewarakul,[2] Kulkanya Chokphaibulkit,[3] Pirom Noisumdaeng,[2] Pathom Sawanpanyalert,[1] and Pilaipan Puthavathana[2]*

AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical and Vaccine Immunology

Kinetics, Longevity, and Cross-Reactivity of Antineuraminidase Antibody after Natural Infection with Influenza A Viruses

Don Changsom,[a] Li Jiang,[a] Hatairat Lerdsamran,[b] Sopon Iamsirithaworn,[c] Rungrueng Kitphati,[c] Phisanu Pooruk,[d] Prasert Auewarakul,[a] Pilaipan Puthavathana[a,b]

OPEN ACCESS Freely available online | PLoS one

Serological Response to the 2009 Pandemic Influenza A (H1N1) Virus for Disease Diagnosis and Estimating the Infection Rate in Thai Population

Hatairat Lerdsamran[1], Chakrarat Pittayawonganon[2], Phisanu Pooruk[1], Anek Mungaomklang[3], Sopon Iamsirithaworn[2], Prasert Thongcharoen[1], Uraiwan Kositanont[1], Prasert Auewarakul[1], Kulkanya Chokephaibulkit[4], Sineenat Oota[5], Warin Pongkankham[6], Patummal Silaporn[2], Supaloek Komolsiri[1], Pirom Noisumdaeng[1], Tawee Chotpitayasunondh[7], Chariya Sangsajja[8], Witthawat Wiriyarat[9], Suda Louisirirotchanakul[1], Pilaipan Puthavathana[1]*

Veterinary Microbiology 146 (2010) 346–349
Contents lists available at ScienceDirect
Veterinary Microbiology
journal homepage: www.elsevier.com/locate/vetmic

Short communication

Erythrocyte binding preference of 16 subtypes of low pathogenic avian influenza and 2009 pandemic influenza A (H1N1) viruses

Witthawat Wiriyarat[a], Hatairat Lerdsamran[b], Phisanu Pooruk[b], Robert G. Webster[c], Suda Louisirirotchanakul[b], Parntep Ratanakorn[a], Kridsada Chaichoune[a], Kannika Nateerom[b], Pilaipan Puthavathana[b,*]

DOI 10.1186/s13756-016-0120-9
Antimicrobial Resistance and Infection Control

SHORT REPORT Open Access

Lack of transmission among healthcare workers in contact with a case of Middle East respiratory syndrome coronavirus infection in Thailand

Surasak Wiboonchutikul[1*], Weerawat Manosuthi[1], Sirirat Likanonsakul[1], Chariya Sangsajja[1], Paweena Kongsanan[1], Ravee Nitiyanontakij[1], Varaporn Thientong[1], Hatairat Lerdsamran[2] and Pilaipan Puthavathana[2,3]

APJAI Asian Pacific Journal of Allergy and Immunology

ORIGINAL ARTICLE

Cross-reactive antibodies against H7N9 and H5N1 avian influenza viruses in Thai population

Li Jiang,[1] Don Changsom,[1] Hatairat Lerdsamran,[1] Wanibtisam Masamae,[1] Anan Jongkaewwattana,[1] Sopon Iamsirithaworn,[2] Sineenat Oota,[3] Suda Louisirirotchanakul,[1] Prasert Auewarakul,[1,4] Pilaipan Puthavathana[1,4,5]

OPEN ACCESS Freely available online | PLoS one

A Novel Pathogenic Mechanism of Highly Pathogenic Avian Influenza H5N1 Viruses Involves Hemagglutinin Mediated Resistance to Serum Innate Inhibitors

Jutatip Panaampon[1,2], Nathamon Ngaosuwankul[2,3], Ornpreya Suptawiwat[1,2], Pirom Noisumdaeng[1,2], Kantima Sangsiriwut[4], Bunpote Siridechadilok[5], Hatairat Lerdsamran[1,2], Prasert Auewarakul[1,2], Phisanu Pooruk[1], Pilaipan Puthavathana[1,2]*

JCVI JournalsASM.org

International Laboratory Comparison of Influenza Microneutralization Assays for A(H1N1)pdm09, A(H3N2), and A(H5N1) Influenza Viruses by CONSISE

Karen L. Laurie,[a] Othmar G. Engelhardt,[b] John Wood,[c] Alan Heath,[c] Jacqueline M. Katz,[d] Malik Peiris,[e] Katja Hoschler,[f] Olav Hungnes,[g] Wenqing Zhang,[h] Maria D. Van Kerkhove,[i] the CONSISE Laboratory Working Group participants

Comparative Study

Int J STD AIDS. 2016 Aug;27(9):761-8. doi: 10.1177/0956462415594061. Epub 2015 Jul 2.

Long-term seroprotective response of trivalent seasonal influenza vaccine in HIV-infected children, regardless of immunogenicity before immunisation

Visal Moolasart [1], Weerawat Manosuthi [2], Jarurnsook Ausavapipit [2], Suthat Chottanapund [2], Sirirat Likanonsakul [2], Sumonmal Uttayamakul [2], Somkid Srisopha [2], Hatairat Lerdsamran [3], Pilaipan Puthavathana [3]

Safety and immunogenicity of a live attenuated influenza H5 candidate vaccine strain A/17/turkey/Turkey/05/133 H5N2 and its priming effects for potential pre-pandemic use: a randomised, double-blind, placebo-controlled trial

Punnee Pitisuttithum, Kobporn Boonnak, Supat Chamnanchanunt, Pilaipan Puthavathana, Viravarn Luvira, Hatairat Lerdsamran, Jaranit Kaewkungwal, Saranath Lawpoolsri, Vipa Thanachartwet, Udomsak Silachamroon, Wanibtisam Masamae, Alexandra Schuetz, Ponthip Wirachwong, Sit Thirapakpoomanunt, Larisa Rudenko, Erin Sparrow, Martin Friede, Marie-Paule Kieny

PLOS ONE

RESEARCH ARTICLE

Seroprevalence of anti-SARS-CoV-2 antibodies in Thai adults during the first three epidemic waves

Hatairat Lerdsamran[1], Anek Mungaomklang[2], Sopon Iamsirithaworn[3], Jarunee Prasertsopon[1], Witthawat Wiriyarat[4], Suthee Saritsiri[5], Ratikorn Anusorntanawat[6], Nirada Siriyakorn[7], Poj Intalapaporn[7], Somrak Sirikhetkon[2], Kantima Sangsiriwut[8], Worawat Dangsakul[9], Suteema Sawadpongpan[1], Nattakan Thinpan[1], Kuntida Kitidee[1], Pilailuk Okada[9], Ranida Techasuwanna[3], Noparat Mongkalangoon[3], Kriengkrai Prasert[10], Pilaipan Puthavathana[1]*