Why is antiviral drug resistance testing necessary?

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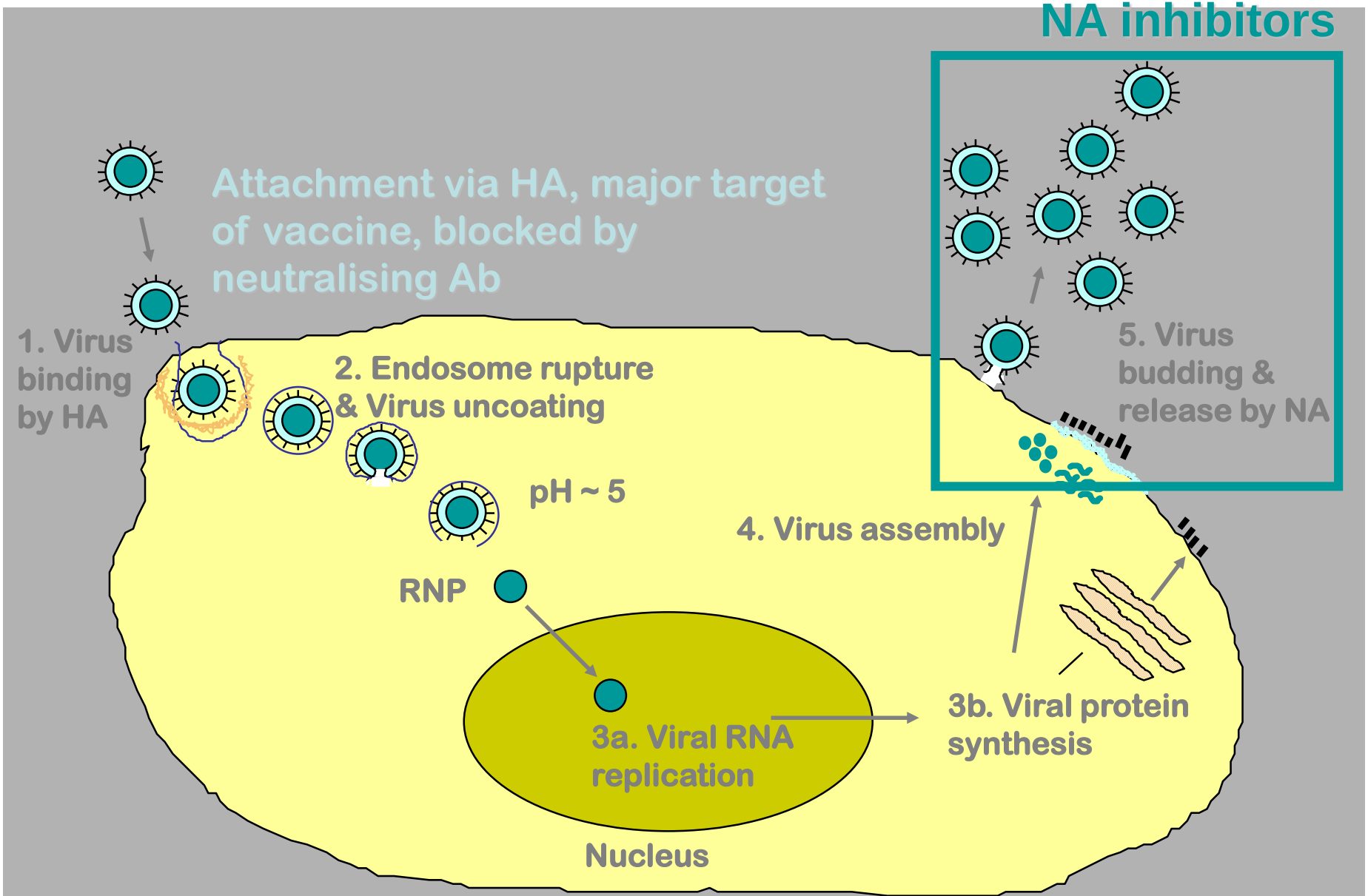
Reasons why influenza antiviral resistance monitoring might be worthwhile in your country

- Has become a basic property of influenza viruses that is widely reported nationally/internationally especially during pandemics
- Gives public health authorities valuable information in this current era when some antivirals will be ineffective or less effective due to drug resistance in some influenza subtypes
- Gives an idea of the potential effectiveness of antivirals stockpiled for pandemic use
- Helps track the spread/fitness of resistant strains in GISN
- ? others

Influenza Control Measures

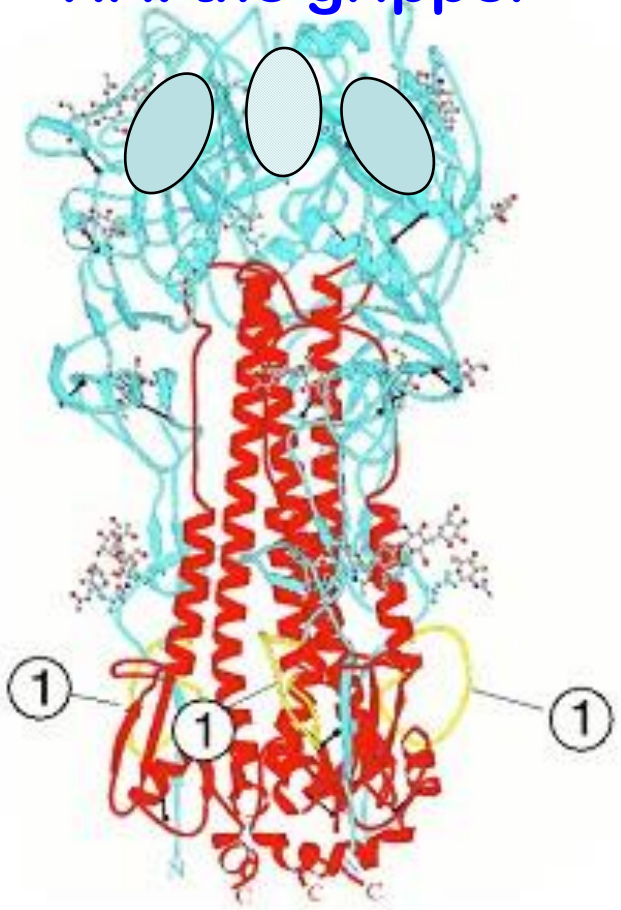
- Symptomatic (paracetamol, cough mixtures etc.)
- Vaccination (preventative)
- Antivirals (therapeutic/preventive)
  - M2 ion channel inhibitors
    - Amantidine and Rimantidine
  - NA inhibitors
    - Zanamivir and Oseltamivir
- Public health measures

Replication cycle of influenza virus



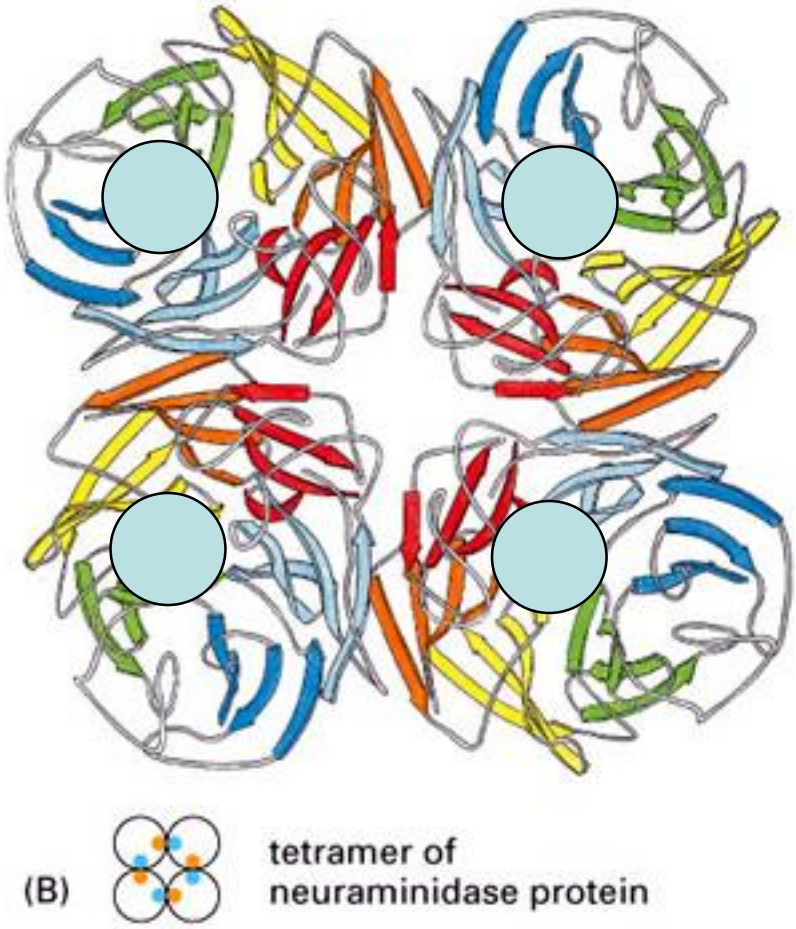
HA and NA interact with sialic acid-containing receptors on cells

HA: the gripper



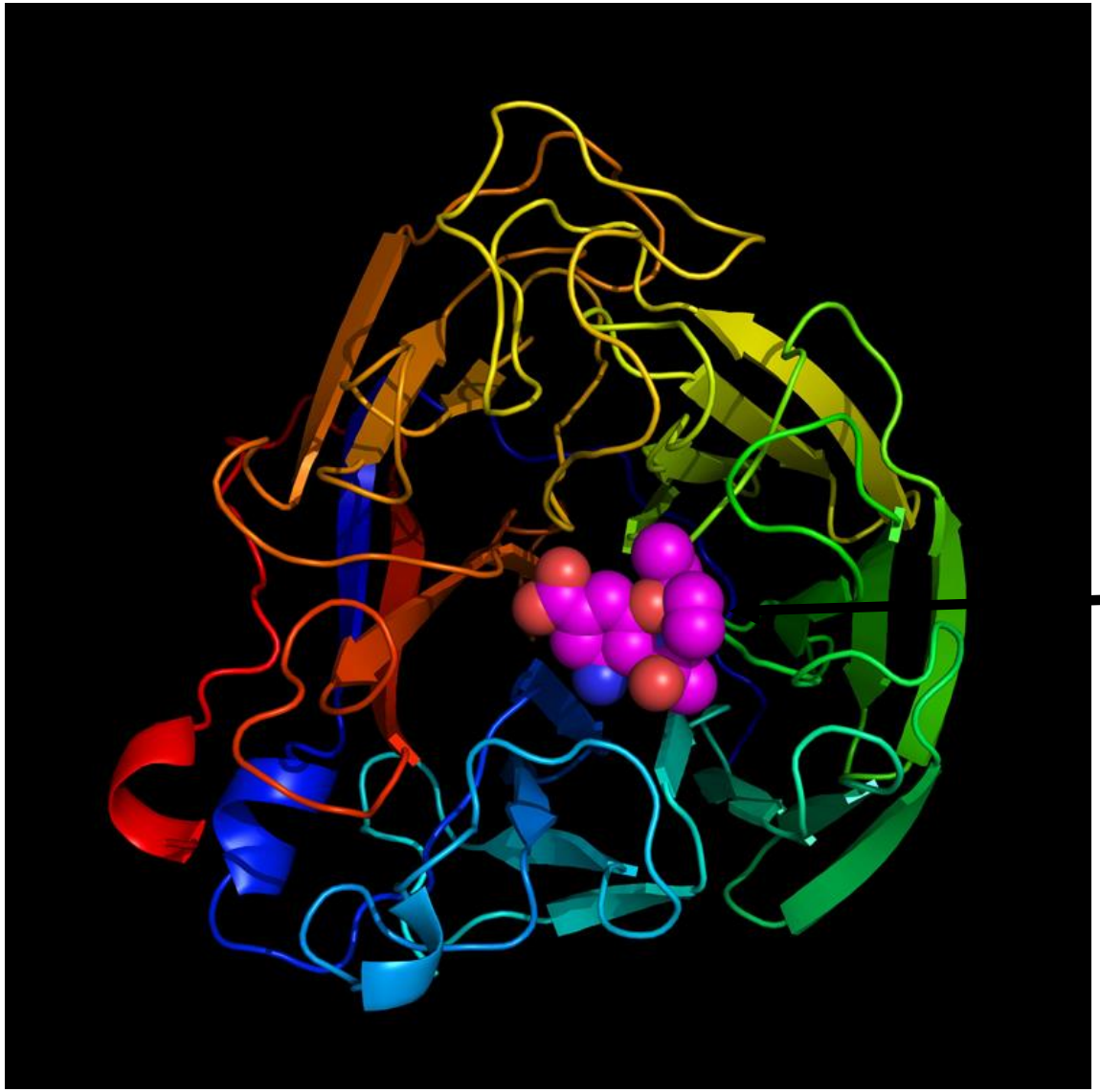
Trimer of hemagglutinin
side view

NA: the snipper



Tetramer of NA
top view

Oseltamivir (carboxylate) binds to the active site of influenza neuraminidase



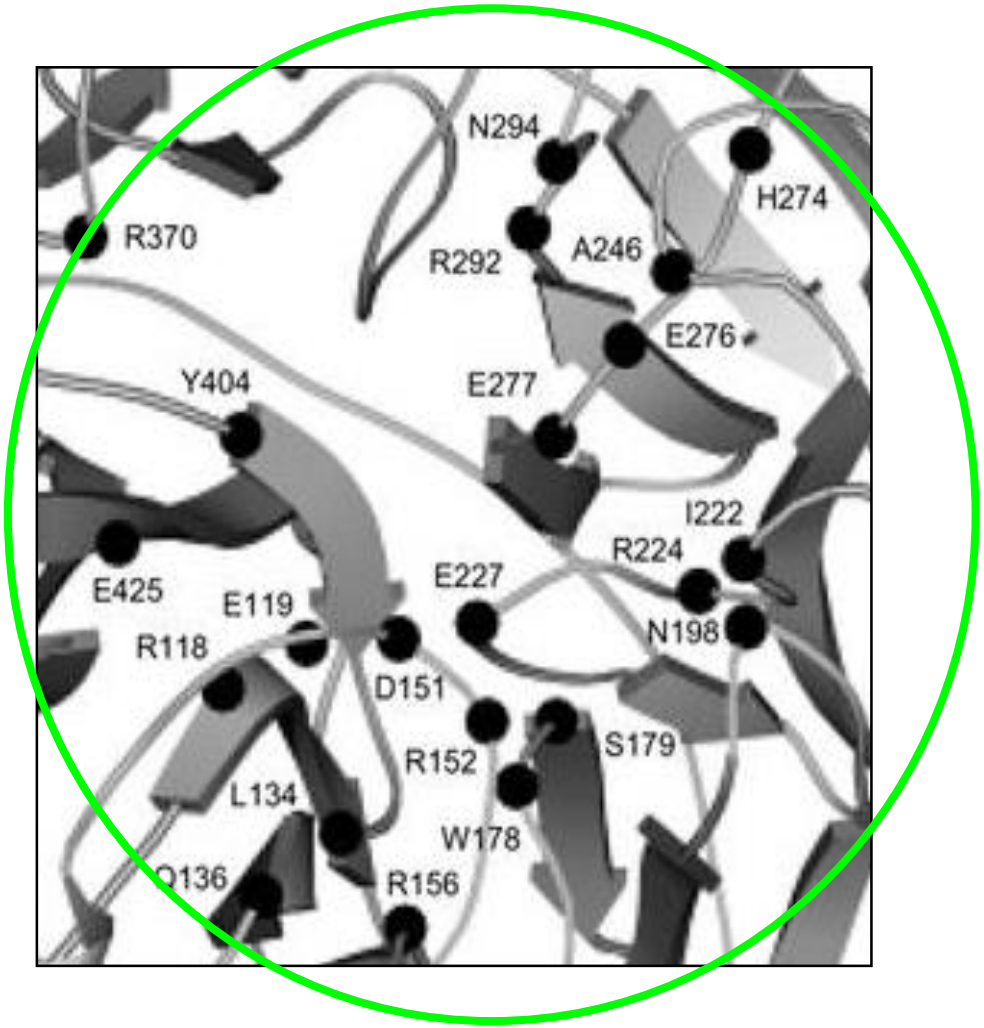
How do we determine influenza virus sensitivity to neuraminidase inhibitors?

- By molecular means examining amino acid substitutions in the NA gene
  - Most common current mutation H275Y in N1
  - Use full gene sequencing of NA
  - Pyrosequencing
    - need several primers sets to cover all known mutations
  - Real time PCR assays (one assay per substitution)
  - DNA melting curve differences (one assay per substitution)

- Functional assays
  - Viral plaque reduction +/- drug
  - Enzyme inhibition assays +/- drug
    - Fluorescence based – MUNANA substrate

      Chemiluminescence based – NA star substrate

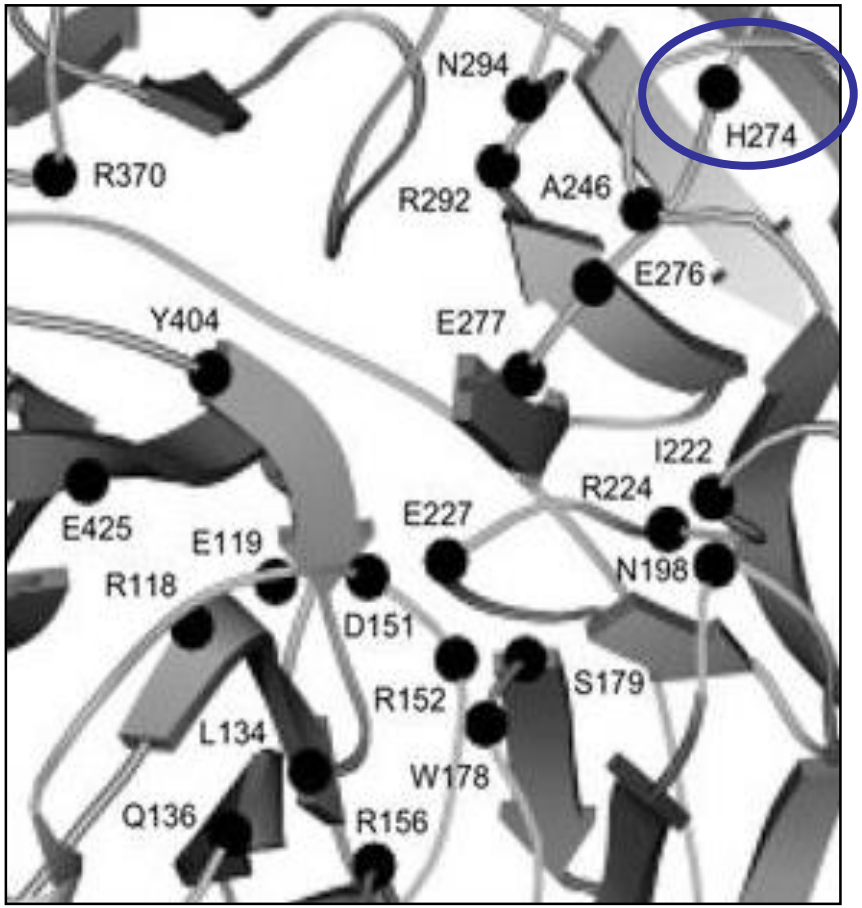
NA enzymatic site



Mutations in the enzymatic site

Prevent NA inhibitor binding (resistance)

H274Y



- **Framework amino acid**
- **H274Y mutants previously observed under oseltamivir treatment in N1**
- **H274Y Mutation affects ability of E276 to reorientate which is required for oseltamivir binding**
- **H274Y mutants are sensitive to zanamivir**

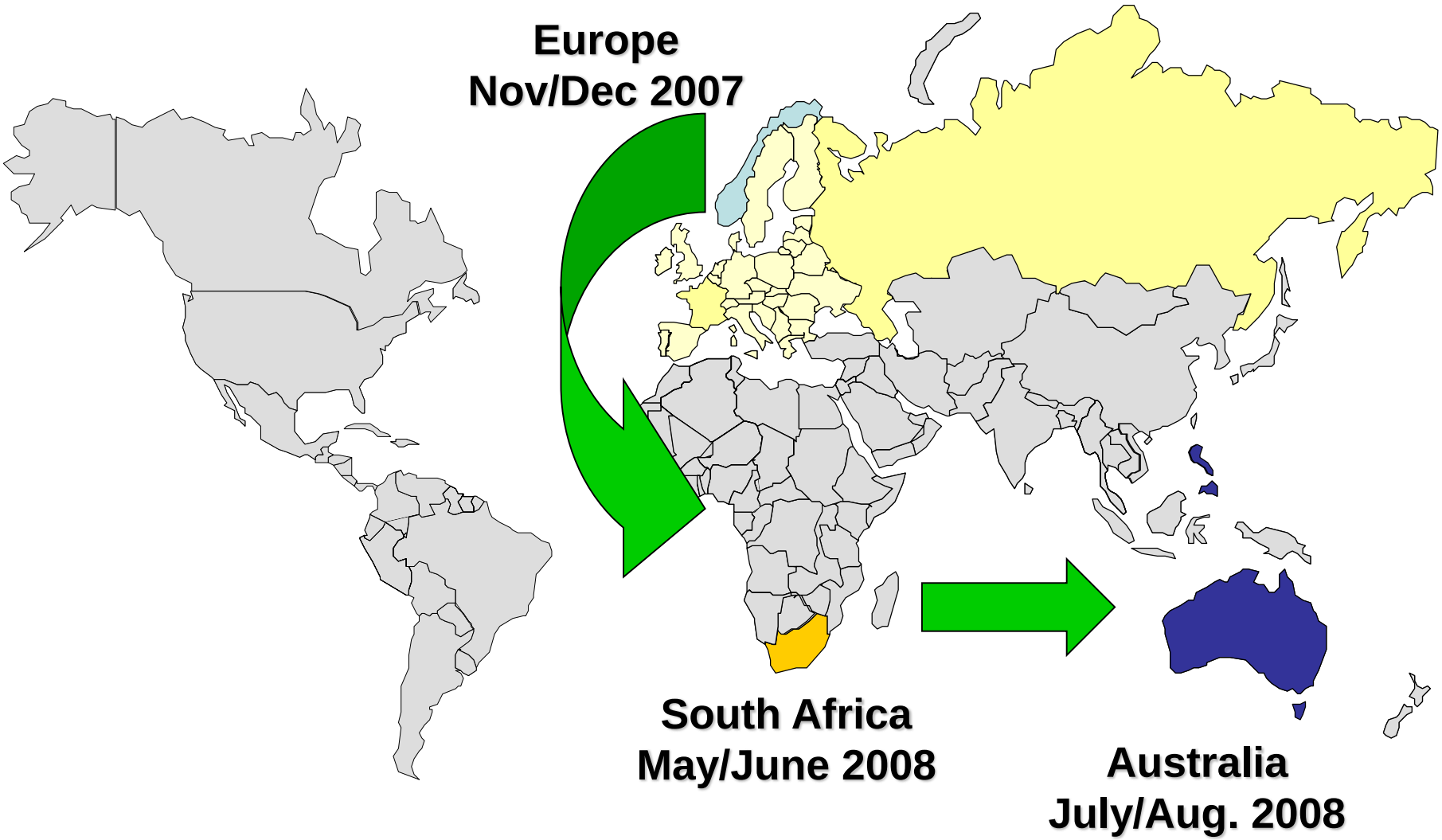
H274Y or H275Y ?

- **Traditionally N1 residues have been numbered based on the equivalent residue in the N2 neuraminidase**

- **However, either H274Y or H275Y are acceptable for reporting, publication, etc**

- **Important that:**
  - **State which numbering system you are using**
    - **eg H274Y (based on N2 numbering)**
  - **Ensure that you are looking at the correct residue!!!**

Rapid movement of H274Y seasonal H1N1 Oseltamivir resistant viruses 07-8



First global spread of fully fit NAI resistant viruses

Summary

- Influenza antivirals are an important part of the worlds weapons against seasonal and pandemic influenza (along with vaccines)
- Two classes– adamantanes (M2 inhibitors) & NI (neuraminidase inhibitors)
- NI first choice of influenza antiviral drugs currently
- NI drugs have variable resistance profiles
- Prior to 2007 no significant resistance to NI drugs
- Since 2007, Oseltamivir resistant H1N1 viruses have emerged (still sensitive to zanamivir)
- Currently Pandemic A(H1N1) 2009, A(H3N2), B, (& H5N1) viruses are virtually all sensitive to oseltamivir & zanamivir
- New drugs in development eg T705 (Toyama) which are not NI will need new assays to determine resistance in influenza viruses