# The Laboratory & TARRANT

By

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Clinical Virologist

**Provincial Laboratory**

# Themes

- Laboratory Tests for Influenza
- Laboratory Surveillance

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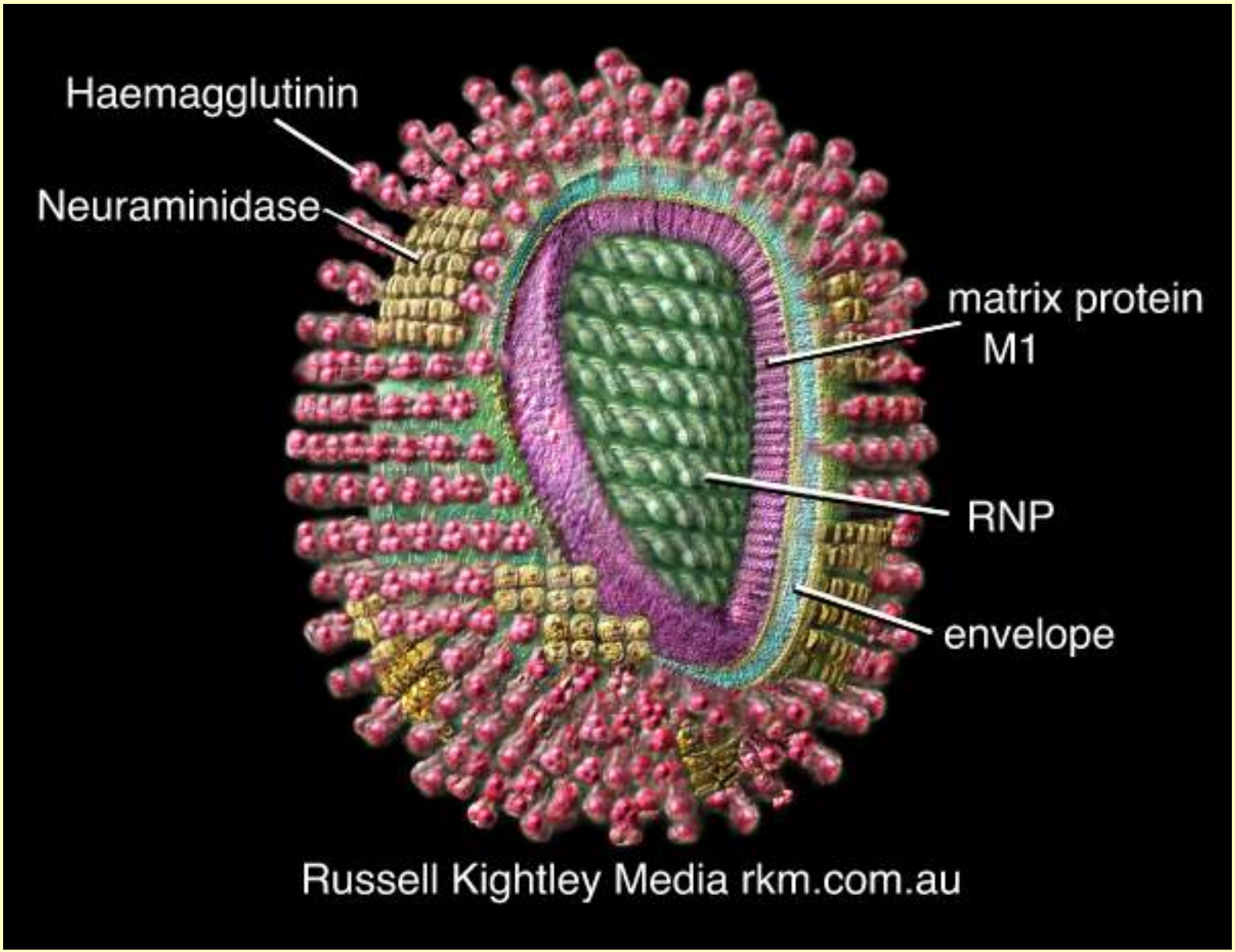


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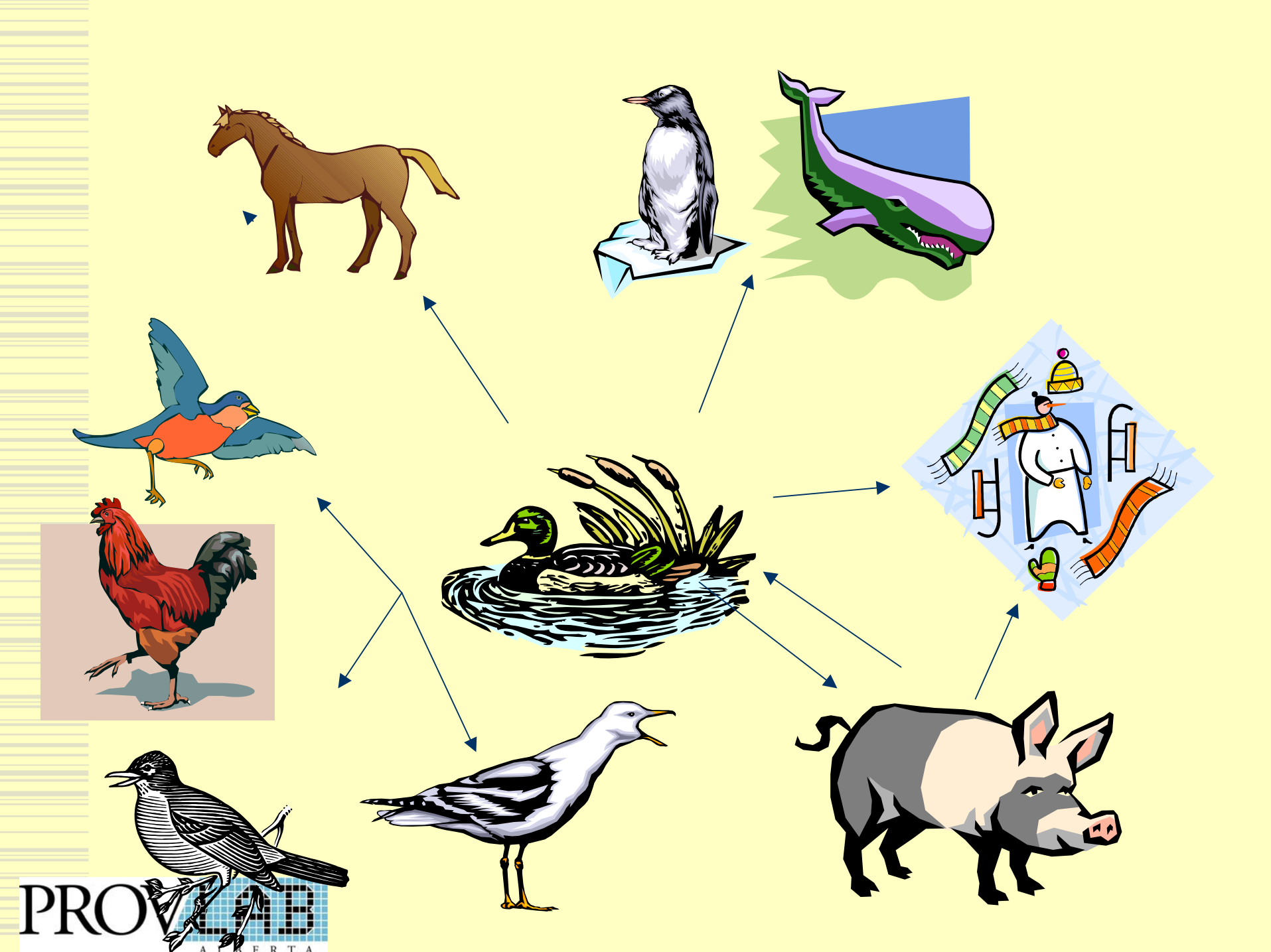
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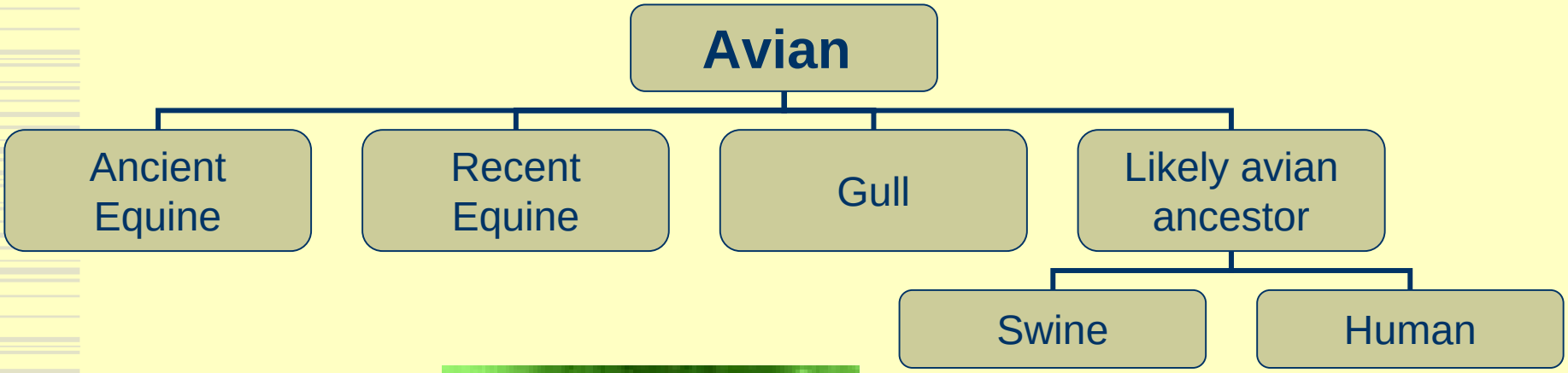
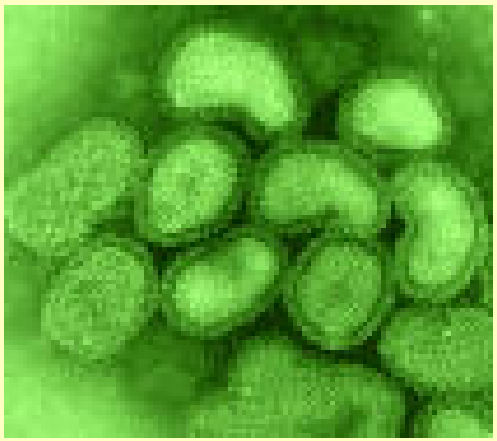
# Prototypic lineages of Influenza (based on Nucleoprotein gene)



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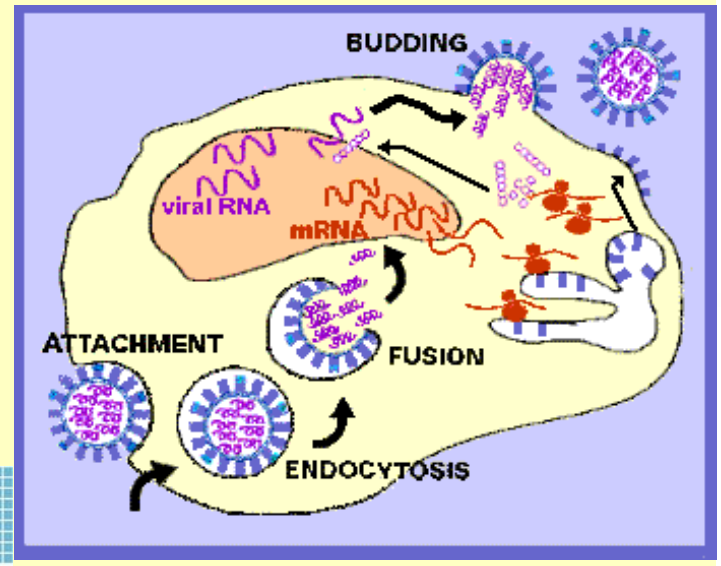
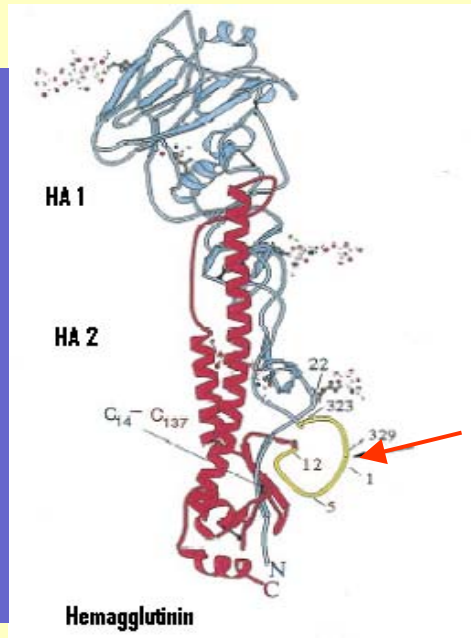
# Key Roles of Haemagglutinin

- Key role in virus attachment and entry to the host cell
- Activation of the virus requires HA cleavage into 2 pieces by host protease




Cleavage site

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# Subtypes of Influenza A

**H1** - swine, human
**H2** - human
**H3** - human
**H4** - avian
**H5*** - avian, (human)
**H6** - avian
**H7*** - equine,avian, (human)
**H8** - avian
**H9** - avian, (human)
**H10 – H15** - avian

**N1** - human
**N2** - human
**N3** - avian
**N4** - avian
**N5** - avian
**N6** - avian
**N7** - equine
**N8** - equine
**N9** - avian

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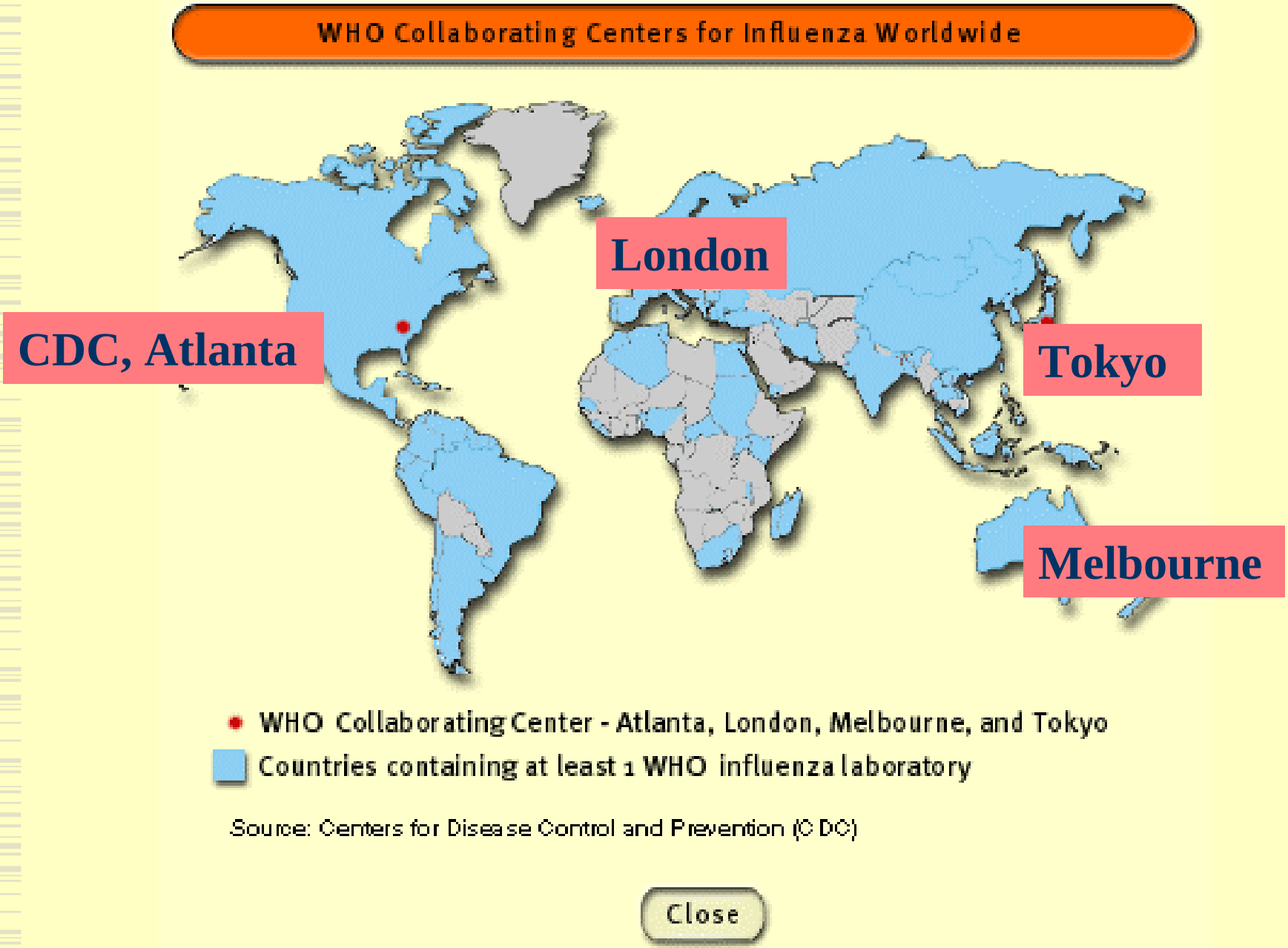


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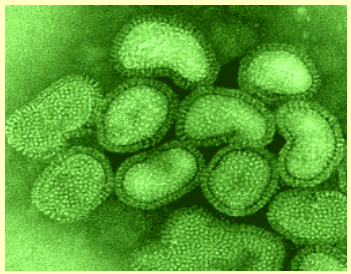
# Classification of Isolates



**A/Fujian/411/02-like [H3N2]**

**Type** **Geographical Origin** **Isolate Number** **Year of Isolation** **HA component** **NA component**

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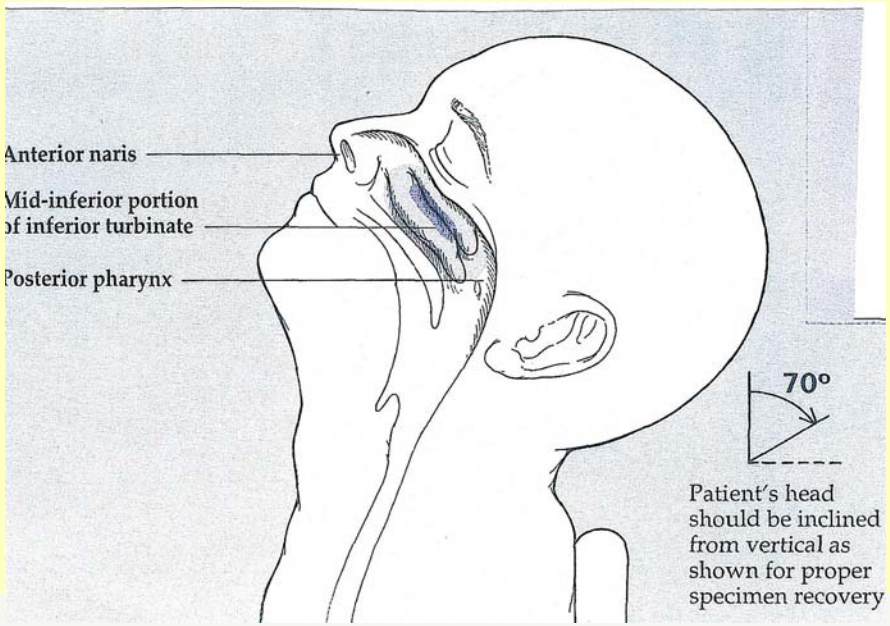


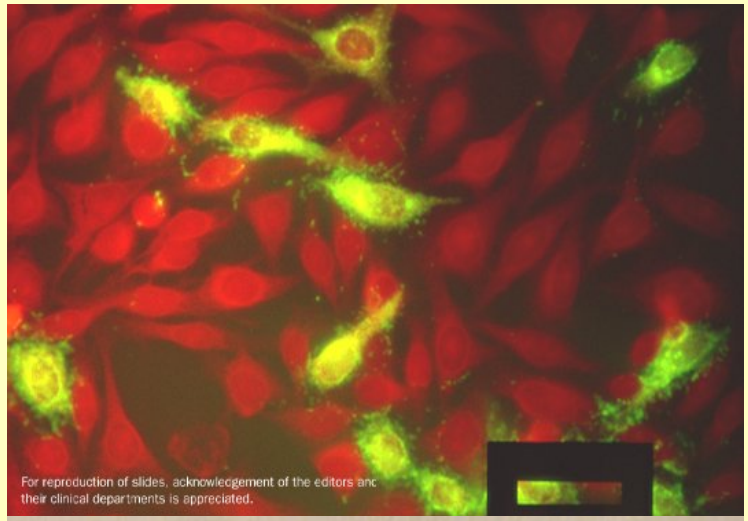


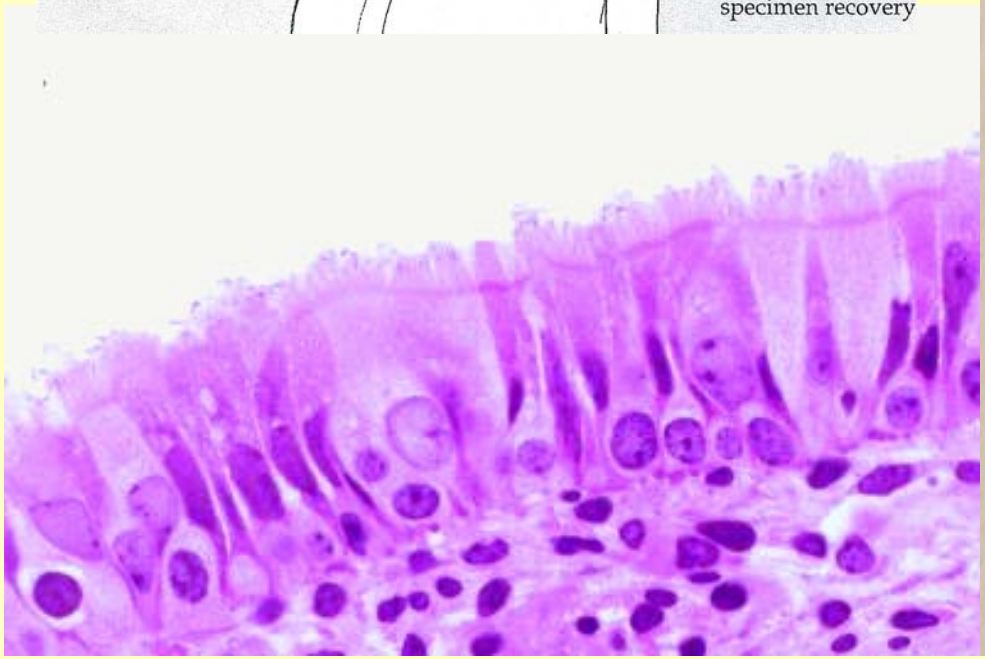

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# Indications for Nasopharyngeal Swabs

- Diagnosis of RSV Infection, especially young children
- Detection of respiratory agents by DFA
- Outbreak Investigations
- In Acute Care settings, BMT, ICU patients
- Severe Respiratory Infections (SRI)

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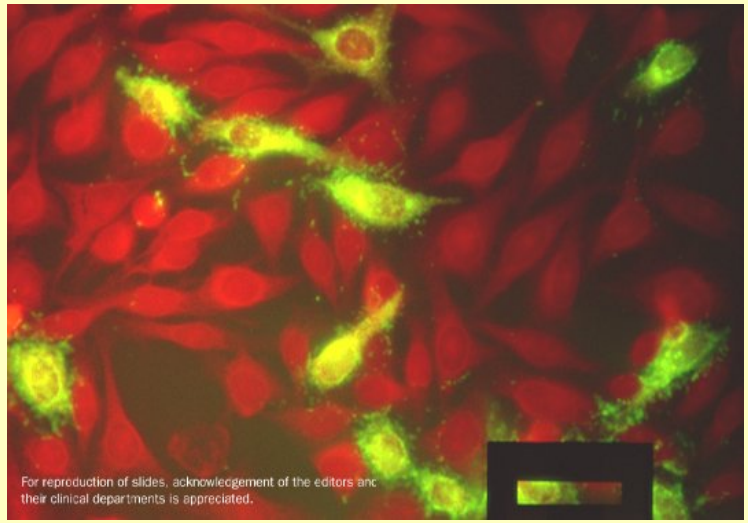
# Laboratory Tests for Diagnosing Influenza

- Rapid antigen/DFA testing




- Rapid Culture
- Standard Culture
- Serology
- Molecular assays

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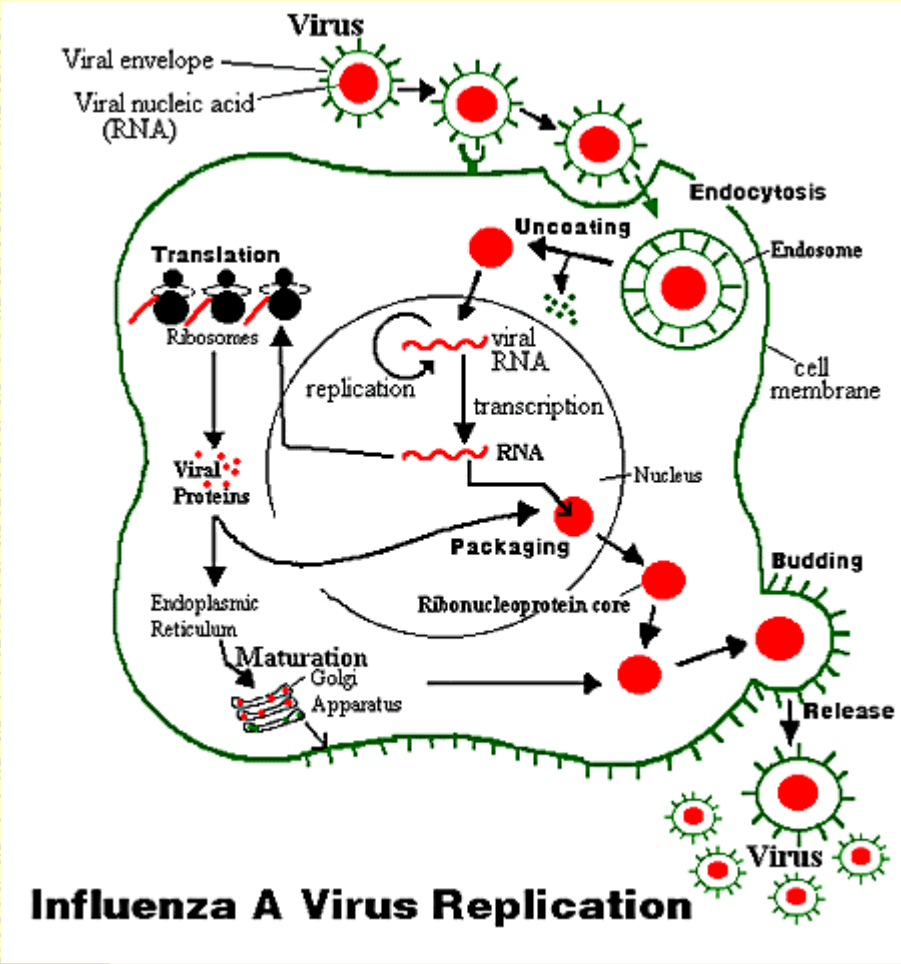
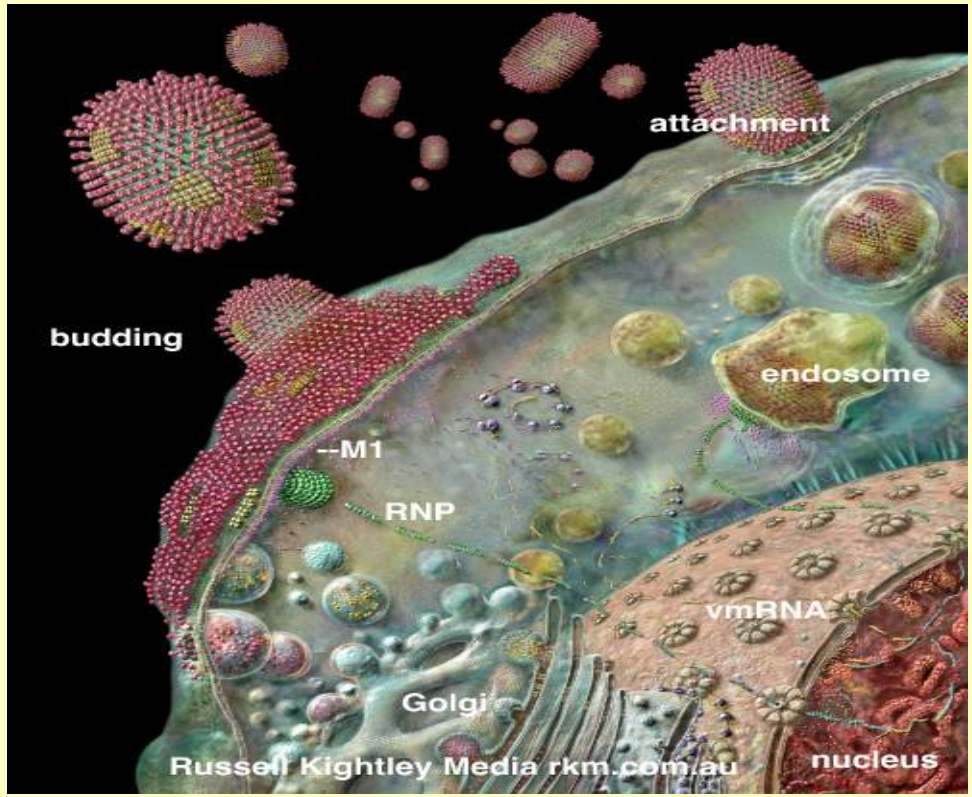
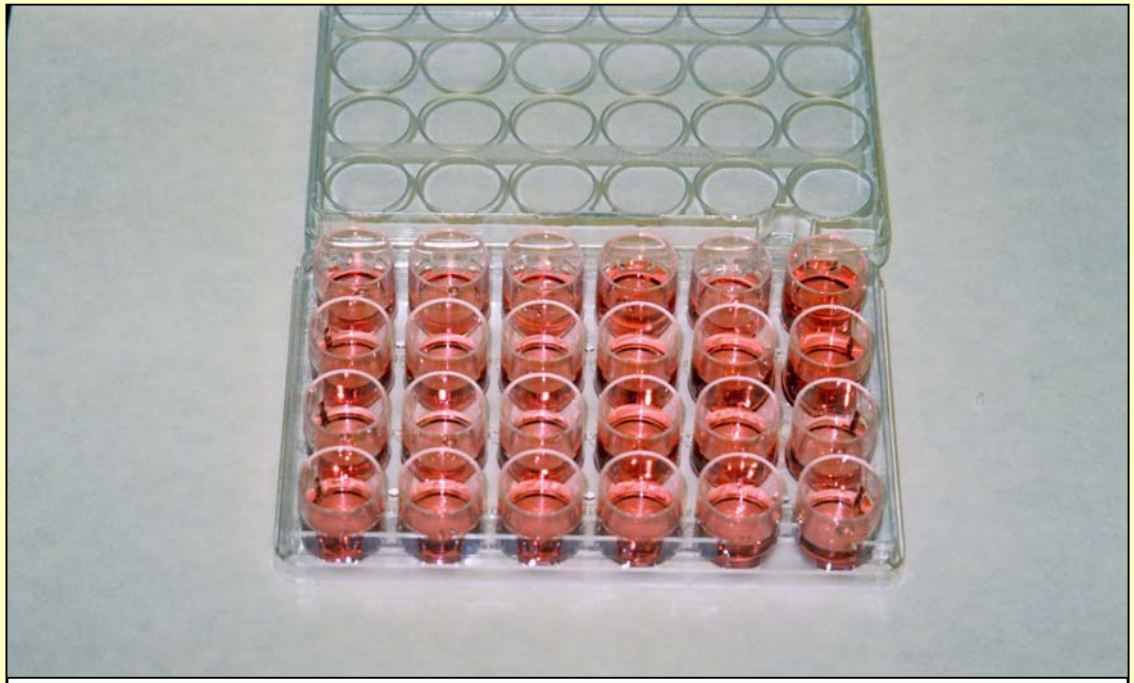
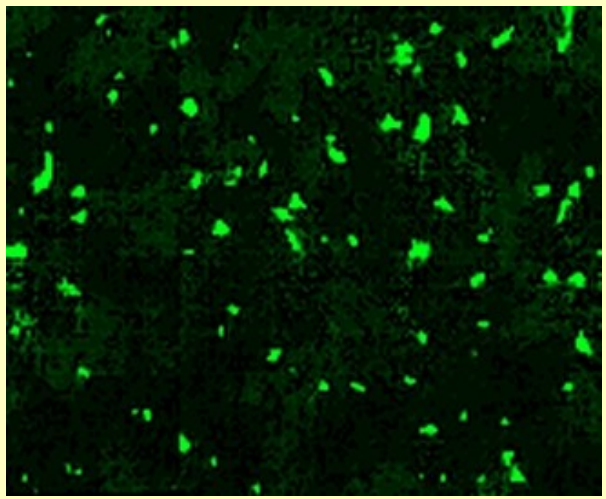
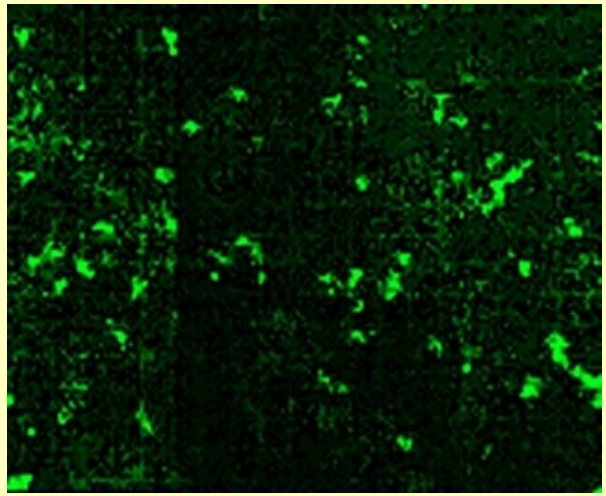

Virus
Viral envelope
Viral nucleic acid (RNA)
Endocytosis
Uncoating
Endosome
Translation
Ribosomes
viral RNA
replication
transcription
cell membrane
RNA
Nucleus
Viral Proteins
Packaging
Budding
Endoplasmic Reticulum
Ribonucleoprotein core
Maturation
Golgi Apparatus
Release
Virus
Influenza A Virus Replication


attachment
budding
endosome
--M1
RNP
vmRNA
Golgi
Russell Kightley Media rkm.com.au
nucleus



Each well contains Mink Lung & NCI cells





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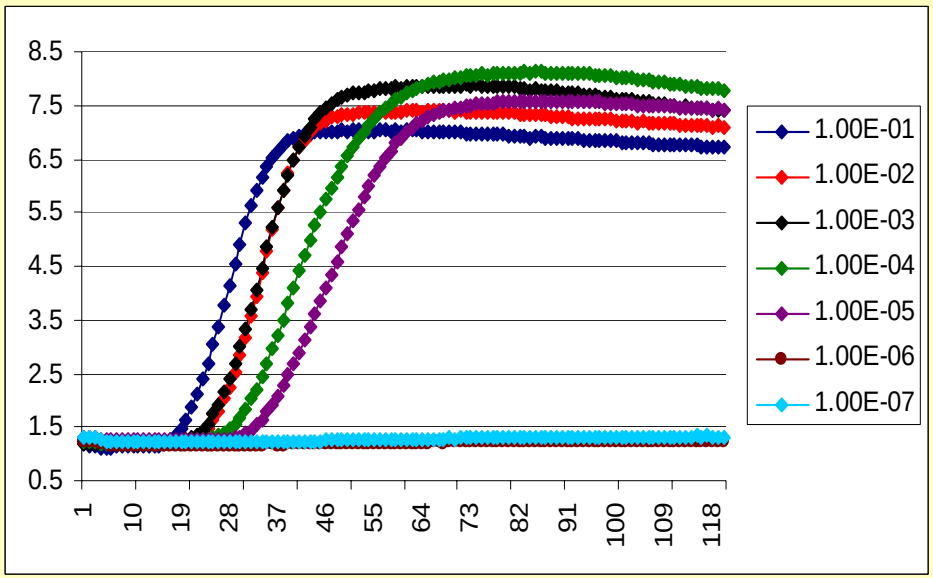
# Molecular Assays

- **Detection** - PCR or NASBA
- **Subtyping** – sequencing
- **Antiviral resistance** - amantadine




Influenza A
Influenza B
RSV (A+B)
Parainfluenza 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4a+4b
SARS coronavirus
Metapneumovirus
Respiratory adenoviruses
Enteroviruses

Rhinoviruses
Coronavirus 229E/OC43
Coronavirus NL63

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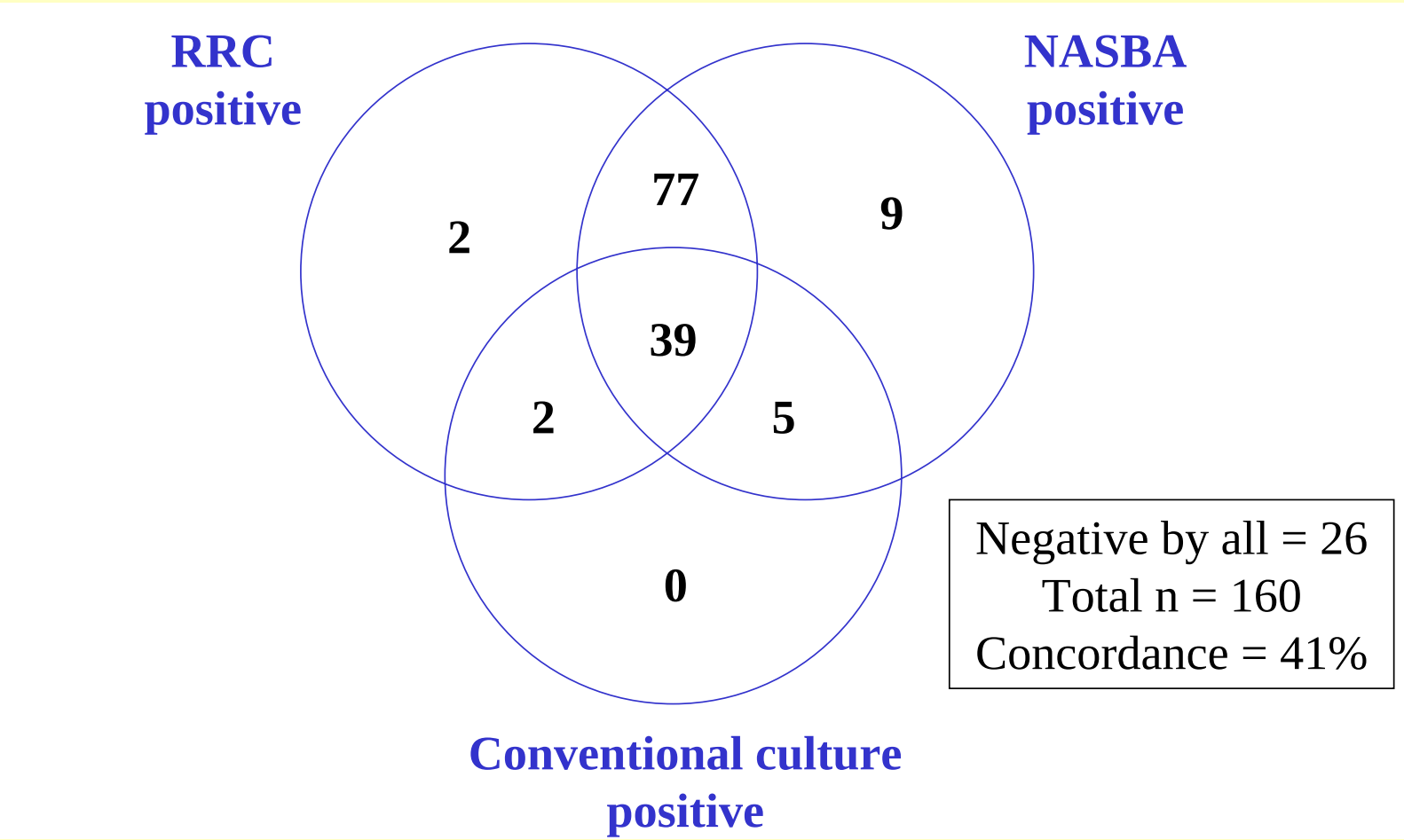
# Comparison of assays for detection of influenza A

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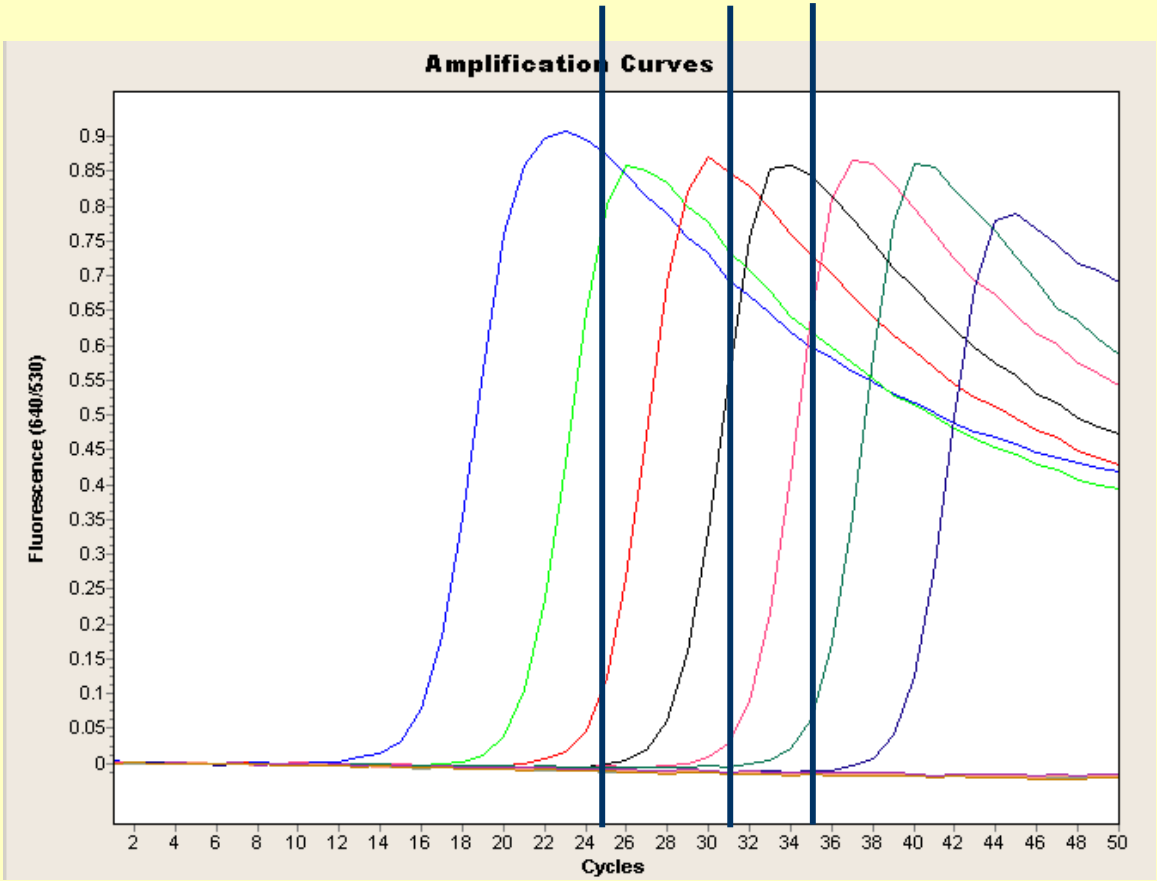
# *B. pertussis* screen assay dilutions




**PCR assay from: Reischl *et al*. 2001. *J. Clin. Micro.* 39: 1963.**

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# Patient at Emergency Department

- Male 47 yrs and companion
- Recent travel abroad on holiday to Thailand with a tour company
- Fever, cough, sore throat, myalgia for about two days since his return
- Not vaccinated to influenza

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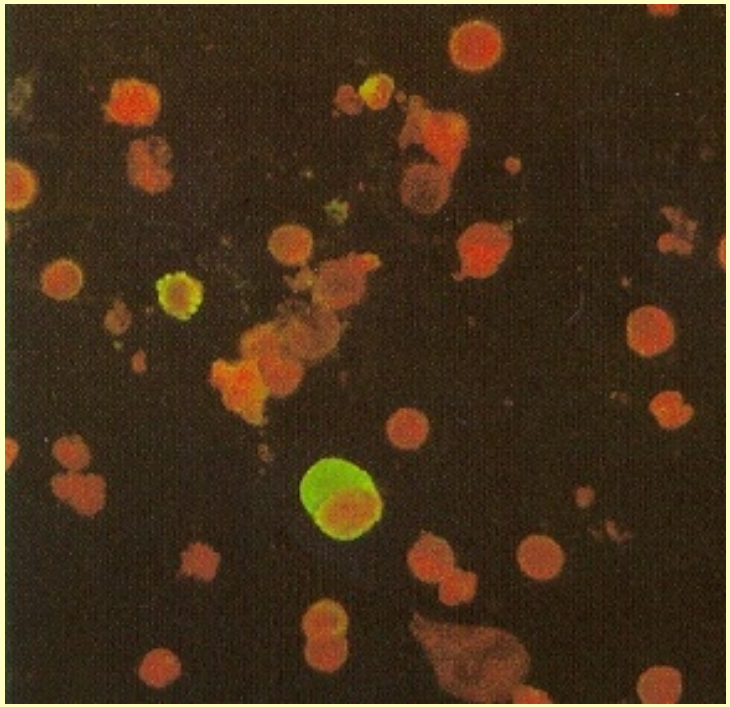
# Lab results



- DFA – Positive for Influenza A (2 hrs later)
- Sample sent to Level 3 in Edmonton for culture
  - What do we do now ??
  - Patient is not too unwell
- PCR confirmed presence of influenza A (5 hrs later)
- Subtyped as H3 (next day)

- Rapid culture (24 hrs)
- Result from NML 7 days later – A/Fujian

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# Sources of Respiratory Samples

- Rural & Acute Care (PLC, ACH, FMC, RVH) Sites
- Emergency Departments (Severe Respiratory Investigations – SRI)
- Outbreak Investigations
- TARRANT
- Community Physicians

- **Reporting: Copies of influenza A & B positives Lab reports are sent to MOH:**
  - **Patient name, address, gender, age**
- **Aggregate data to AHW and other agencies**

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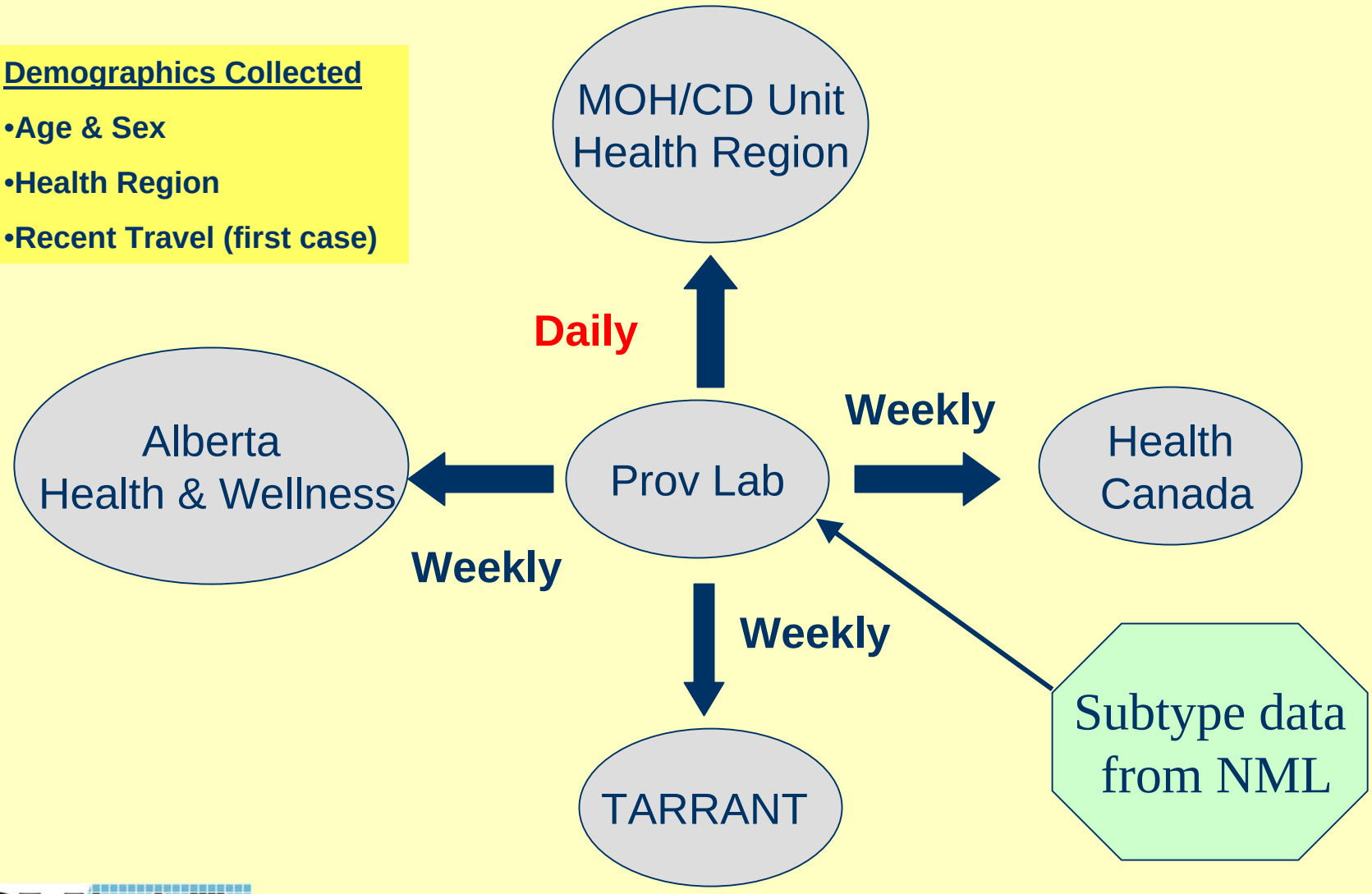
# Surveillance Data – What & Where

**Demographics Collected**

- **Age & Sex**
- **Health Region**
- **Recent Travel (first case)**

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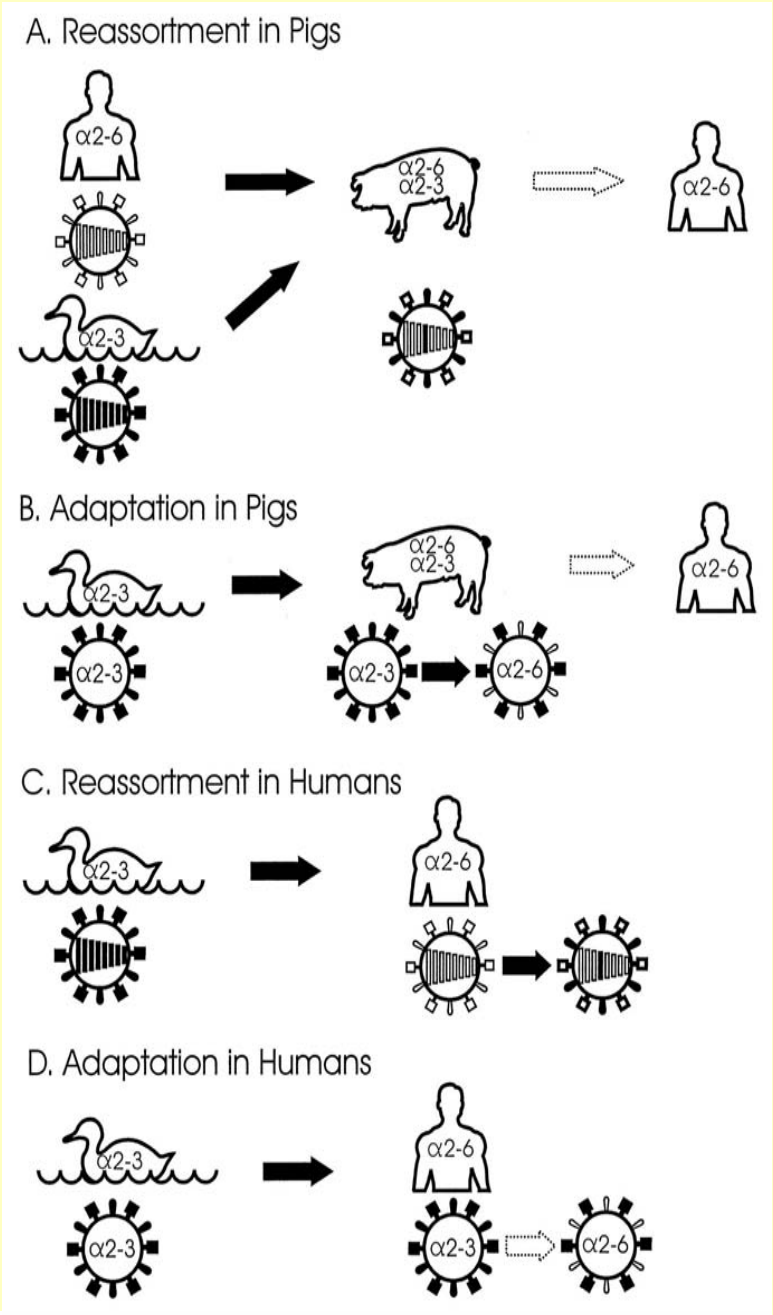


# Possible Mechanisms for the Emergence of Potential Pandemic strains

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# Pandemics of Influenza A

| Year | | Subtype | Deaths x$10^6$ | Origin |
|---|---|---|---|---|
| 1889 | | H2N2 | 6 | Europe |
| 1898 | | H3N2 | 0.5 | Europe |
| 1918 | Spanish flu | H1N1 | 20-40 | Europe |
| 1957 | Asian flu | H2N2 | 4 | Asia |
| 1968 | Hong Kong flu | H3N2 | 2 | Asia |
| 1977 | Russian flu | H1N1 | ? | Asia /lab |

*JS Oxford: Rev Med Virol:2000:10;119-33*

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# Partnership Strengths Between TARRANT & Laboratory

- Incidence of influenza and respiratory pathogens
- Value of subtype analysis influenza
- Detection of possible new subtypes in returning Canadians or visitors
- Out-of-season outbreaks or sporadic events
- May be a SARS-like case !!

✓ **Keep Recording & Swabbing !!!**

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# Acknowledgements


- Dr. Jim Dickinson, Pin Cai & the TARRANT network
- Dr. Julie Fox – Director MOD Department
- Dr. Yan Li – NML
- Pat Morin @ AHW
- Staff of the Prov Lab Virology Departments (Calgary & Edmonton)


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# Definition of a Virus

- **“Trouble wrapped up in protein”**

***Sir Peter Medawar***

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