Laboratory Diagnosis of Highly Pathogenic Avian Influenza

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Topic Outline

- Work of RITM in Influenza
- Laboratory aspects of testing for Avian Influenza
- Laboratory assays currently available for testing of human samples

Disclaimer

- No conflicts of interest.
- Mention of specific assays and certain products are meant for educational/ information purposes only.

RITM as National Reference Laboratory for DOH

Department Order 393-E s. 2000
(November 14, 2000)
RITM as National Reference Laboratory for Infectious Diseases:

1) Dengue
2) Influenza
3) Tuberculosis and other Mycobacteria
4) Malaria and other parasites
5) Bacterial enteric diseases
6) Measles and other viral exanthems
7) Mycology
8) Enteroviruses (incl Polio)
9) Antimicrobial resistance
10) Emerging Infectious Diseases
11) NVBSP

AIM:
To improve the quality of health services and establish effective public health laboratory network in the country

- **Provide laboratory referral services (e.g. confirmatory testing, surveillance, research)**
- **Train laboratory personnel**
- **Maintain QA program for laboratory tests *(with DoH-BHFS)***
- **Evaluate test kits and reagents *(with DoH-BHDT)***

Research Institute for Tropical Medicine
Philippine National Influenza Center

2000 – Designation of RITM as the **NRL for Influenza**

2003 – Designation of RITM as the **National Influenza Center by WHO**

Inclusion in the WHO Network

2005-2009 – 1st Cooperative Grant of US-CDC for NIC

Influenza-like Illness Surveillance

2009 – Pandemic H1N1
Establishment of Subnational Laboratories for Influenza

2009-2014 – 2nd Cooperative Grant of US-CDC for NIC

Expansion of ILI Surveillance

And

Reactivation of Subnational Laboratories

2014 – Official designation as **Philippine National Influenza Center by DOH**

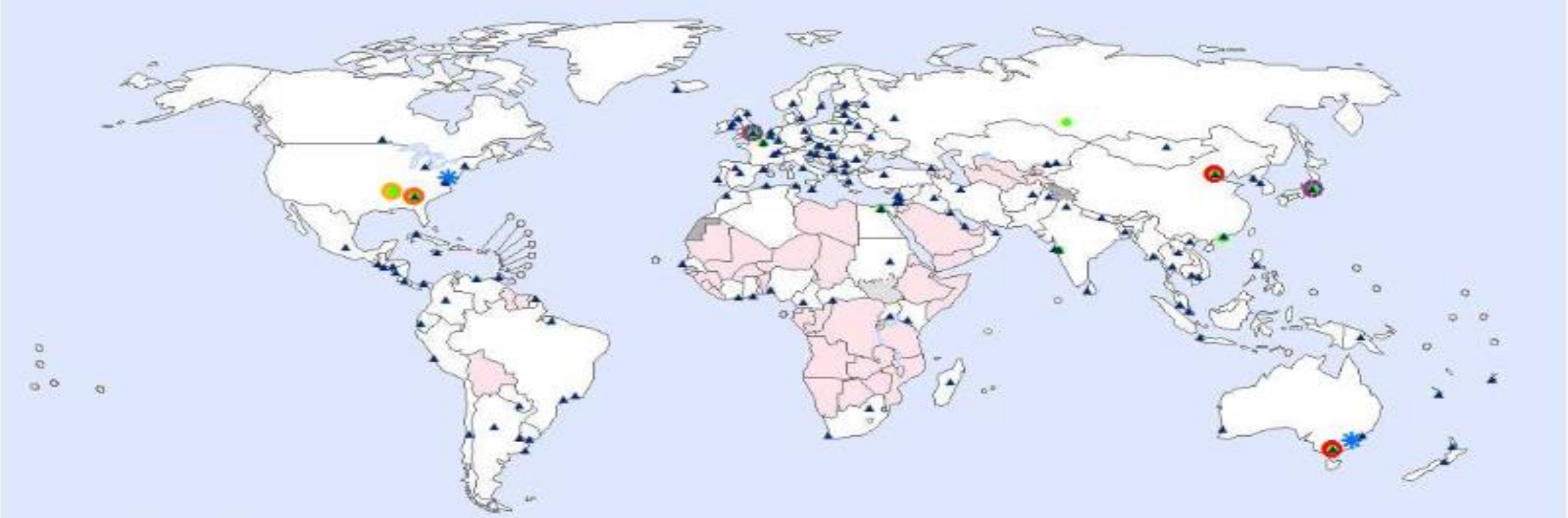
Framework for response to MERS-COV, Avian Influenza and other Novel Emerging Diseases

2015-present

- Continuation of ILI Surveillance
- Establishment of **Severe Acute Respiratory Infection (SARI) surveillance**

CAPACITY BUILDING → **SUSTAINABILITY**

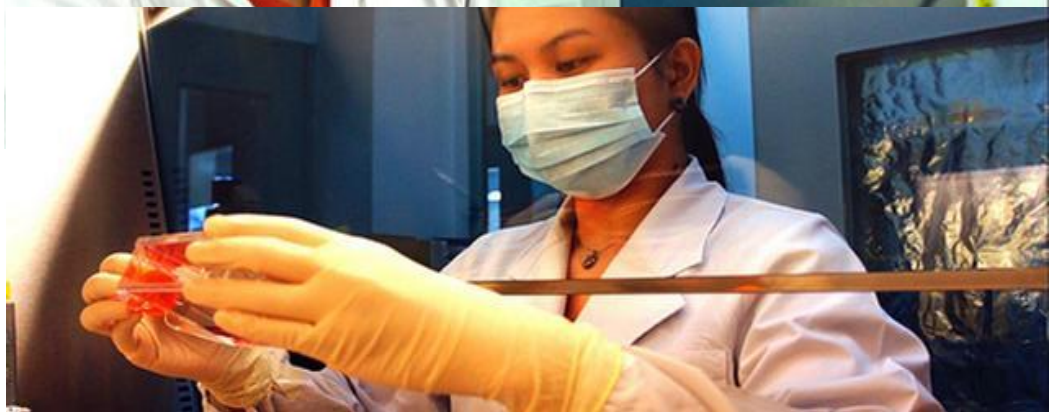
WHO GLOBAL INFLUENZA SURVEILLANCE AND RESPONSE SYSTEM (GISRS)



- **Monitors impact and evolution of influenza viruses and emergence of novel influenza viruses with pandemic potential.**
- **Provides recommendations on suitable virus strains for inclusion in vaccines**
- **Provides recommendations on diagnostic tests and antiviral drug sensitivity**

RITM
NATIONAL INFLUENZA CENTER

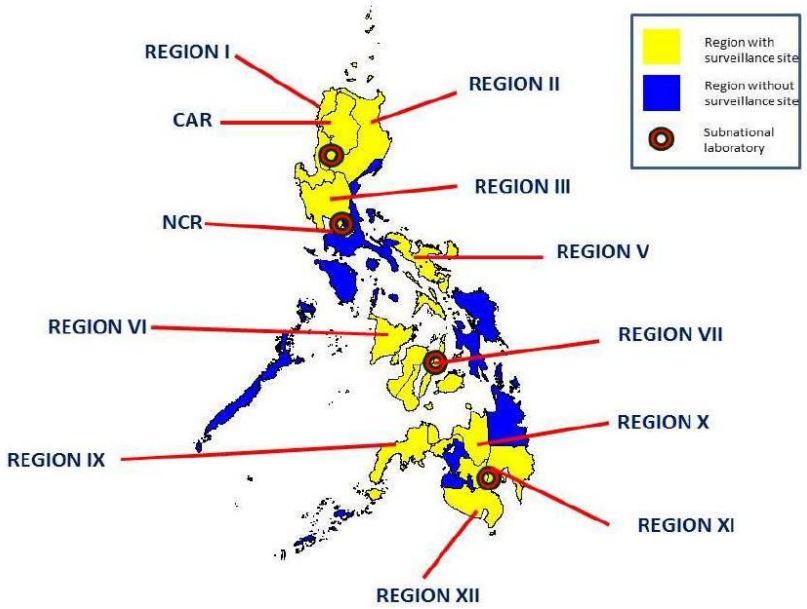
1. Describe **local virus circulation** in a timely manner and providing virus isolates for vaccine development
2. Define the local epidemiology of influenza, patterns of circulation, clinical manifestations and high risk groups **to make better recommendations for prevention and control**
3. Provide a framework and support mechanism for broader pandemic **early/rapid warning and monitoring systems**



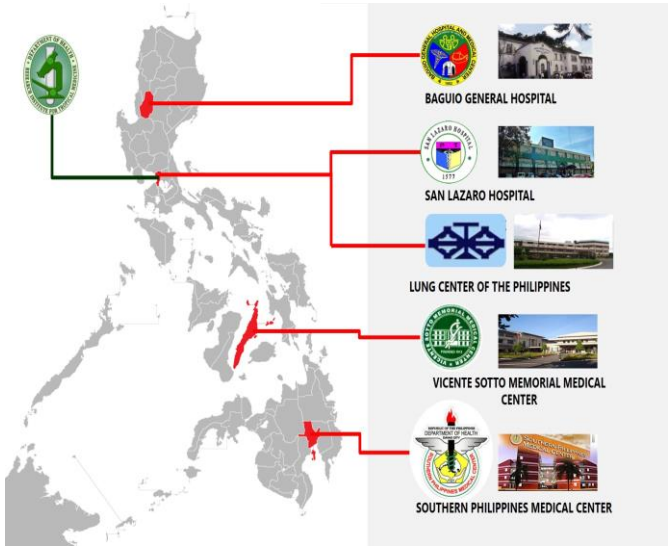
INFLUENZA VIROLOGIC SURVEILLANCE

MILD ILLNESS — SEVERE DISEASE

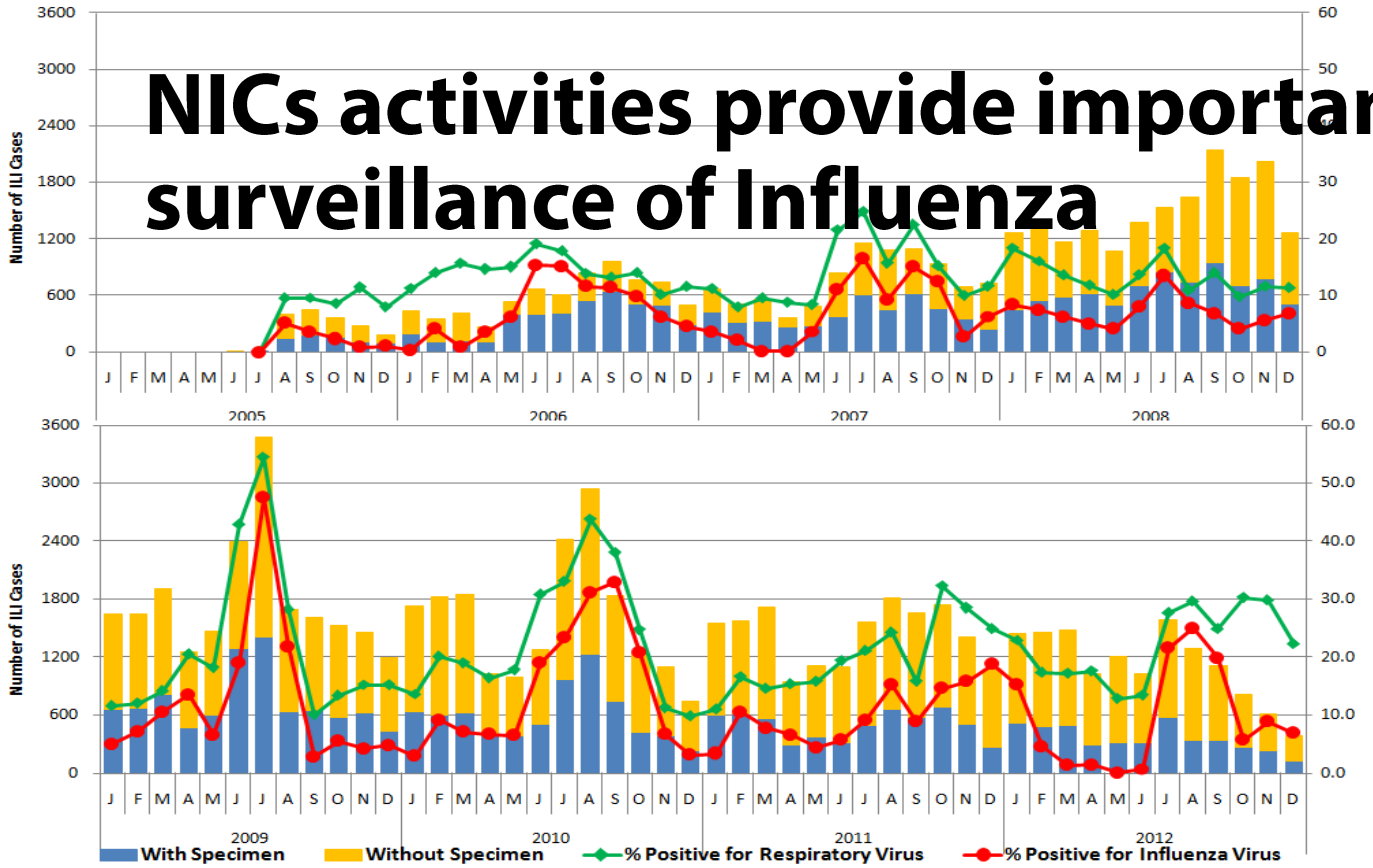
Health Center Based Sentinel surveillance for Influenza Like Illness (ILI) monitors persons seeking care in ambulatory facilities.

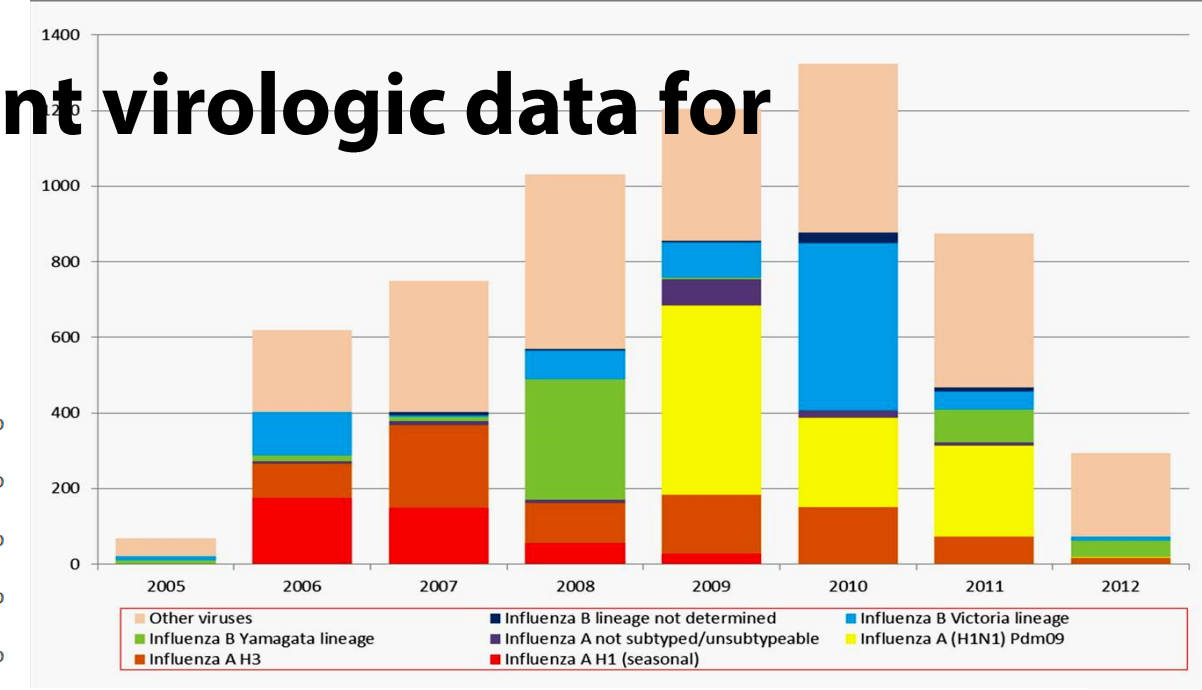


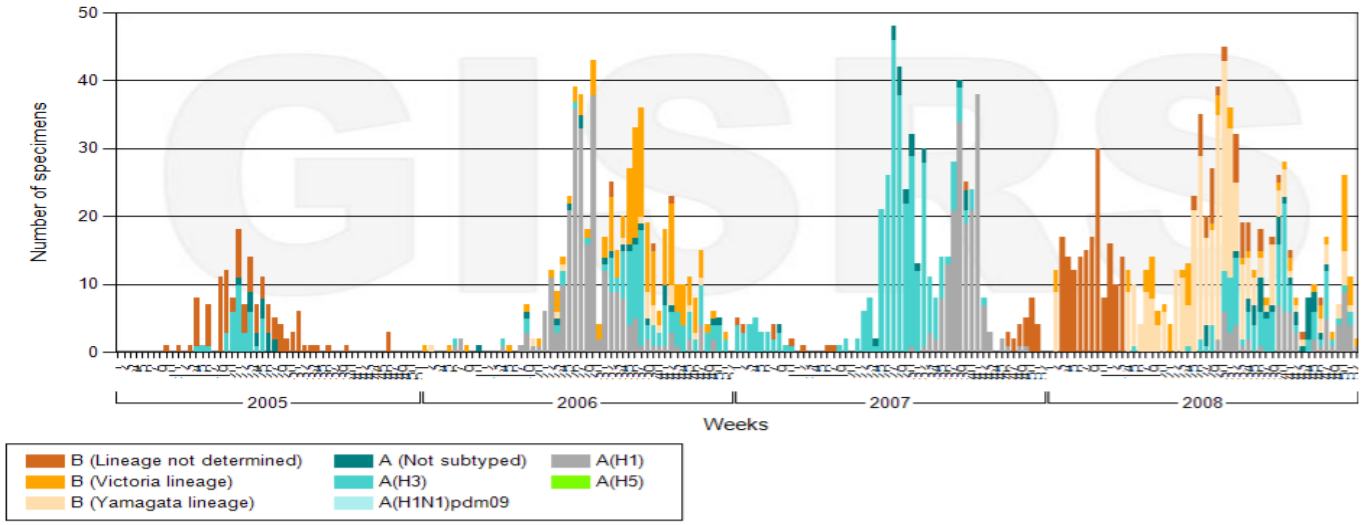
Hospital Based Sentinel surveillance for Severe Acute Respiratory Illness (SARI) monitors persons with more severe illness who have been admitted to hospitals for treatment

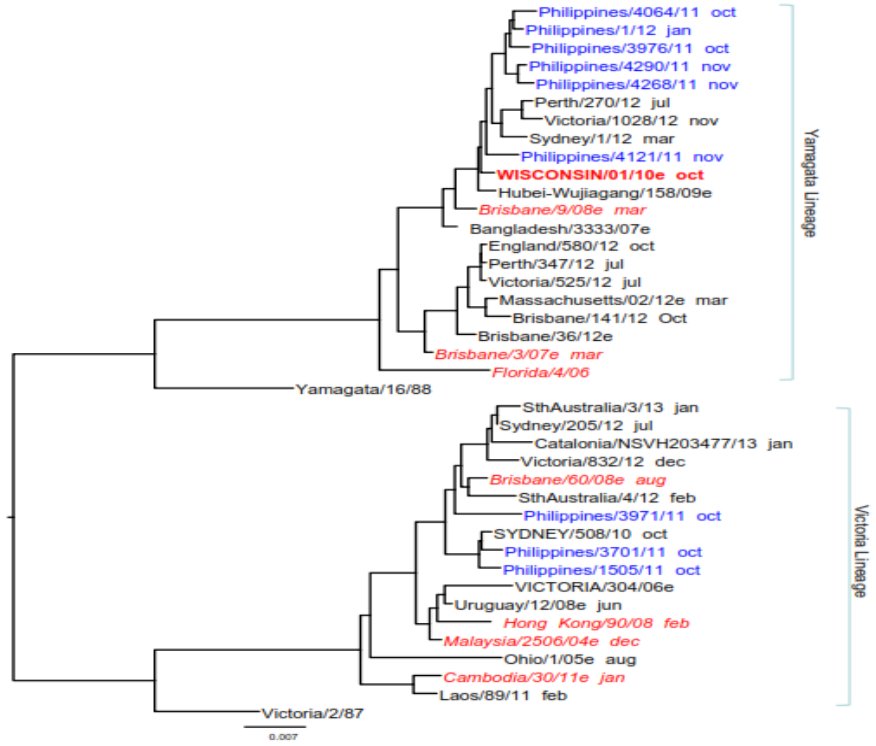


NICs activities provide important virologic data for surveillance of Influenza









These activities and data are published in BMC in 2016.

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National Influenza Surveillance in the Philippines from 2006 to 2012: seasonality and circulating strains

Marilla G. Lucero,[✉1] Marianette T. Inobaya,[1] Leilani T. Nillos,[1] Alvin G. Tan,[1] Vina Lea F. Arguelles,[1] Christine Joy C. Dureza,[1] Edelwisa S. Mercado,[1] Analisa N. Bautista,[1] Veronica L. Tallo,[1] Agnes V. Barrientos,[1] Tomas Rodriguez,[2] and Remigio M. Olveda[1]

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Abstract

Go to:

Background

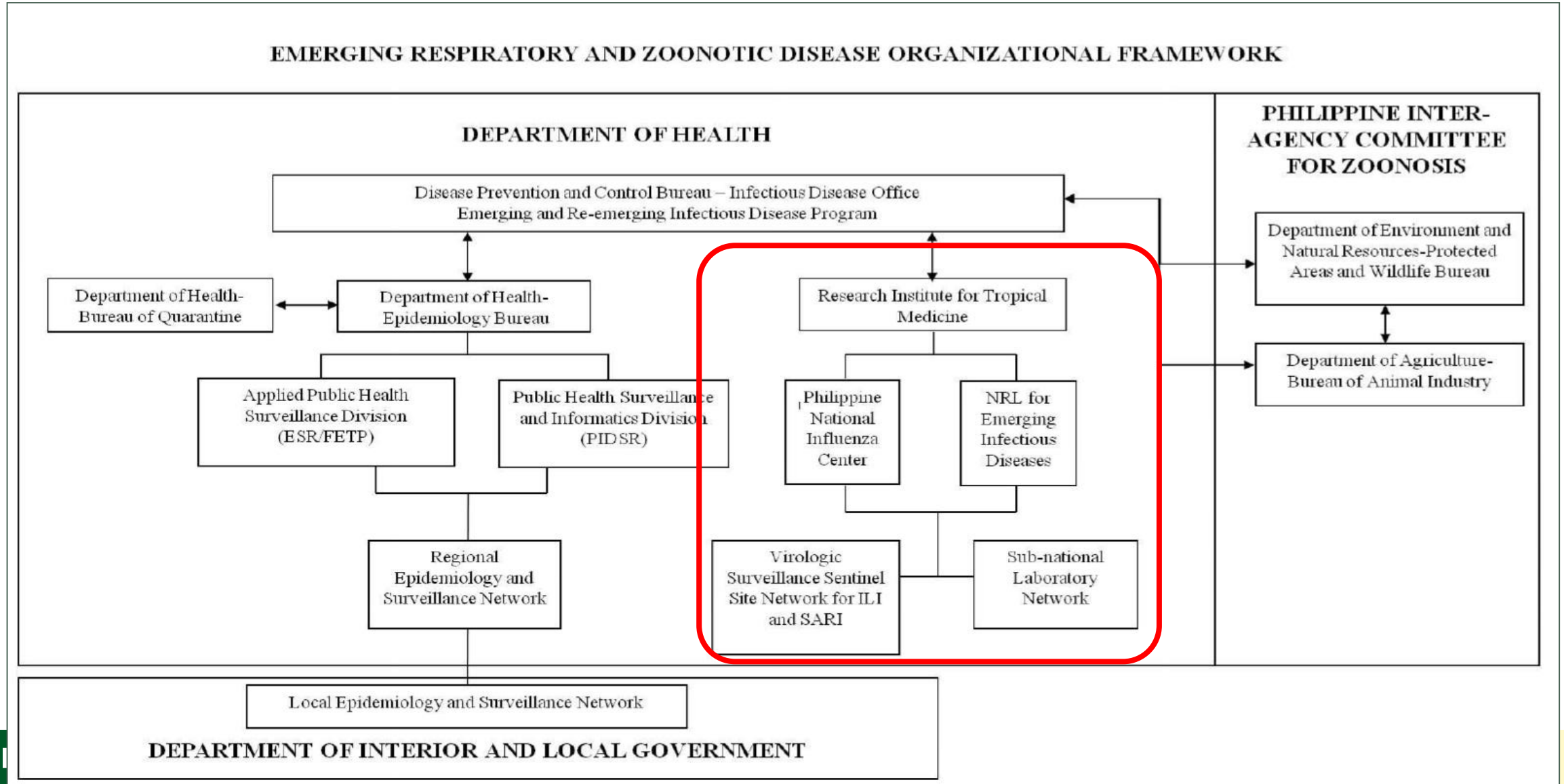
RESEARCH ARTICLE Open Access

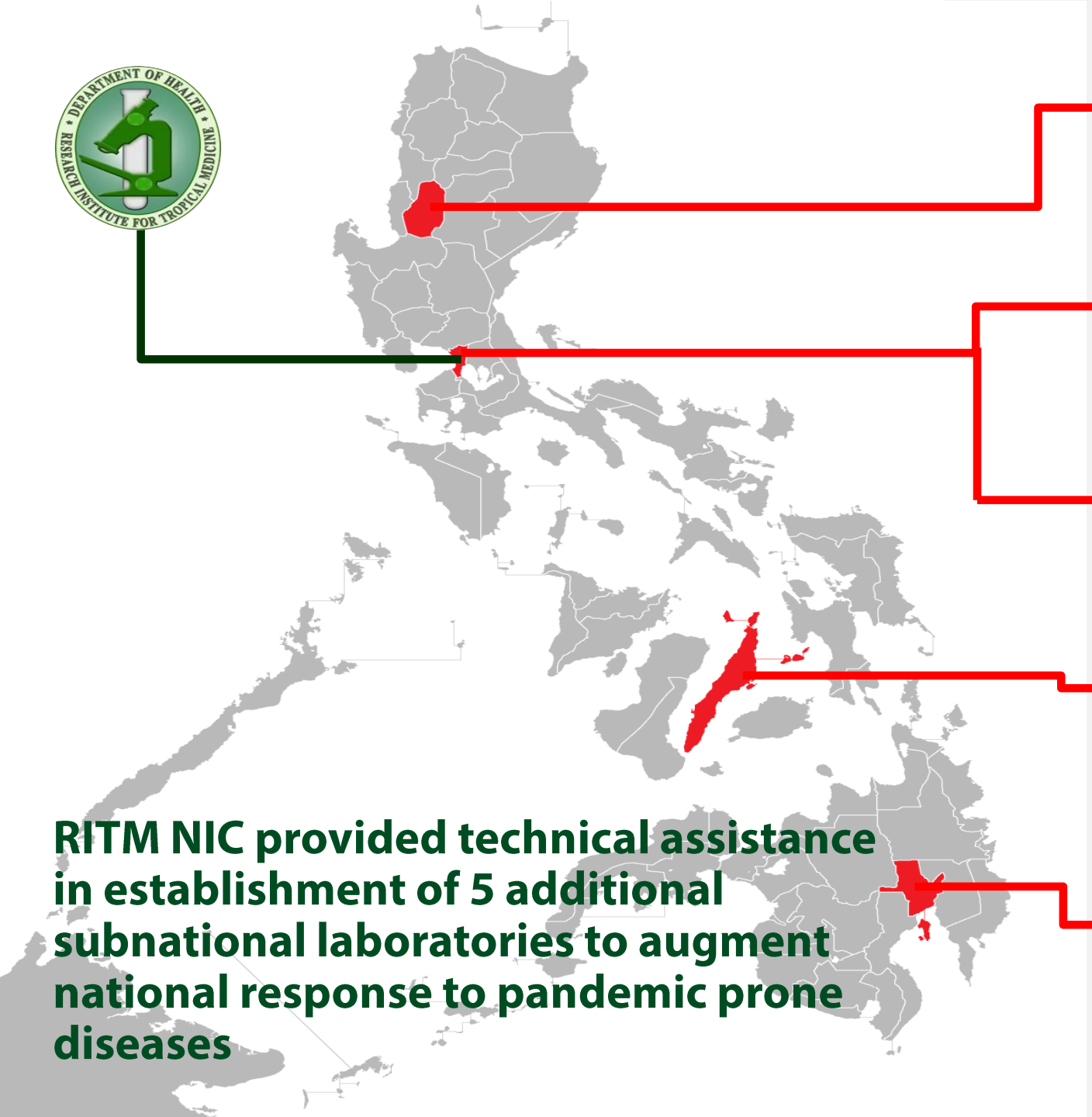
National Influenza Surveillance in the Philippines from 2006 to 2012: seasonality and circulating strains

Marilla G. Lucero[1*], Marianette T. Inobaya[1], Leilani T. Nillos[1], Alvin G. Tan[1], Vina Lea F. Arguelles[1], Christine Joy C. Dureza[1], Edelwisa S. Mercado[1], Analisa N. Bautista[1], Veronica L. Tallo[1], Agnes V. Barrientos[1], Tomas Rodriguez[2] and Remigio M. Olveda[1]

- Influenza seasonality in the Philippines is from **June to November**.
- **AH1N1, AH3N2, and 2 types of Influenza B viruses** circulate in varying proportions every year.
- The ideal time to administer **Southern Hemisphere Influenza Vaccine** should be from **April to May**.
- With 2 lineages of Influenza B circulating annually, **quadrivalent vaccine** might have more impact on influenza control than trivalent vaccine.
- Establishment of thresholds and average epidemic curve provide a tool for policy makers to assess the intensity or severity of influenza epidemics even early in its course, to help plan (for outbreak response)
- Influenza surveillance activities should be continued in the Philippines.

RITM NIC provides a framework for prevention, control and response for Influenza through collaboration with other agencies





RITM NIC provided technical assistance in establishment of 5 additional subnational laboratories to augment national response to pandemic prone diseases

BAGUIO GENERAL HOSPITAL

SAN LAZARO HOSPITAL

LUNG CENTER OF THE PHILIPPINES

VICENTE SOTTO MEMORIAL MEDICAL CENTER

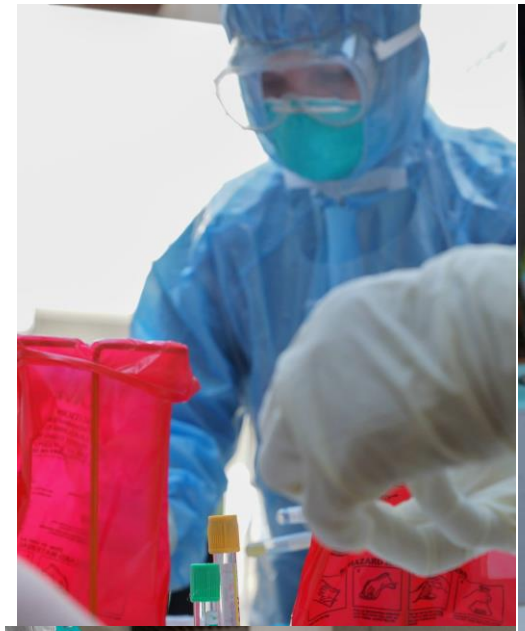
SOUTHERN PHILIPPINES MEDICAL CENTER

RITM Technical Assistance to DOH and Regions include logistics for surveillance and outbreak response

Educational materials, guidelines and technical advise to standardize and ensure safety for specimen collection

Training on Specimen Collection

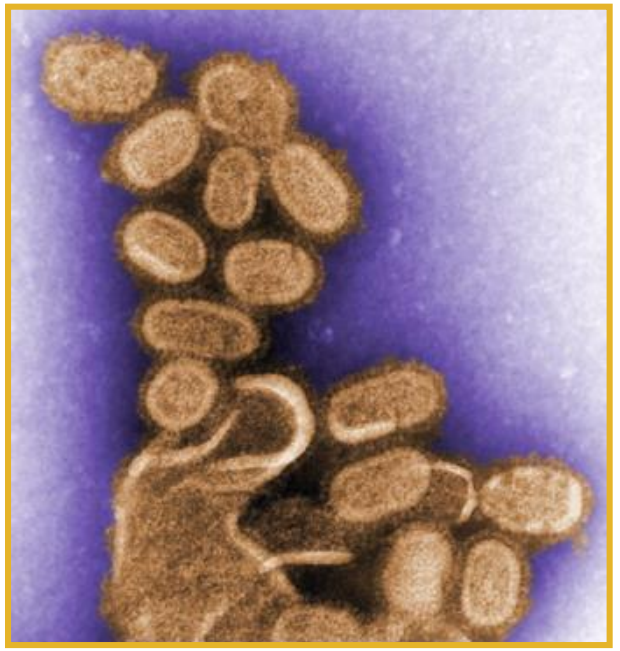
Figure 7a-c: The NIC provides trainings on respiratory specimen collection, storage and transport, to ensure that quality samples from cases in the field reaches RITM in optimal conditions fit for testing.



Training on Personal Protective Equipment for High Risk Pathogens

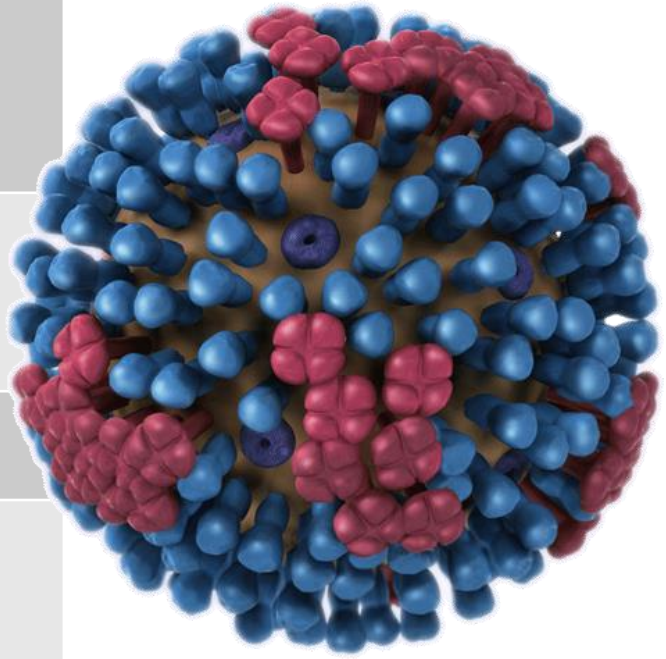
Laboratory Testing for HPAI

- **In view of the nonspecific nature of the illness, laboratory confirmation of HPAI influenza virus is essential….**
  - Severe illness and high mortality
  - Guide for treatment and clinical management
  - Disease control and prevention, public health measures
- **…. but challenging.**
  - H5N1 - Predominantly respiratory
    - Non-respiratory symptoms: diarrhea, vomiting and abdominal pain
    - May also present with CNS involvement
  - It requires a high index of suspicion and the most sensitive detection methods available
  - May require the testing of multiple specimens
- **…. and extremely risky**

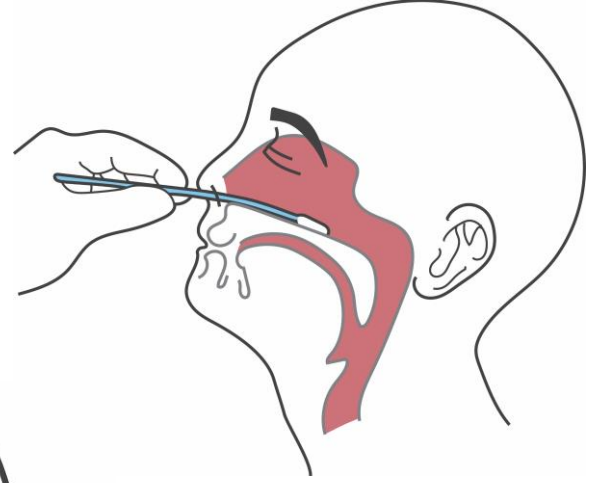


Testing for Influenza in General

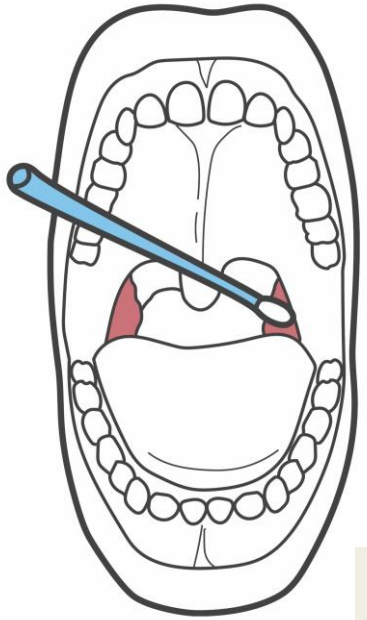
Target	Rapid	Not so Rapid
Whole virus	Electron Microscopy	Virus Culture
Antigen	Point of Care Antigen test	
Antibody		Antibody tests
Nucleic Acid	Realtime PCR	Conventional PCR
	POC Molecular	



Clinical Specimens for Virus Detection



- For screening purposes, **respiratory specimens remain the first choice.**
  - Virus has been isolated and viral RNA has been detected in respiratory specimens obtained from H5N1-infected patients for **to 16 days** after the onset of illness, indicating that virus is shed and can be detected for prolonged periods.
  - Diagnostic yield: Nasopharyngeal aspirates, Nasopharyngeal swa > throat swabs > nasal swabs
  - Upper respiratory symptoms: NPA, NPS, TS, NS
  - Lower respiratory symptoms: ETA, BAL
- H5N1 virus has also been isolated and viral RNA has been detected in feces, sera and CSF
- Transport: in ice and tested as soon as possible; for long term storage for virus detection or isolation, freeze in -80C; OR place in virus transport medium

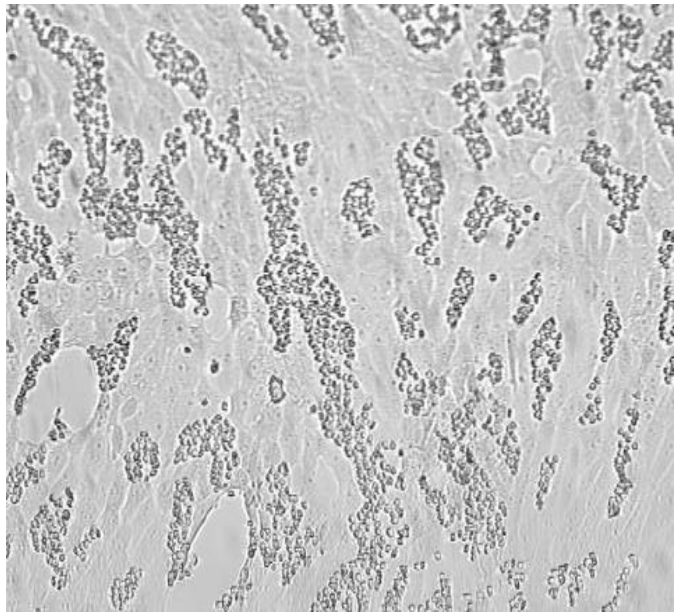


Virus Isolation

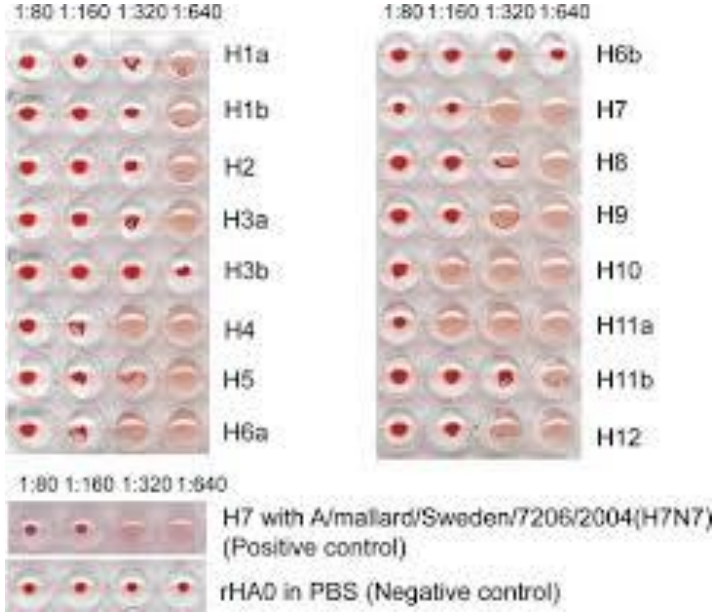
- Cell lines used for the isolation of respiratory viruses:
  - **MDCK cells** – is a diploid cell line from the Madin-Darby Canine Kidney. It is recommended for the isolation of Influenza Parainfluenza viruses.
  - **Hep 2C cells** – heteroploid cell line derived from the carcinoma of the human larynx. **(Adeno,RSV, HSV-1 Rhino, Entero)**
- Still represents the "Gold Standard" for diagnosis of Influenza **HOWEVER, HPAI shall only be manipulated in BSL3 facilities**

Virus Isolation (MDCK)

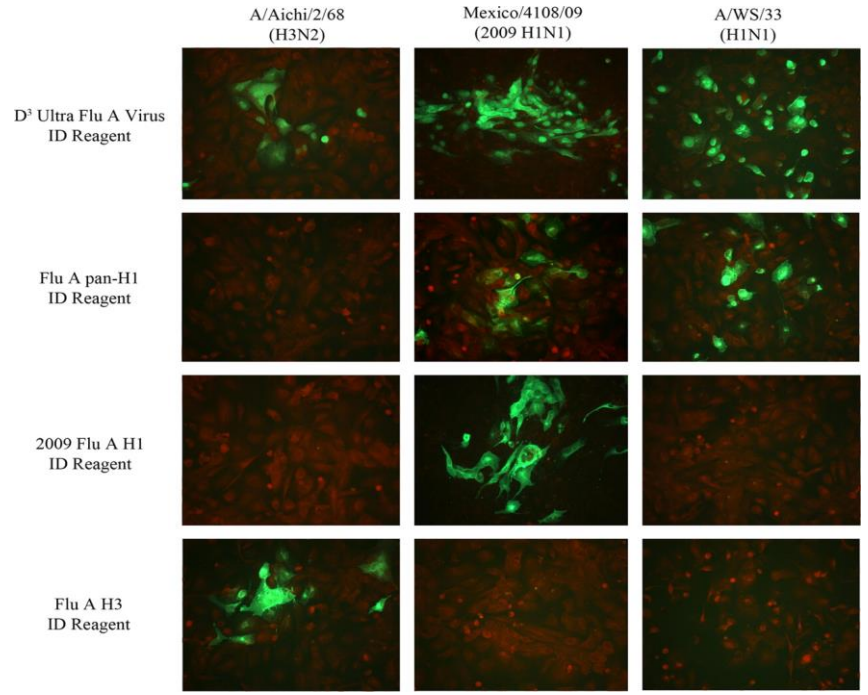
Hemadsorption



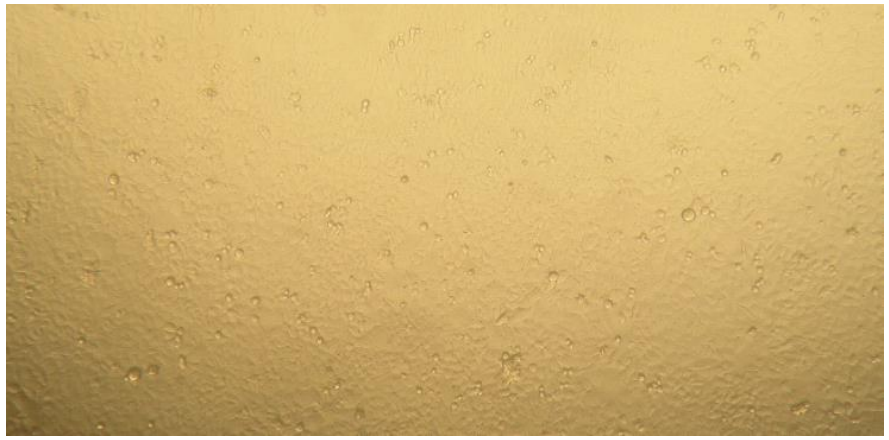
Hemagglutination



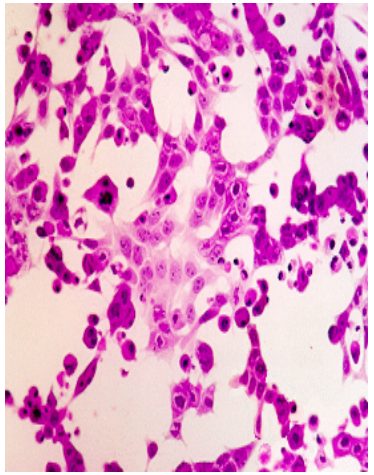
Immunofluorescence



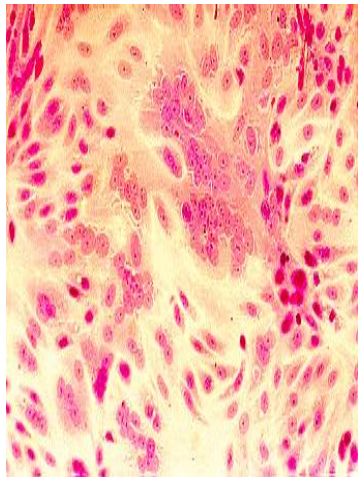
Virus Isolation (HEP2)



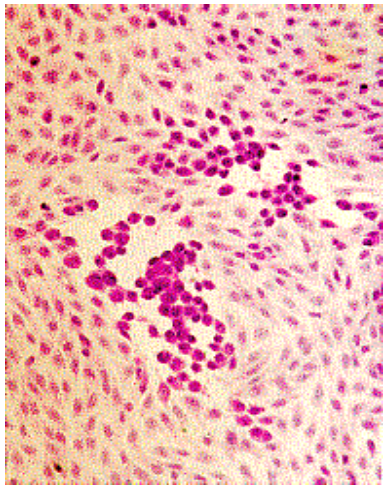
Normal HEP2



ADENOVIRUS

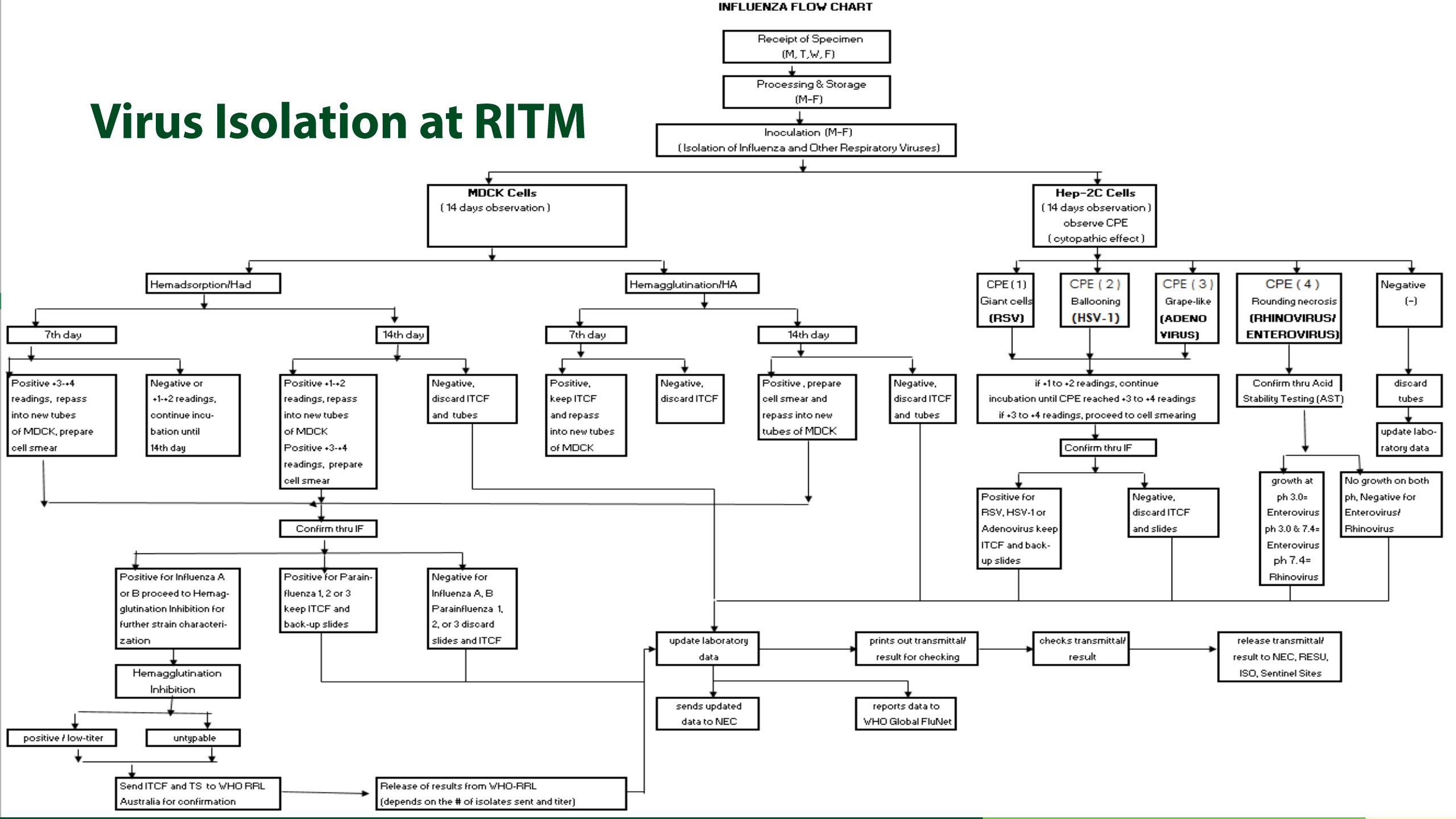


RSV



HSV

Virus Isolation at RITM

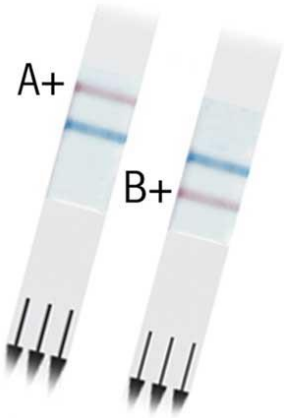


Antigen Detection

- Enzyme immunoassay format (simple and convenient to use)
- Directed at conserved viral antigens (e.g., nucleoprotein and matrix protein)
- Commercially available for human influenza virus strains only
- Does not differentiate human from avian influenza virus subtypes.
- Requires additional subtype specific methods
- **Currently, limited clinical utility for HPAI testing in humans**

Read by eye

QuickVue



Read by machine

Directigen

Sofia

Veritor

Molecular Testing

Real-time and Conventional RT-PCR

- Influenza Type detection – A and B
  - RT-PCR assays need to be targeted at genes (e.g., matrix gene) that are relatively conserved in order to detect all influenza A viruses and, separately, at the HA or NA genes to identify specific influenza A virus subtypes.
- Subtyping of Influenza A (H1/H3/Pandemic H1, H5, H7)
- Lineage detection of Influenza B (Victoria and Yamagata lineage)

Sequencing of Influenza samples

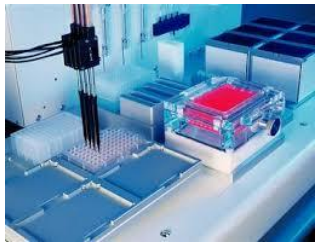
- Conventional/Sanger sequencing
- Next generation sequencing

PCR for Influenza Detection

RNA extraction → RT-PCR → Detection → Sequencing

Manual

Automated



One-step
(combines RT and PCR)

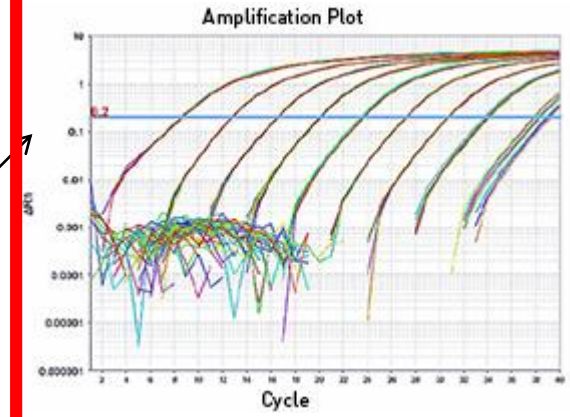
Two-step
(separate RT and PCR)

Conventional PCR

Real-time PCR

Extra step

Gel electrophoresis



Florescence chart

Job No.: #REF!

Run Filename:

Machine:

Date Performed:

Performed by: Jonjee Morin

Validated by: Vina Arguelles

Test Done	Rnase P ☐	InfA ☐	Pdm InfA ☐	Pdm H1 ☐	InfA/H3 ☐	InfA/H5 ☐	InfB ☐	InfB/Vic ☐	InfB/Yam ☐
(+) Control Batch No.									

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												

A panel of such RT-PCR assays, which includes generic influenza A virus detection plus specific detection of H5, H3, and H1 subtypes, is used to investigate suspected human H5N1 disease. Strategy helps overcome potentially false-negative PCR results due to the mutation of the HA gene because a specimen with a positive matrix gene that is negative for H5, H3, and H1 would flag that specimen for more detailed investigation.

Molecular Based Tests (Rapid, point-of-care)

- **Sensitivity (nearly) as good as RT-PCR!**
- **Speed of testing (nearly) as fast as a rapid test!**

Genexpert (Cepheid)

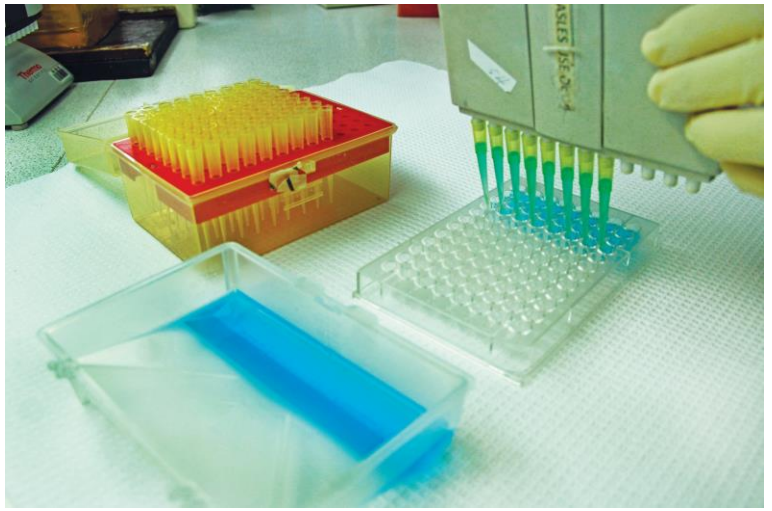
Cobas Liat (Roche)

Alere I (Alere)

Solana (Quidel)

Antibody detection

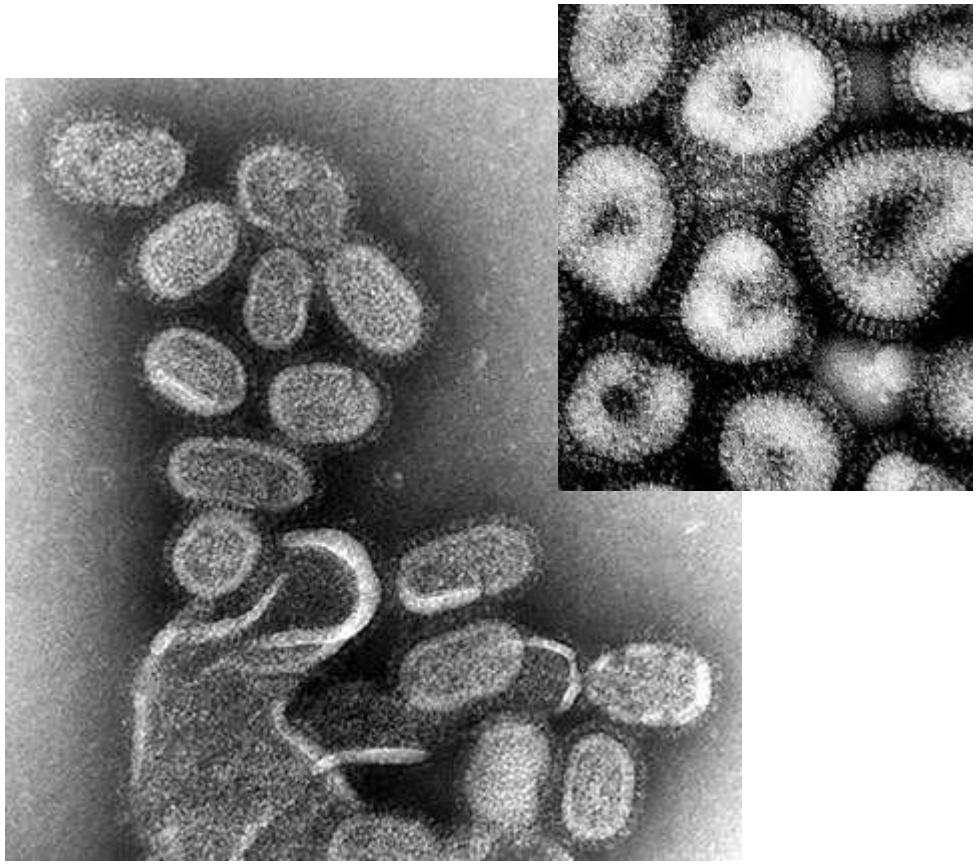
- Because of the delayed seroconversion and the need for paired sera, serology can provide **retrospective confirmation of H5N1 infection.**
- Detection of H5-specific neutralizing antibodies in humansns.
- Antibodies against H5N1 virus generally detected 14 or more days after the onset of symptoms in patients infected
- Requires BSL3 if working on avian viruses



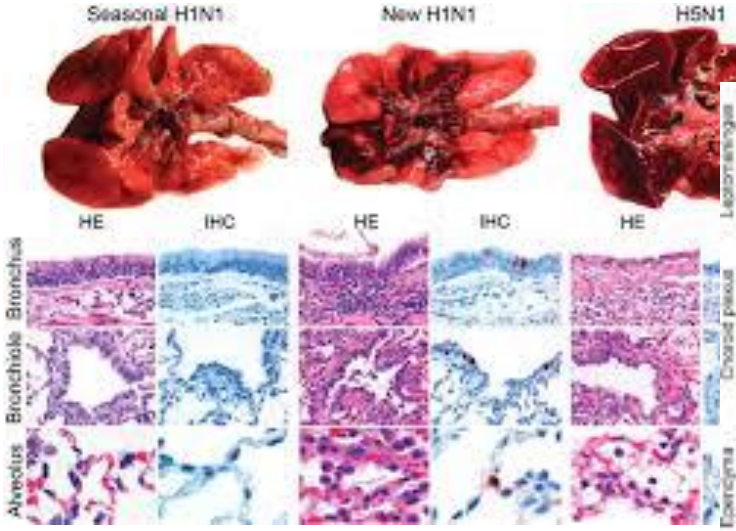
Pathogen	IgM ELISA	IgG ELISA	HAI	IFA IgM	IFA IgG	Luminex (IgG)
Inf. A (H5N1)*		✓				✓
Inf.A (H5N6)*		✓	✓			
Inf. A (H7N9)*						✓
SARS-CoV+	✓	✓				
MERS-COV		✓		✓		

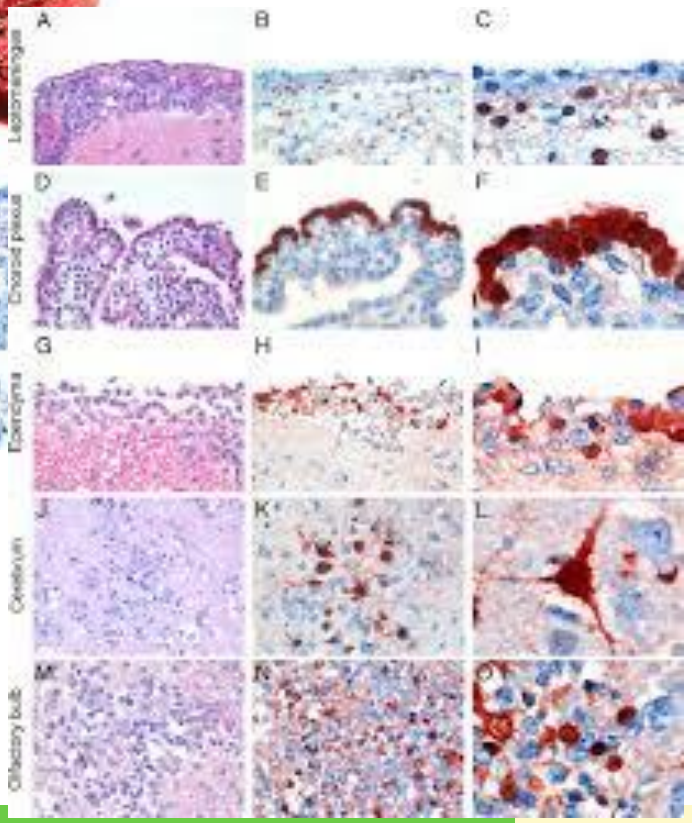
Others

Electron Microscopy



Histopathology and Immunohistochemistry





Biosafety Considerations

- Laboratory procedures that involve virus culture (virus isolation and neutralization tests) should be carried out in BSL-3 laboratory facilities.
- In view of the potential presence of infectious virus in stools and blood, it would also be prudent to perform any tests on such specimens within BSL-2 containment unless agents that reliably inactivate the virus are added in the course of the procedure.
- **Tests with serum or plasma samples are best done after heat inactivation for 30 min at 56°C.**

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