ASLM 2014
AFRICAN SOCIETY FOR LABORATORY MEDICINE
Innovation and Integration of Laboratory and Clinical Systems
Reshaping the Future of HIV, TB, Malaria, Flu,
Neglected Tropical Diseases and Emerging Pathogens in Africa

30th November – 04th December 2014
Cape Town International Convention Centre (CTICC)
Cape Town, South Africa

Influenza: the role of a WHO CC within GISRS and provision of candidate vaccine viruses

Dr Rod Daniels
Deputy Director,
WHO Collaborating Centre for Reference and Research on Influenza
MRC National Institute for Medical Research,
The Ridgeway, Mill Hill, London NW7 1AA

Symposium 03rd December: **Evidence to Support Influenza Vaccination in Africa**

GISRS

Global influenza virologic surveillance has been conducted through WHO's Global Influenza Surveillance and Response System (GISRS) for 62 years

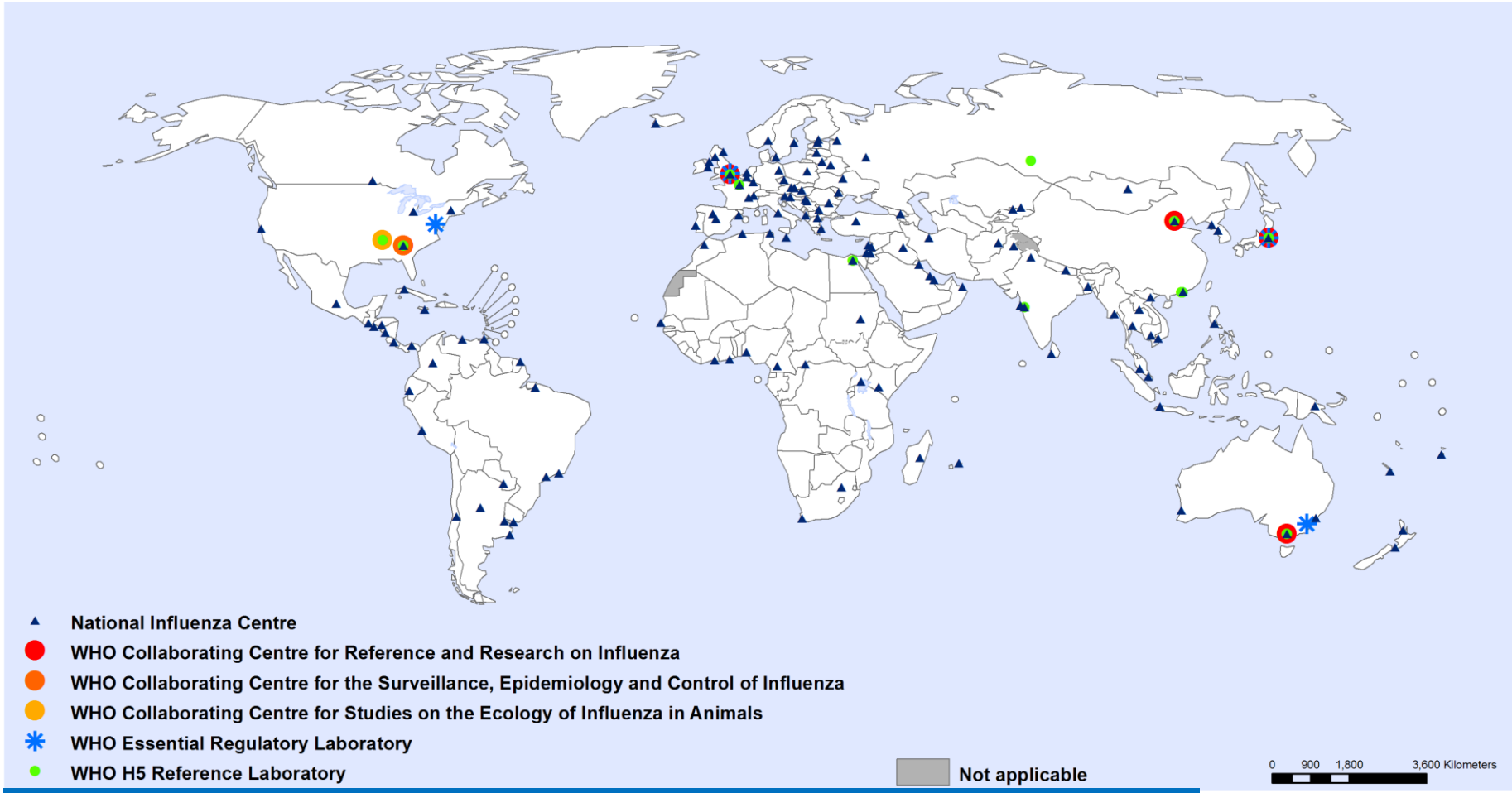
Formerly known as the Global Influenza Surveillance Network (GISN), the new name came into effect following the adoption of the Pandemic Influenza Preparedness (PIP) Framework in May 2011

- **WHO GISRS monitors the evolution of influenza viruses and provides recommendations in areas including laboratory diagnostics, vaccines, antiviral susceptibility and risk assessment**
- **WHO GISRS also serves as a global alert mechanism for the emergence of influenza viruses with pandemic potential**

GISRS: http://www.who.int/influenza/en/

WHO Global Influenza Surveillance and Response System

25 April 2013



141 National Influenza Centres in 114 countries/territories
6 Collaborating Centres: 5 Human & 1 Influenza Ecology
13 H5N1 Reference Labs (inclusive of 6 CCs)
4 Essential Regulatory Labs (Australia, Japan, UK, USA)
As of 30 October 2014

World Health Organization

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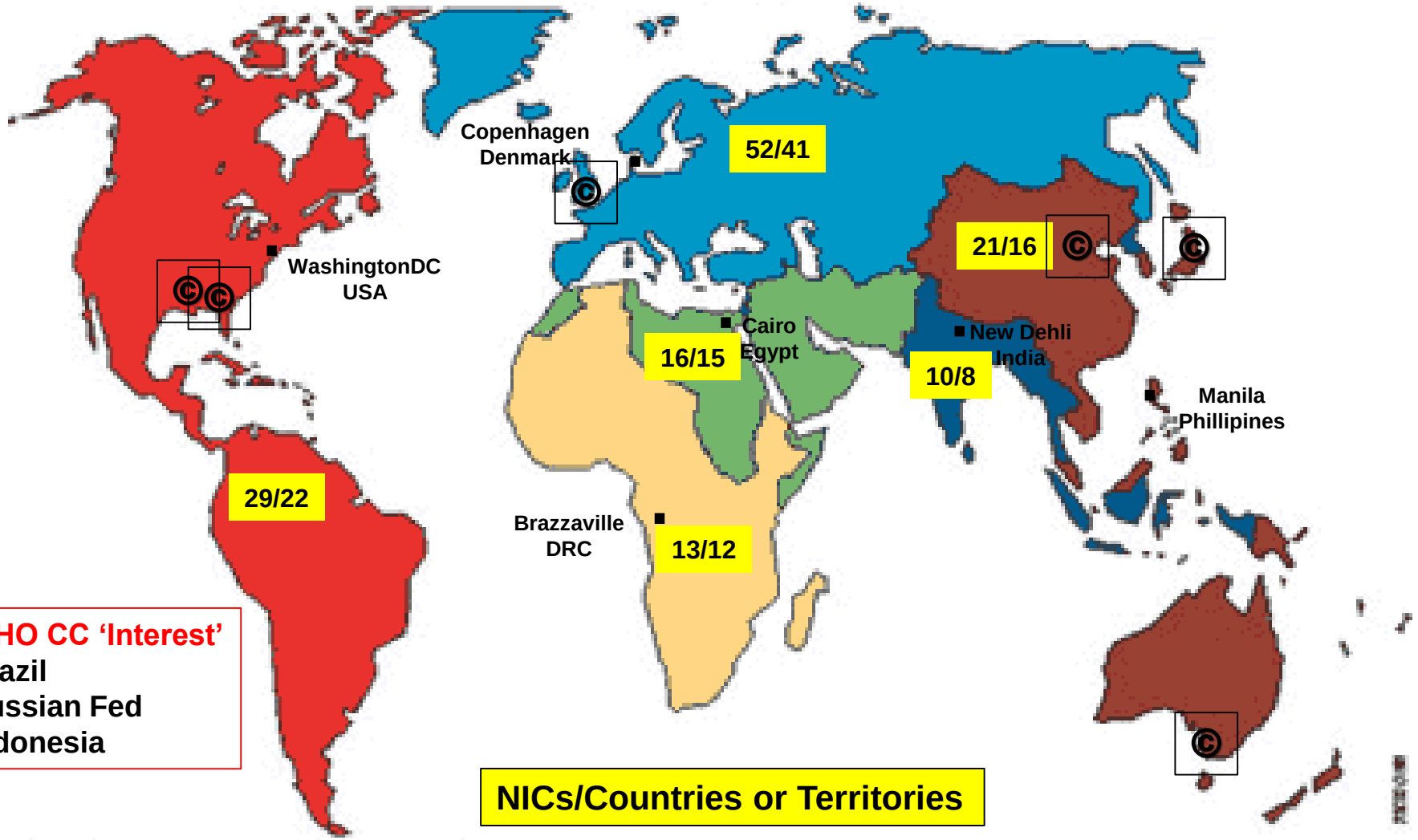
The WHO Regions and Locations of Regional Offices



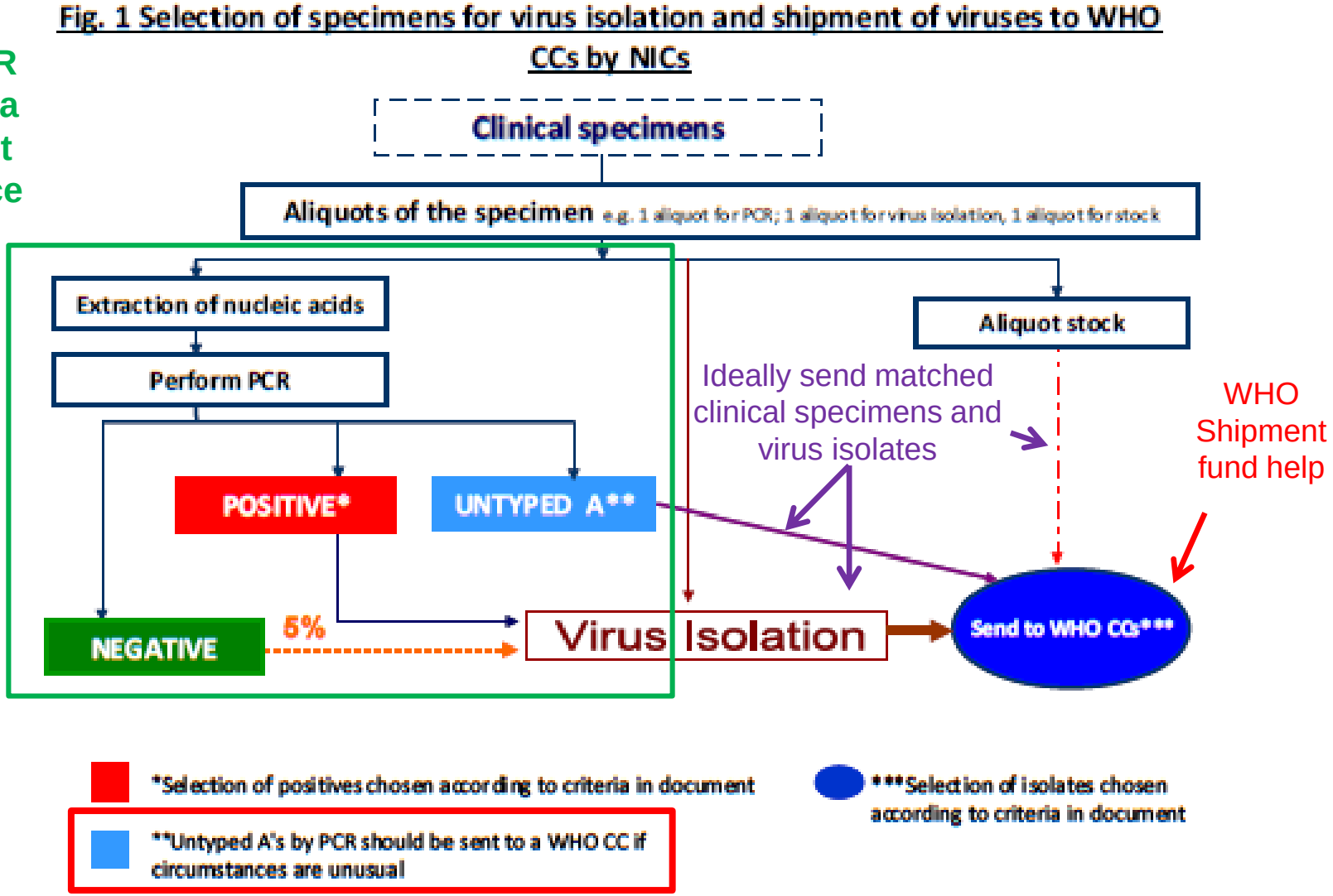
WHO CC 'Interest'
Brazil
Russian Fed
Indonesia

1+1CC **1CC** **3CC**

Advice on NIC Web-page Regarding Shipments to WHO CC

(Ideally specimens collected within 1-2months of the date of shipping)

CDC-IRR Influenza Reagent Resource



http://www.who.int/influenza/gisrs_laboratory/national_influenza_centres/20101206_specimens_selected_for_virus_isolation_and_shipment.pdf

The WHO CC in London is well served by International Flights, many direct, to Heathrow (as well as airports at Gatwick, Luton, Stansted, London City)

During the 2009-2010 pandemic, and 2010-2011
Received ~ 3500 viruses/clinical samples each year from 55 countries

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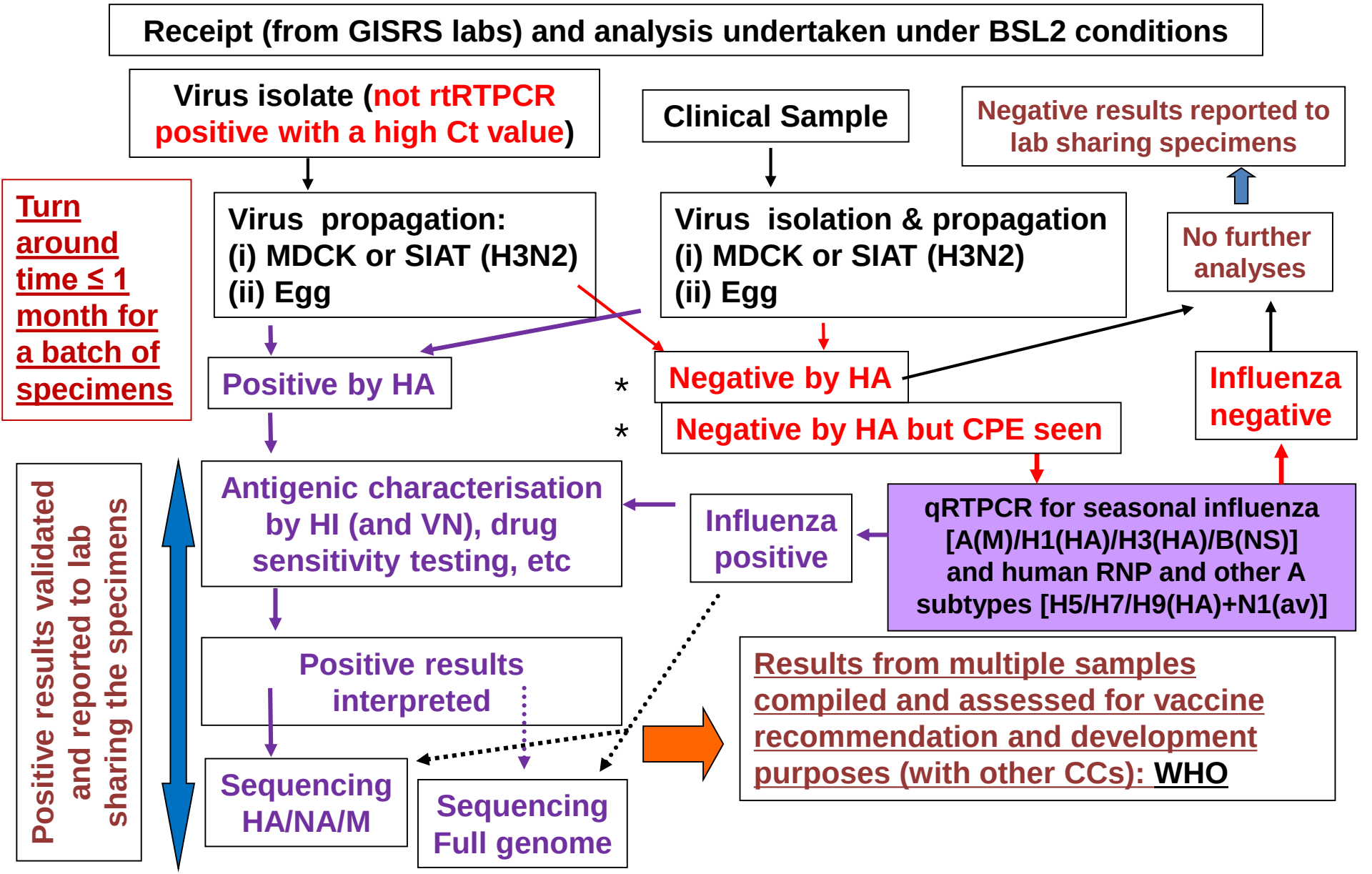
Seasonal Influenza Specimen Receipt and Analysis at WHO CC



* A significant number of recent H3N2 isolates quantified by MUNANA-based neuraminidase activity

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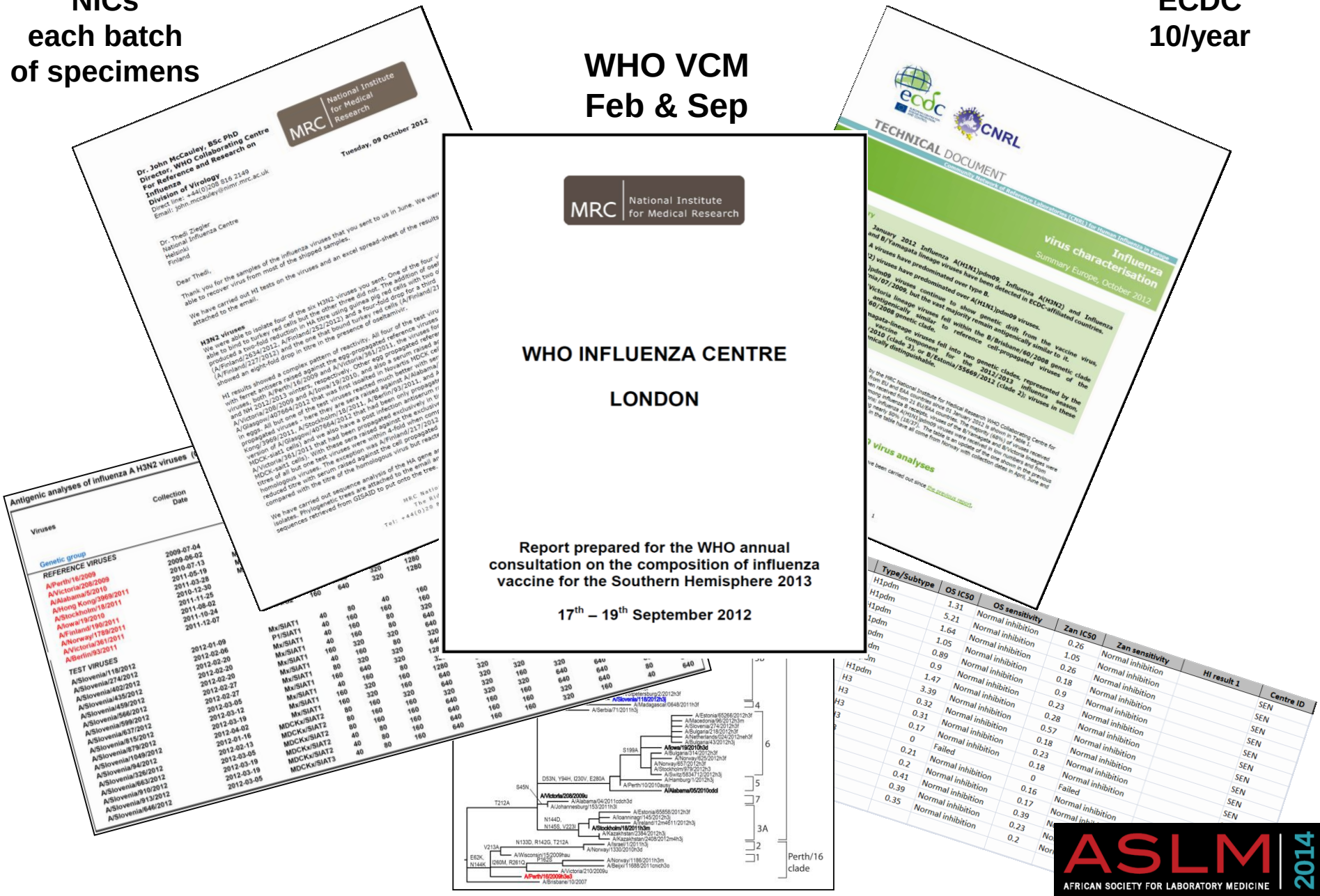
Reports prepared include antigenic, genetic and drug susceptibility data

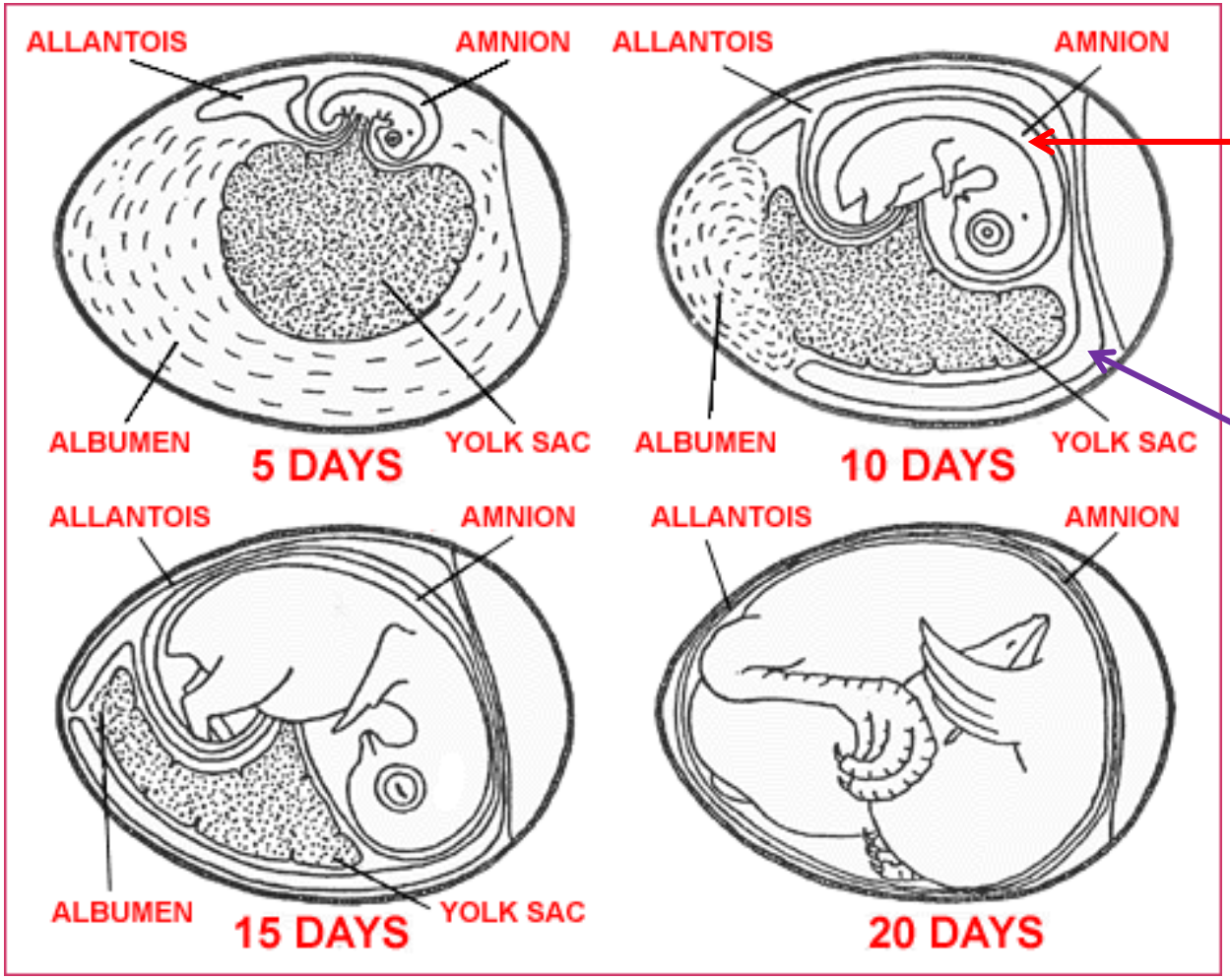
NICs
each batch
of specimens

WHO VCM
Feb & Sep

ECDC
10/year



The Bulk of Influenza Vaccine Production (~95%) is still Dependent on Propagation in Chicken Eggs



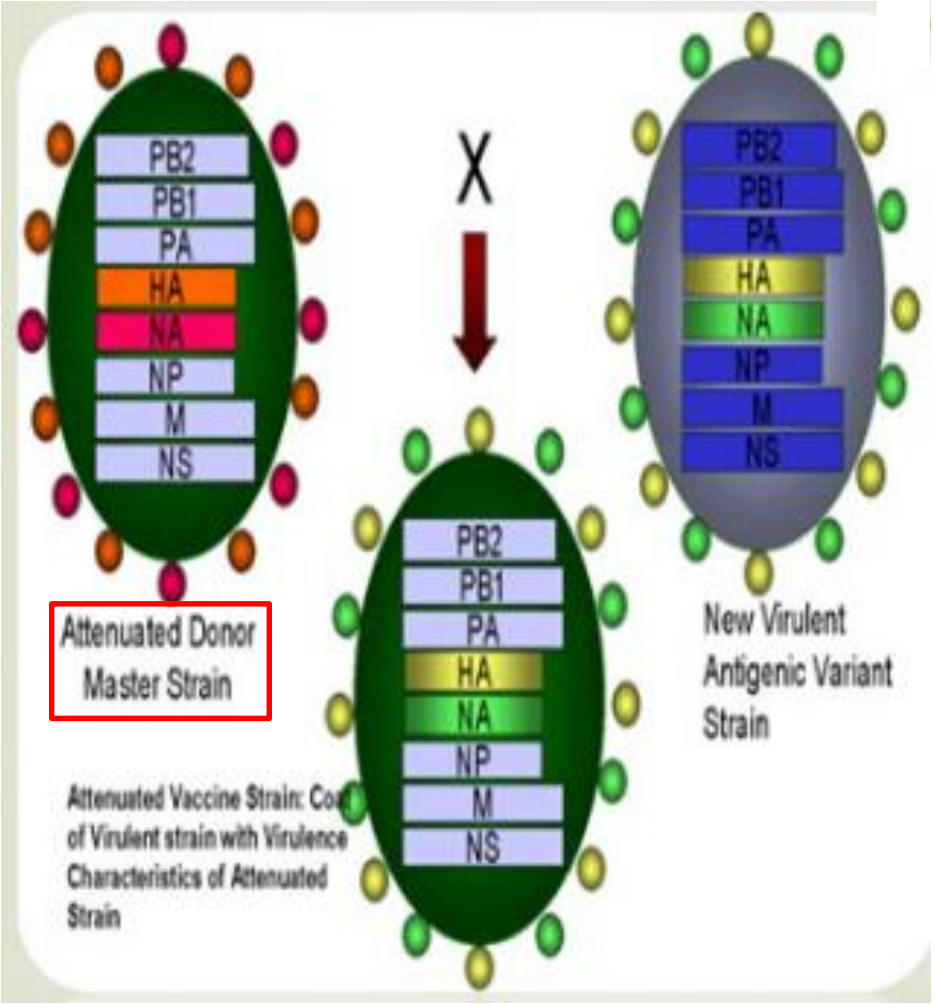
AMNIOTIC INOCULATION
Commonly the first route on inoculation in adapting influenza viruses to propagate in eggs – notably for A(H3N2) and type B viruses

ALLANTOIC INOCULATION
Following amniotic isolation viruses are propagated to high titre (and volume) in the allantoic cavity – as used in large-scale vaccine virus production

For Influenza Isolation/Propagation, and development of potential vaccine candidates, 10-15 day Embryonated Chicken Eggs are Commonly Used

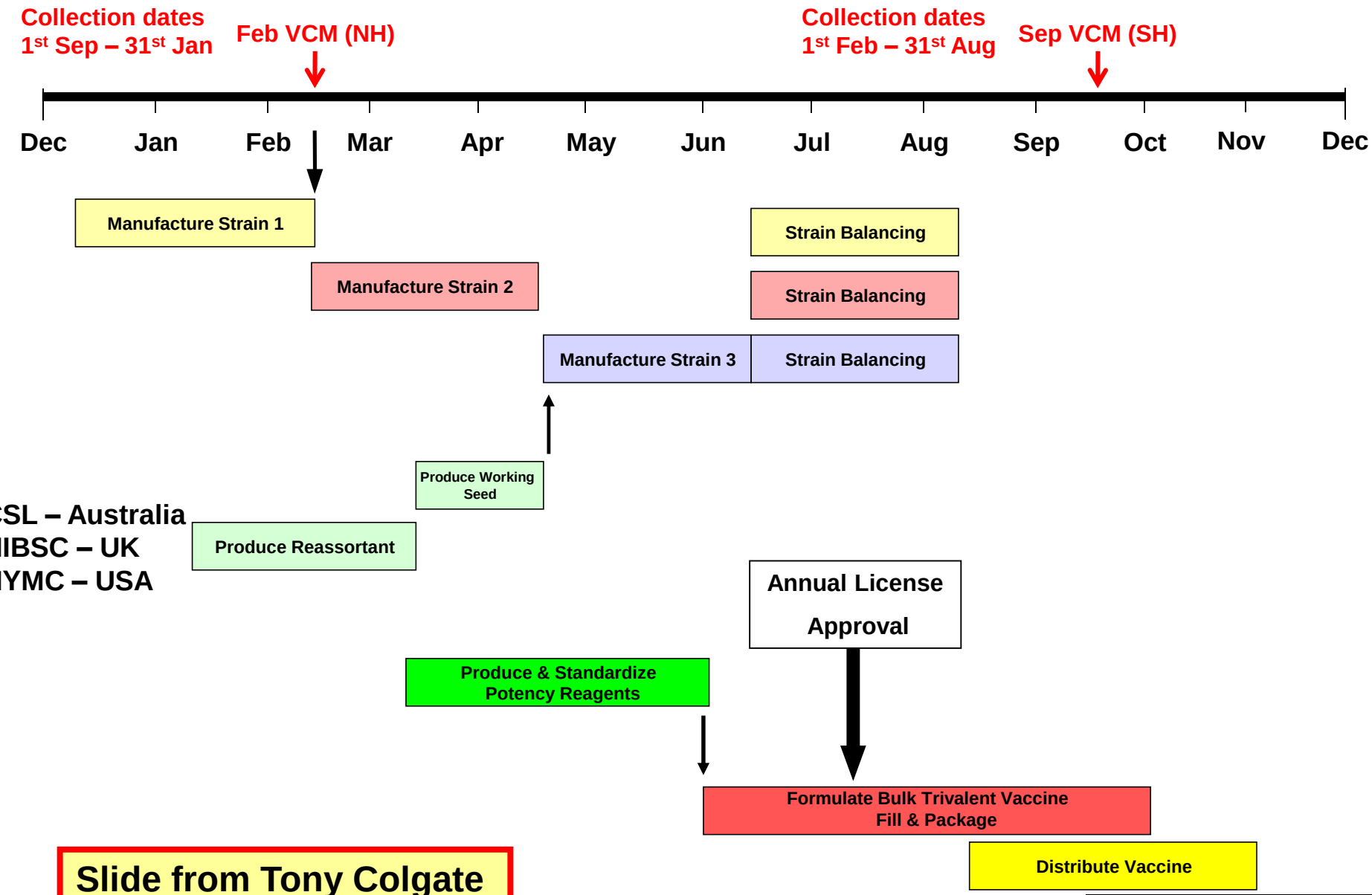
Generation of High Growth Reassortants (HGRs) in Eggs for Vaccine Production

- The egg is inoculated with a standard ‘attenuated’ virus (green) and the epidemic virus (grey) of interest.
- Both viruses replicate and their gene segments become mixed producing hybrid viruses known as reassortants.
- Reassortants with the HA and NA of the epidemic virus, but other genes from the attenuated virus are assessed for the correct antigenic properties and growth potential for vaccine production.



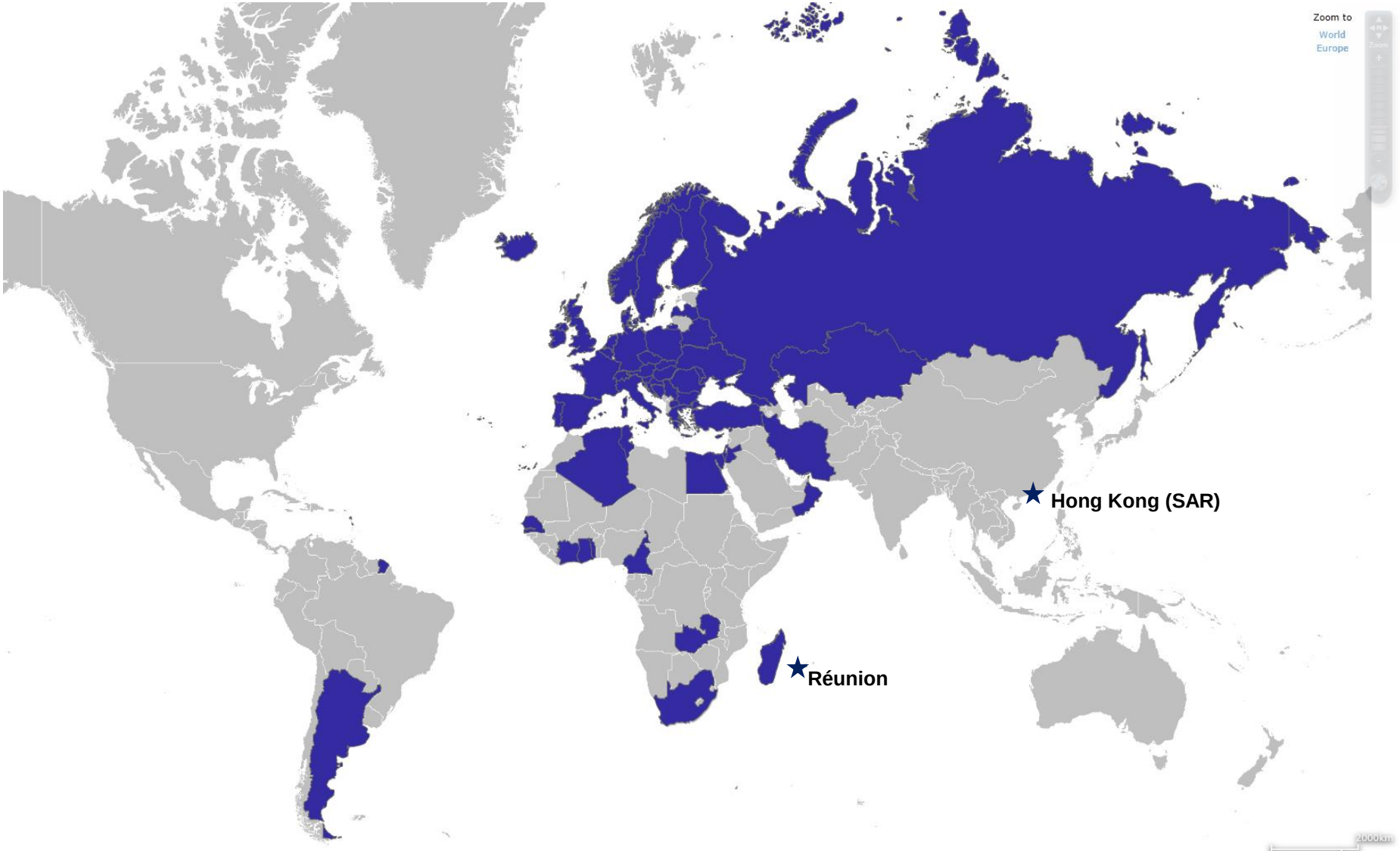
For type A influenza: commonly A/Puerto Rico/8/34 (H1N1)
For type B influenza: commonly B/Lee/40

Vaccine production Time Lines for TIV (Northern Hemisphere)



Slide from Tony Colgate Novartis Vaccines.

Countries/Territories sharing Influenza-positive specimens with WHO CC, London: 2013-2014 season



Just under 3000 specimens received in the 2013-2014 season

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African Influenza Specimens Shared with WHO CCs since the Emergence of A(H1N1)pdm09 viruses (based on sequences available in GISAID)

African Country	WHO Member State	WHO Region	WHO NIC	Shared with WHO CC:GISAID Sequences available (calendar year) 2009	2010	2011	2012	2013	2014	
Northern										
Algeria	Y	AFRO	Algiers	L	L	L	L	L		
Egypt	Y	EMRO	Cairo/Cairo	A, L	A, L	A, L	A, L	L		
Libya										
Morocco	Y	EMRO	Rabat	L	L	L				
South Sudan	Y	AFRO								
Sudan	Y	EMRO	Khartoum							
Tunisia	Y	EMRO	Tunis	L	L	L	L	L		
Western Sahara										
Western										
Benin	Y	AFRO								
Burkina Faso	Y	AFRO				A	A	A	A	
Cape Verde	Y	AFRO								
Côte d'Ivoire	Y	AFRO	Abidjan	A	A, L	L	A, L	A, L	L	
Gambia, The	Y	AFRO					L	L		
Ghana	Y	AFRO	Accra	L	L	L	L	A, L	L	
Guinea	Y	AFRO								
Guinea-Bissau	Y	AFRO								
Liberia	Y	AFRO								
Mali	Y	AFRO								
Mauritania	Y	AFRO								
Niger	Y	AFRO			L					via France (Paris)
Nigeria	Y	AFRO	Ibadan	A	A	A	A	A		
Saint Helena										
Senegal	Y	AFRO	Dakar	L	L	L	A, L	A, L	L	
Sierra Leone	Y	AFRO								
Togo	Y	AFRO						L		via Ghana
Central										
Angola	Y	AFRO								
Cameroon	Y	AFRO	Yaoundé	L	L	L	L	A, L	L	
Central African Republic	Y	AFRO	Bangui							
Chad	Y	AFRO								
Congo, Republic	Y	AFRO	Brazzaville							
Congo, Democratic Republic	Y	AFRO								
Equatorial Guinea	Y	AFRO								
Gabon	Y	AFRO								
São Tomé & Principe	Y	AFRO								
Eastern										
Burundi	Y	AFRO								
Comoros	Y	AFRO								
Djibouti	Y	EMRO		A						
Eritrea	Y	AFRO								
Ethiopia	Y	AFRO		A	A	A	A	A	A	
Kenya	Y	AFRO	Nairobi	M, A	M, A	A	A	A	A	
Madagascar	Y	AFRO	Antananarivo	L	L	L	L	L	L	
Malawi	Y	AFRO								
Mauritius	Y	AFRO	Candos	L			L	L		via Senegal
Mozambique	Y	AFRO								
Réunion					L				L	via France (Lyon)
Rwanda	Y	AFRO							A	
Seychelles	Y	AFRO		A						
Somalia	Y	EMRO								
Tanzania	Y	AFRO	Dar es Salaam	A	A	A	A	A		
Uganda	Y	AFRO	Entebbe	A		A	A			
Zambia	Y	AFRO			L	L	L	L		
Zimbabwe	Y	AFRO								
Southern										
Botswana	Y	AFRO								
Lesotho	Y	AFRO								
Namibia	Y	AFRO								
South Africa	Y	AFRO	Cape Town Sandringham	M, L	A, M, L	A, L	L	A, M, L	L	
Swaziland	Y	AFRO								
TOTALS 57	53	47/6	17 (16: 12/4)/2	18 (14/4)	17 (14/3)	17 (14/3)	18 (16/2)	18 (16/2)	11 (11/0)	

A Atlanta WHO CC (CDC)
M Melbourne WHO CC (VIDRL)
L London WHO CC (NIMR)

Location of WHO Regional Offices

Proceed to discuss individual influenza A subtypes and B lineages in relation to the September 2014 Vaccine Consideration Meeting

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Evolution of A(H1N1)pdm09 viruses:2009-2012

WHO CC London VCM Report for February 2012

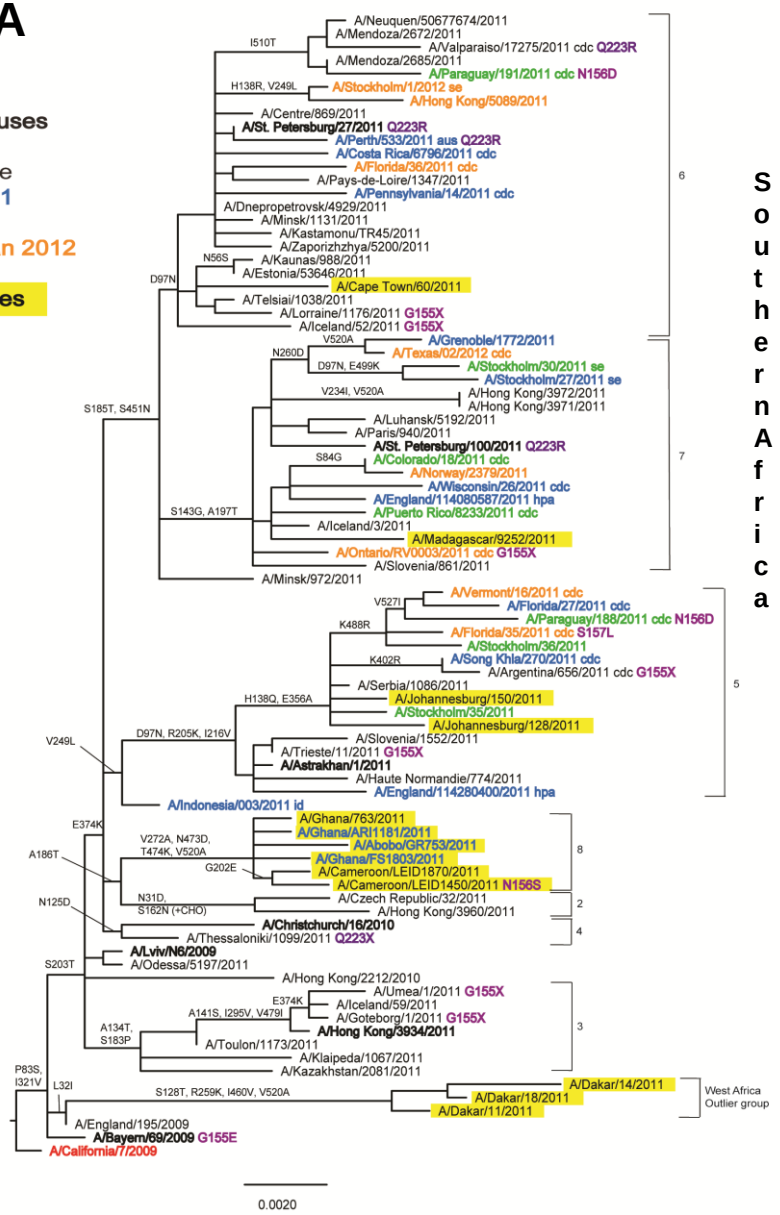
H1p-HA

Vaccine virus

Reference viruses

Collection date
Sep - Oct 2011
Nov 2011
Dec 2011 - Jan 2012

African samples



Southern Africa

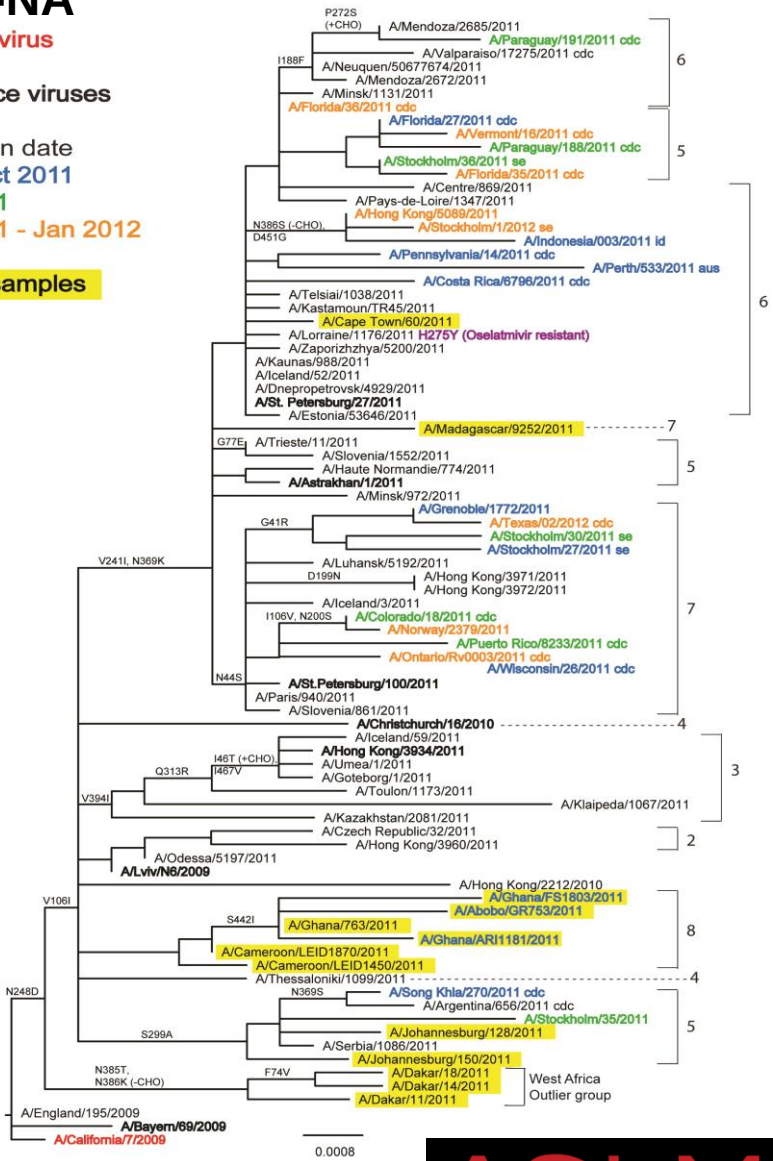
H1p-NA

Vaccine virus

Reference viruses

Collection date
Sep - Oct 2011
Nov 2011
Dec 2011 - Jan 2012

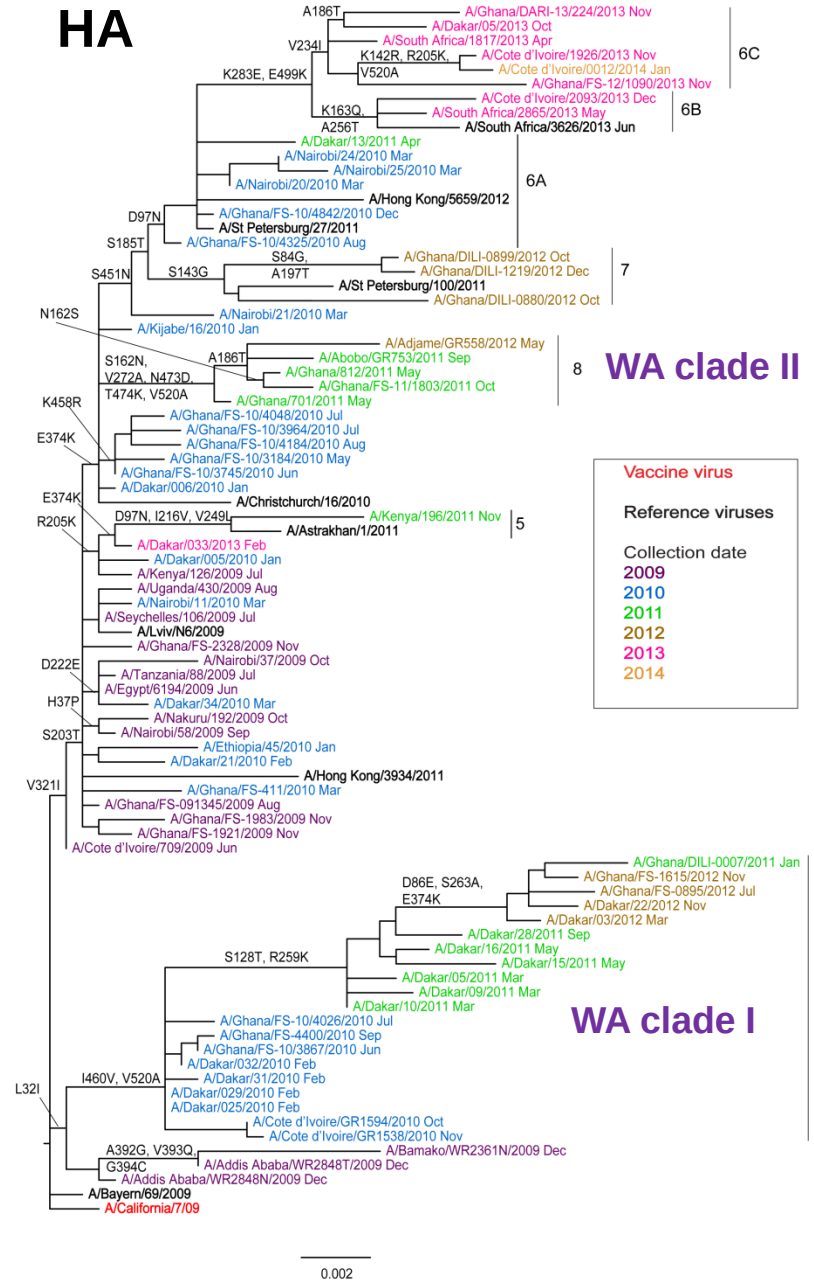
African samples

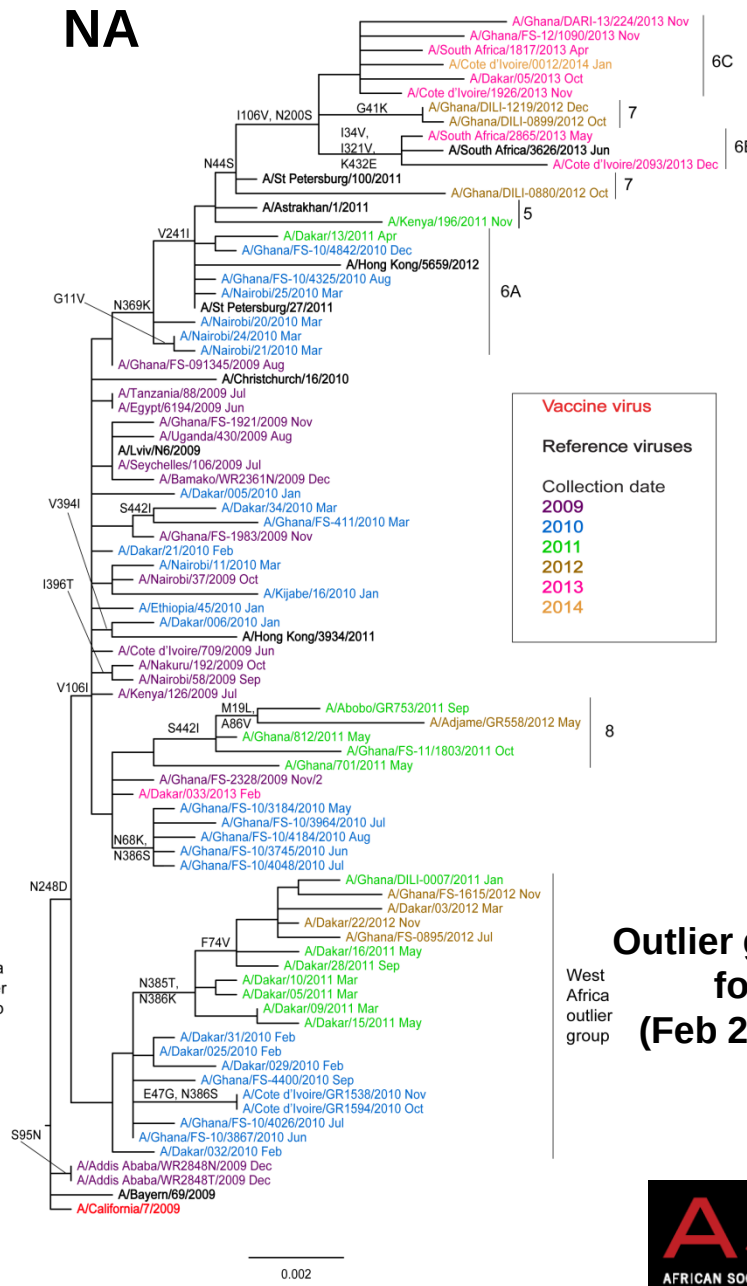


W+CA Africa

Eight genetic groups and a West Africa 'outlier' group had emerged

Divergent Evolution of A(H1N1)pdm09 viruses in Africa (1)





Nelson M I *et al* *J Inf Dis* (2014), 210, 121-125

The potential for novel influenza virus lineages to evolve within Africa warrants intensified influenza surveillance in Africa and other understudied areas

Outlier group persisted for ~3 years (Feb 2010-Nov 2012)

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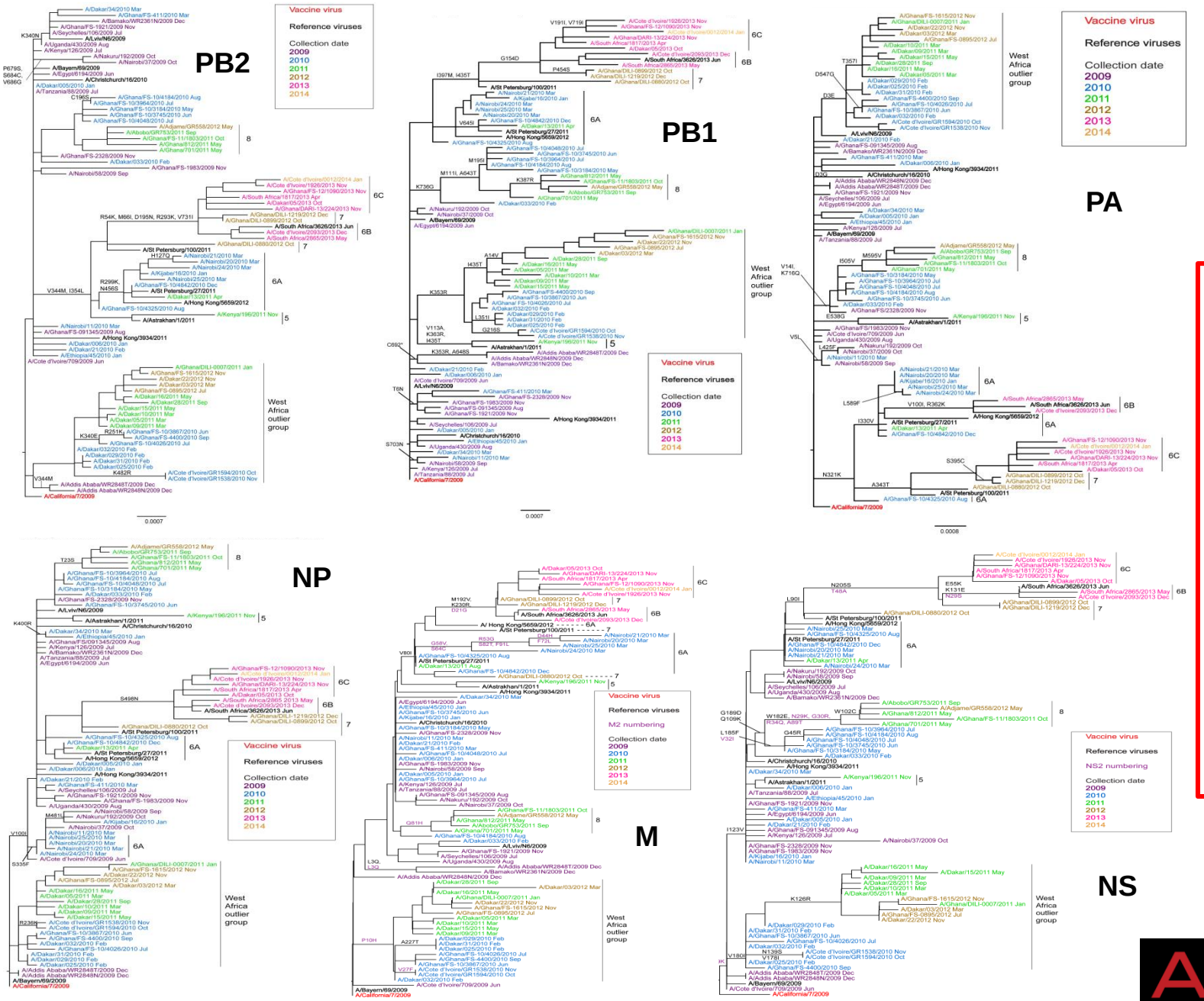
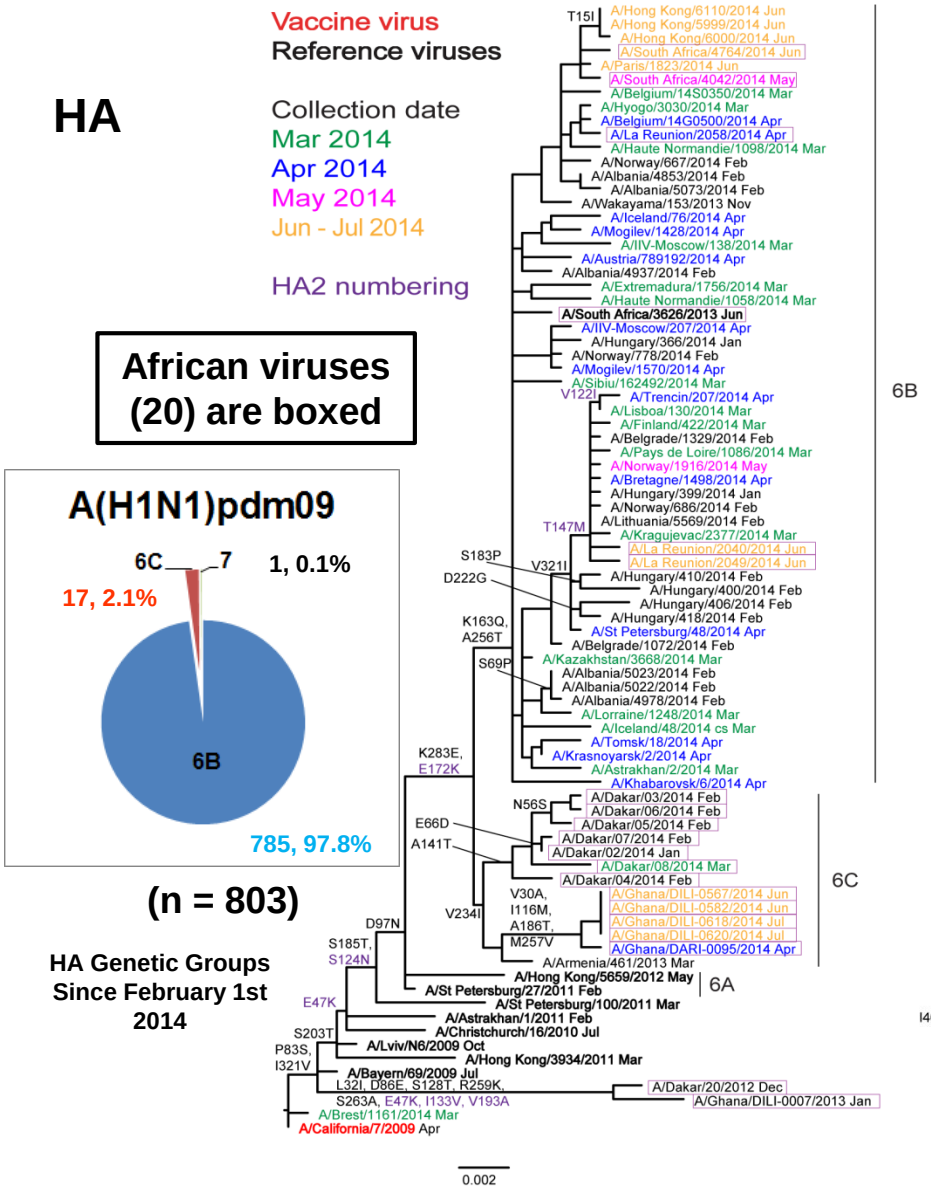
Divergent Evolution of A(H1N1)pdm09 viruses in Africa (2)

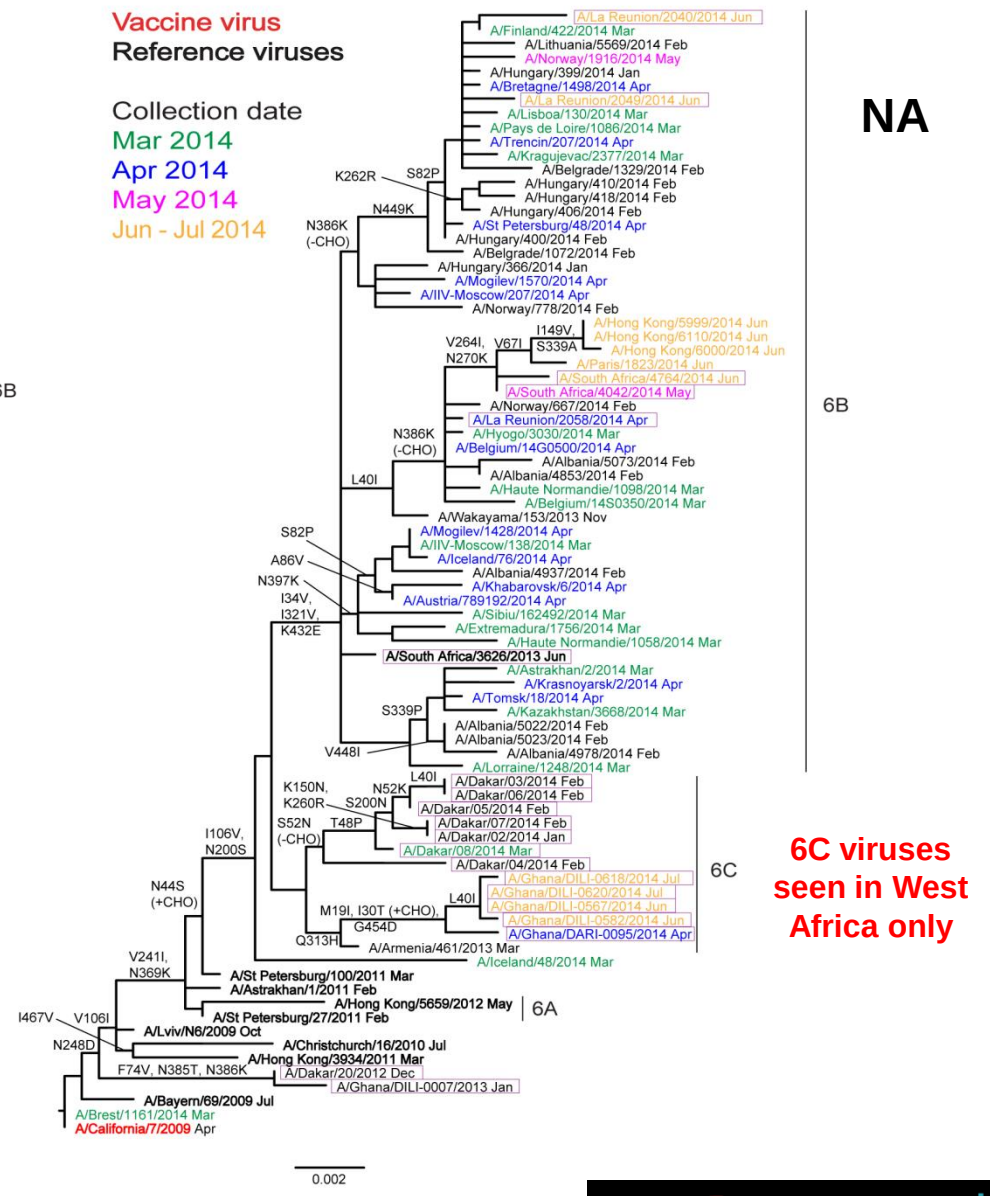


Full genome analyses conducted at WHO CC London show:

Genetic groups defined for HA are seen in all other genes – evidence for low level reassortant events in humans

A(H1N1)pdm09 Phylogenetic Analyses – September 2014 VCM





6C viruses seen in West Africa only

http://www.nimr.mrc.ac.uk/documents/about/NIMR-VCM-report-Sep-14-web.pdf

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Antigenic analyses (HI/TRBC) of influenza A(H1N1)pdm09 viruses

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre[1] — Post infection ferret antisera: A/Cal 7/09 F30/11	A/Bayern 69/09 F11/11	A/Lviv N6/09 F14/13	A/Chch 16/10 4	A/HK 3934/11 3	A/Astrak 1/11 5	A/St. P 27/11 6	A/St. P 100/11 7	A/HK 5659/12 6A	A/Sth Afr 3626/13 6B	X-243 NIBSC F48/14 6B	
REFERENCE VIRUSES															
A/California/7/2009		2009-04-09	EP1/E2	1280	1280	1280	320	320	320	640	1280	320	640	320	
A/Bayern/69/2009		2009-07-01	MDCK5/MDCK2	320	640	640	80	80	80	160	160	80	160	40	G155E
A/Lviv/N6/2009		2009-10-27	MDCK4/S1/MDCK3	640	1280	2560	160	80	160	320	160	320	160	80	G155E>G, D222G
A/Christchurch/16/2010	4	2010-07-12	E1/E3	1280	2560	2560	5120	2560	2560	2560	5120	2560	2560	2560	
A/Hong Kong/3934/2011	3	2011-03-29	MDCK2/MDCK3	640	320	640	640	1280	1280	1280	2560	1280	1280	1280	
A/Astrakhan/1/2011	5	2011-02-28	MDCK4/MDCK1	2560	1280	1280	1280	2560	2560	2560	5120	5120	2560	2560	
A/St. Petersburg/27/2011	6	2011-02-14	E1/E3	1280	1280	1280	1280	1280	2560	2560	5120	2560	2560	1280	
A/St. Petersburg/100/2011	7	2011-03-14	E1/E3	1280	1280	1280	1280	1280	2560	2560	5120	2560	1280	2560	
A/Hong Kong/5659/2012	6A	2012-05-21	MDCK4/MDCK2	640	320	640	640	1280	2560	2560	5120	2560	1280	1280	
A/South Africa/3626/2013	6B	2013-06-06	E1/E2	1280	1280	1280	640	1280	1280	1280	5120	1280	2560	2560	
X-243 (A/South Africa/3626/2013)	6B		EX/E1	2560	1280	2560	2560	5120	5120	5120	5120	5120	2560	5120	
TEST VIRUSES															
A/Estonia/85899/2014		2014-03-25	MDCK2/MDCK1	2560	1280	2560	1280	2560	2560	2560	5120	5120	2560	2560	
A/Estonia/86382/2014	6B	2014-04-16	MDCK2/MDCK1	2560	1280	2560	2560	5120	5120	5120	5120	5120	5120	5120	
A/Estonia/85829/2014	6B	2014-03-21	MDCK2/MDCK1	640	640	640	640	1280	1280	1280	2560	2560	1280	1280	
A/Estonia/85847/2014		2014-03-20	MDCK2/MDCK1	1280	1280	1280	1280	2560	5120	2560	5120	5120	2560	2560	
A/Estonia/85792/2014		2014-03-19	MDCK1/MDCK1	2560	1280	2560	2560	5120	5120	5120	5120	5120	5120	2560	
A/Estonia/85739/2014	6B	2014-03-18	MDCK2/MDCK1	1280	640	640	640	1280	1280	2560	5120	2560	2560	1280	
A/Estonia/85759/2014		2014-03-18	MDCK2/MDCK1	1280	1280	1280	1280	2560	2560	2560	5120	5120	2560	2560	
A/Estonia/85729/2014		2014-03-17	MDCK1/MDCK1	2560	1280	2560	1280	2560	2560	5120	5120	5120	5120	2560	
A/Estonia/85629/2014		2014-03-13	MDCK2/MDCK1	2560	1280	2560	2560	5120	5120	5120	5120	5120	5120	2560	
A/Estonia/85660/2014		2014-03-13	MDCK2/MDCK1	1280	1280	1280	1280	2560	5120	5120	5120	5120	5120	2560	
A/Estonia/85519/2014	6B	2014-03-10	MDCK1/MDCK1	1280	640	1280	1280	1280	2560	2560	5120	2560	2560	1280	
A/Estonia/85422/2014		2014-03-06	MDCK2/MDCK1	2560	1280	2560	2560	5120	5120	5120	5120	5120	5120	5120	
A/Estonia/85408/2014		2014-03-05	MDCK2/MDCK1	2560	1280	2560	2560	2560	5120	5120	5120	5120	2560	5120	
A/Estonia/85353/2014		2014-03-04	MDCK1/MDCK1	2560	1280	2560	2560	5120	5120	5120	5120	5120	5120	5120	
A/Estonia/85246/2014		2014-02-28	MDCK1/MDCK1	2560	640	1280	2560	2560	2560	2560	5120	5120	2560	2560	
A/Estonia/85212/2014		2014-02-27	MDCK2/MDCK1	1280	640	1280	1280	2560	2560	2560	5120	5120	2560	2560	
A/Estonia/85108/2014		2014-02-25	MDCK1/MDCK1	1280	640	1280	1280	2560	2560	2560	5120	5120	2560	2560	
A/Estonia/85066/2014		2014-02-21	MDCK2/MDCK1	2560	1280	2560	2560	5120	5120	5120	5120	5120	5120	5120	
A/Estonia/84981/2014		2014-02-20	MDCK2/MDCK1	1280	1280	1280	1280	2560	2560	2560	5120	5120	2560	2560	
A/Estonia/84627/2014		2014-02-10	MDCK2/MDCK1	2560	640	1280	2560	2560	2560	5120	5120	5120	5120	2560	
A/Estonia/84639/2014		2014-02-10	MDCK2/MDCK1	1280	320	640	640	1280	2560	2560	5120	2560	2560	2560	
A/Estonia/84490/2014		2014-02-06	MDCK2/MDCK1	2560	1280	2560	2560	5120	5120	5120	5120	5120	5120	5120	
A/Estonia/84426/2014	6B	2014-02-04	MDCK3/SIAT2	640	320	1280	2560	1280	2560	1280	5120	1280	640	5120	
				Vaccine											

All ferret antisera raised against non-genetic group 1 viruses show at least 4-fold reductions in HI titres with A/California/7/2009 compared to the respective homologous titres

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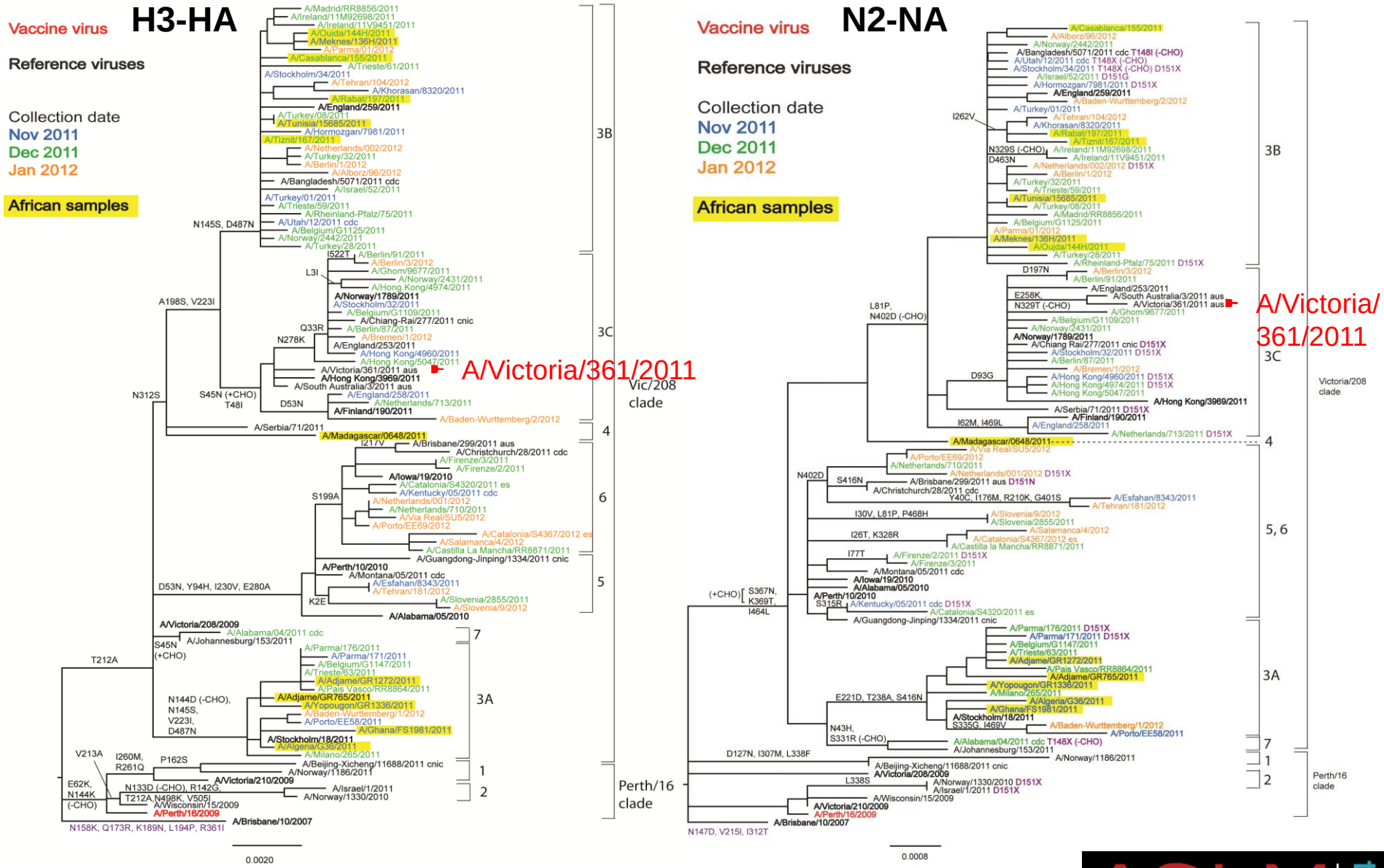
A(H1N1)pdm09 low reactors in HI assays by WHO CC (September 2014 VCM)

WHO CC	A/Cal/07/09	Low (≥ 8 fold)
CDC	662 (99.7%)	2 (0.3%)
CNIC	683 (100%)	0
NIID	51 (100%)	0
NIMR	204 (99.5%)	1 (0.5%)
VIDRL	1061 (99.4%)	6 (0.6%)
Total	2661 (99.7%)	9 (0.3%)

Evolution of A(H3N2) viruses:2009-2012

WHO CC London VCM Report for February 2012



Seven genetic groups had emerged and group 3 was subdividing (A-C)

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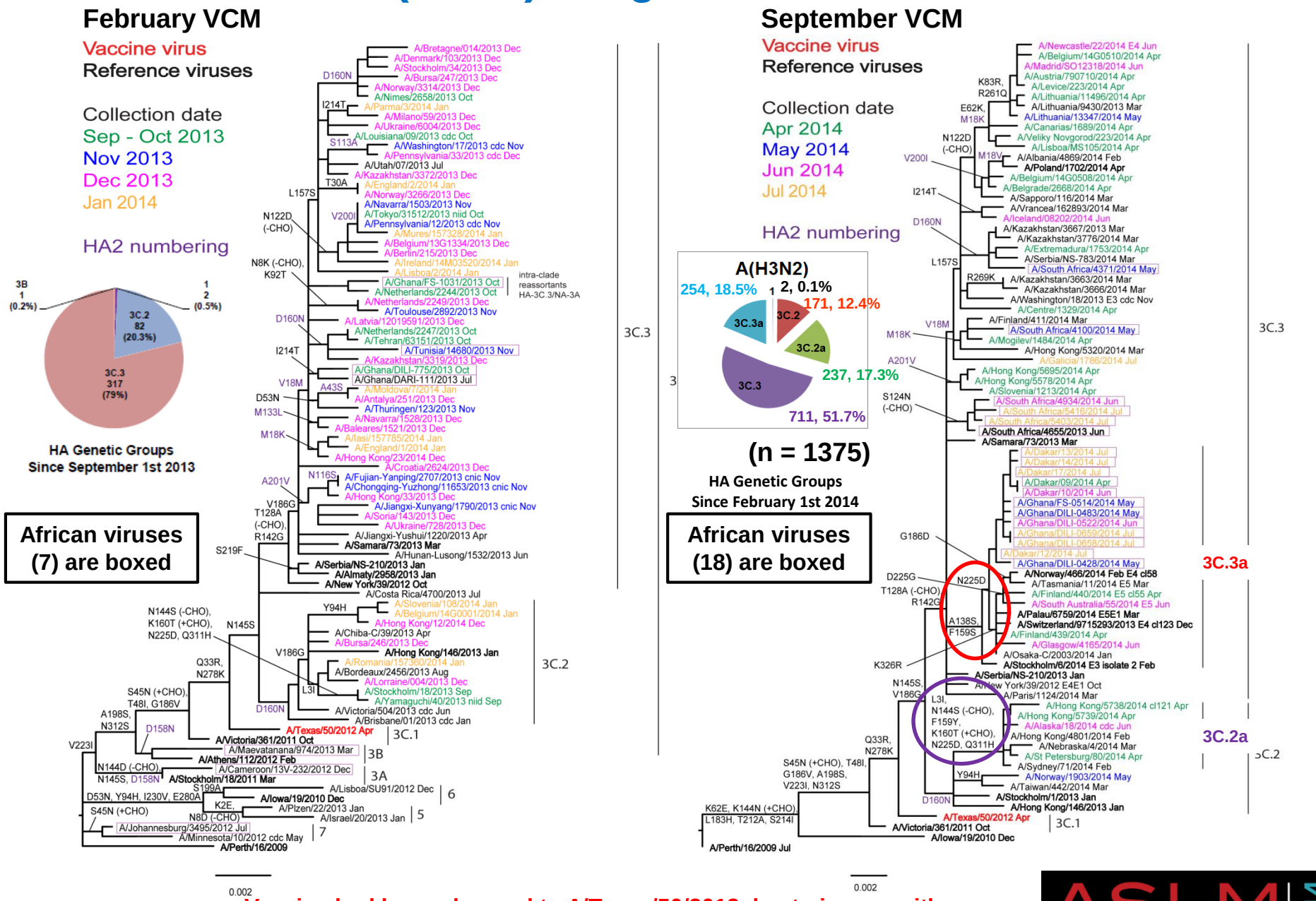
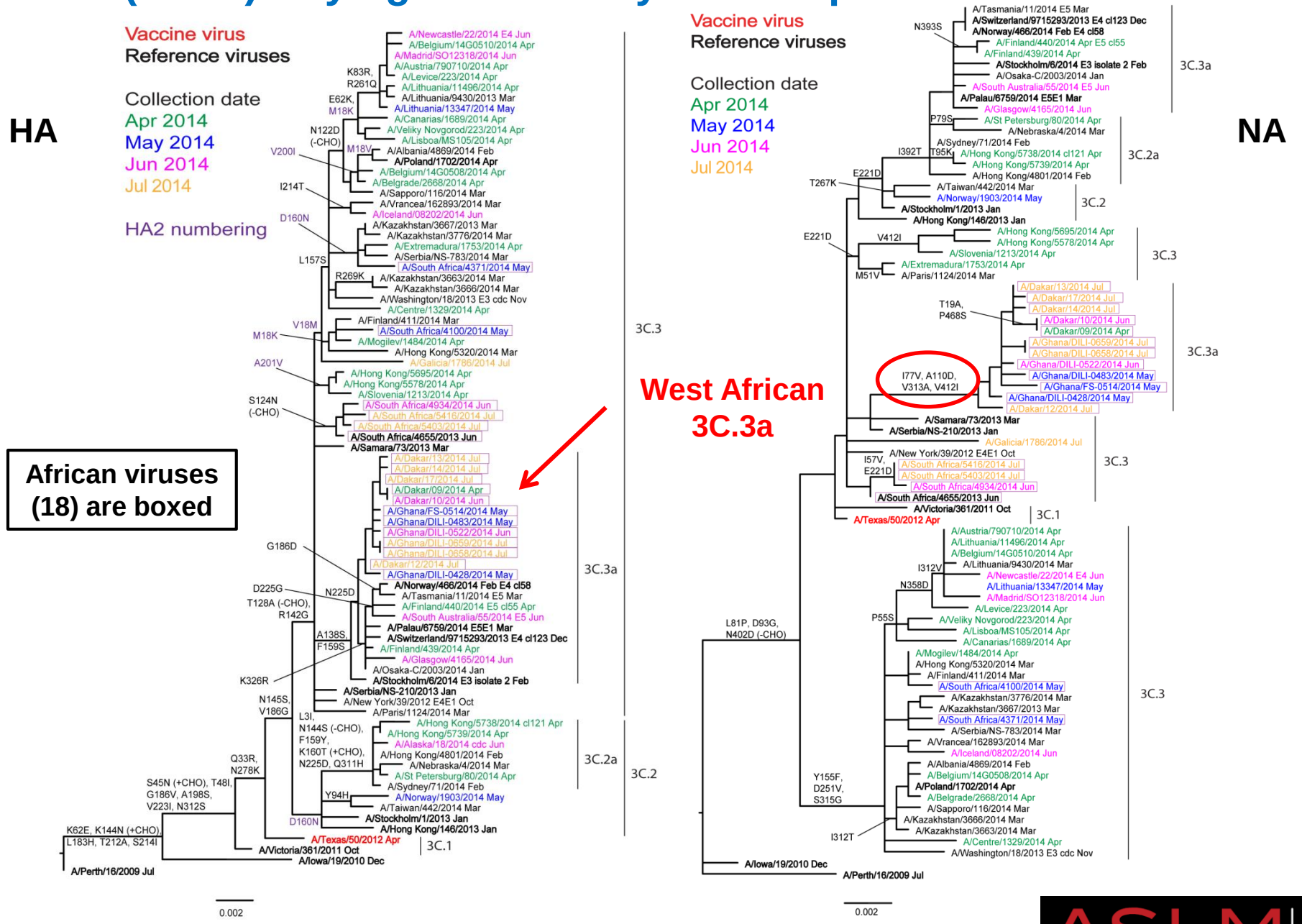
Evolution of A(H3N2) HA genes between VCMS: 2014



A(H3N2) Phylogenetic Analyses – September 2014 VCM



http://www.nimr.mrc.ac.uk/documents/about/NIMR-VCM-report-Sep-14-web.pdf

Antigenic analyses (HI/GPRBC + 20nM oseltamivir) of influenza A(H3N2) viruses

Viruses	Genetic group	Collection Date	Passage History	Haemagglutination inhibition titre[1] Post-infection ferret antisera A/Vic 361/11 T/C F09/12	A/Texas 50/12 Egg F42/12	A/Samara 73/13 F24/13	A/HK 146/13 F40/13	A/Stock 6/14 F14/14	A/Nor 466/14 F13/14
				3C.1	3C.1	3C.3	3C.2	3C.3a	3C.3a
REFERENCE VIRUSES									
A/Victoria/361/2011	3C.1	2011-10-24	MDCK2/SIAT4	320	1280	1280	640	640	320
A/Texas/50/2012	3C.1	2012-04-15	E5/E2	1280	1280	640	640	80	40
A/Samara/73/2013	3C.3	2013-03-12	C1/SIAT2	1280	640	2560	2560	640	640
A/Hong Kong/146/2013	3C.2	2013-01-11	E6	640	640	1280	2560	160	80
A/Stockholm/6/2014	3C.3a	2014-02-06	SIAT2/SIAT1	80	40	160	160	320	320
A/Norway/466/2014	3C.3a	2014-02-03	SIAT2/SIAT1	80	40	160	160	320	320
TEST VIRUSES									
A/Ghana/DILI-0428/2014	3C.3a	2014-05-02	C1/SIAT1	<	<	80	40	320	160
A/Ghana/FS-0514/2014	3C.3a	2014-05-17	C1/SIAT1	80	40	160	80	320	320
A/Ghana/DILI-0479/2014	3C.3a	2014-05-19	C1/SIAT1	40	40	80	80	320	320
A/Ghana/DARI-0101/2014	3C.3a	2014-05-19	C1/SIAT1	40	40	80	80	320	320
A/Ghana/DILI-0483/2014	3C.3a	2014-05-20	C1/SIAT1	40	<	80	80	320	320
A/Ghana/DARI-0104/2014	3C.3a	2014-05-27	C1/SIAT1	80	40	160	80	320	320
A/Ghana/DILI-0522/2014	3C.3a	2014-06-02	C2/SIAT1	160	40	160	160	640	640
A/Dakar/10/2014	3C.3a	2014-06-18	C1/SIAT1	80	40	160	80	320	320
A/Dakar/12/2014	3C.3a	2014-07-07	C2/SIAT1	40	40	160	160	320	320
A/Ghana/DILI-0659/2014	3C.3a	2014-07-22	C1/SIAT1	80	40	160	80	320	320
					Vaccine				

1. < = <40

Low reactivity **Good reactivity**

Problems with growth of 3C.2a viruses to HA titres sufficient for HI assay

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Antigenic analyses of influenza A(H3N2) viruses - Plaque Reduction Neutralisation (MCDK-SIAT)

Viruses	Genetic group	Collection Date	Passage History	Neutralisation titre[1]						
				Post-infection ferret antisera						
				A/Vic 361/11 T/C F09/12	A/Texas 50/12 E F42/13	A/Stock 6/14 T/C F14/14	A/Switz 9715923/13 T/C NIBSC F13/14	A/Switz 9715923/13 E Cl123 F25/14	A/Nor 466/14 T/C F13/14	A/Nor 466/14 E Cl58 F24/14
				3C.1	3C.1	3C.3a	3C.3a	3C.3a	3C.3a	3C.3a
REFERENCE VIRUSES										
A/Victoria/361/2011	3C.1	2011-10-24	MDCK2/SIAT4	320	320	320	80	320	160	80
A/Texas/50/2012	3C.1	2012-04-15	E5/E2	1280	1280	320	80	320	80	160
A/Stockholm/6/2014	3C.3a	2014-02-06	SIAT2/SIAT3/MDCK1	40	40	160	40	80	80	40
A/Switzerland/9715293/2014	3C.3a	2013-12-06	SIAT1/SIAT2	40	40	160	160	80	160	40
A/Switzerland/9715293/2013 CI123	3C.3a	2013-12-06	E4	80	80	160	80	160	80	40
A/Norway/466/2014	3C.3a	2014-02-03	SIAT2/SIAT3	80	80	320	160	80	160	80
A/Norway/466/2014 CI32	3C.3a	2014-02-03	E4	320	320	640	320	1280	320	640
A/Norway/466/2014 CI58	3C.3a	2014-02-03	E4	160	160	640	320	160	160	160
TEST VIRUSES										
A/Hong Kong/5695/2014	3C.3	2014-04-21	SIAT1	320	320	640	320	160	160	160
A/Hong Kong/5578/2014	3C.3	2014-04-04	SIAT1	320	320	320	320	160	80	80
A/Hong Kong/5320/2014	3C.3	2014-03-20	SIAT1	320	640	640	320	160	160	160
A/Nebraska/4/2014	3C.2a	2014-03-11	C2/SIAT1	80	40	160	160	80	80	40
A/Hong Kong/4801/2014	3C.2a	2014-02-26	MDCK2	80	80	320	160	80	80	80
A/Hong Kong/5738/2014	3C.2a	2014-04-30	MDCK2	40	40	160	80	80	80	40
A/Finland/440/2014	3C.3a	2014-04-28	SIAT1	40	40	160	160	80	80	40
A/Finland/439/2014	3C.3a	2014-04-23	SIAT1	40	40	320	320	80	160	80
A/Finland/438/2014	3C.3a	2014-04-03	SIAT1	40	40	320	320	80	320	80
A/Finland/437/2014	3C.3a	2014-03-24	SIAT1	80	40	320	160	80	160	80
A/Finland/428/2014	3C.3a	2014-02-17	SIAT1	80	40	320	320	80	320	80
					Vaccine					

[1] Readings show the antiserum doubling dilution value corresponding to 50% plaque reduction

Antisera raised against 3C.1 viruses show poor reactivity with 3C.2a and 3C.3a viruses

Antisera raised against 3C.3a viruses show cross-reactivity with 3C.2a viruses

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H3 low reactors in HI assays by WHO CC (September 2014 VCM)

WHO CC	A/Texas/50/12	Low (≥ 8 fold)
CDC	85 (41.0%)	120 (59.0%)
CNIC	16 (2.1%)	756 (97.9%)
NIID	23 (29.5 %)	55 (70.5%)
NIMR	47 (13.3%)	307 (86.7%)
VIDRL	96 (24.2%)	301 (75.8%)
Total	267 (14.8%)	1539 (85.2%)

Direct egg isolation of A(H3N2) 3C.3a and 3C.2a viruses to produce candidate vaccine viruses

Clade	Virus	Passage History	HAU GPRBC	HA amino acid position & identity*																										Homol HI titre	Ferret number
				3	92	128	138	140	142	144	156	159	160	183	186	190	194	196	203	219	225	246	311	312	326	489	526				
				L	K	A	S	I	G	N	H	S	K	H	G	D	L	A	T	S	D	N	Q	S	K	D	L				
3C.3a	A/Stockholm/6/2014	SIAT																												640	14/14
		E (Am1AI1)	32-64								R										G									640	19/14
		E (Am2AI1)	32-64												V						N									640	20/14
Pending	NIB-90														V						N	K (-CHO)									
3C.3a	A/Norway/466/2014	SIAT2	32																					R						320	13/14
			64								X				X						G			R							
		E3	32-64												X						X	X (-CHO)		R							
		E4 clone 32	>256												E						G	S (-CHO)		R						5120	23/14
		E4 clone 39	>256												E						G	S (-CHO)		R							
		E4 clone 58	128-256												E						G			R						160	24/14
Failed 1-way	NIB-89										R				E						G			R							
3C.3a	A/Switzerland/9715293/2013	SIAT2	32																					R						160	NIB 13/14
			128					R			X				V									R							
		E2	128-256					X							X						X			R							
		E3 clone 123	128-256					R							V									R						1280 1280	25/14 32/14
2-way pass	NIB-88														V					Y				R						160	NIB 54/14
2-way pass	X-247														V					F				R						640	31/14
		E3 clone 125	64					R							V									R							
		E3 clone 128	32-64					R							V									R							
		E3 clone132	128					R							V									R		F					
		E3 clone 130	32-64																		G	S (-CHO)		R							
		E3 clone 135	64																		G	S (-CHO)		R							
3C.3a	A/Finland/428/2014		64-128							X (-CHO)											G			R							
			128	X	X					X (-CHO)						X		X			G	X (-CHO)		R							
3C.3a	A/Finland/437/2014		64								X										G	X (-CHO)		R							
			32																		G	X (-CHO)		R							
3C.3a	A/Finland/438/2014		64												V						N			R							
			128												V					X	N	X (-CHO)		R							
3C.3a	A/Finland/439/2014		64					X			X				X						X			R							
			64-128					K							V									R							
3C.3a	A/Finland/440/2014	SIAT																						R						160	NIB 47/14
		E3	16-32											X		X					G			R							
		E4 clone 54	32																		G	H (-CHO)		R							
		E4 clone 55	64											F							G			R						160	NIB 42/14
3C.2a	A/Hong Kong/5738/2014	SIAT		I		T (+CHO)	A		R	S (-CHO)		Y	T (+CHO)										H	X		N			160	30/14
		E3	16-32										K (-CHO)				P		X					N							
		E4 clone 121	32										K (-CHO)				P		I					N						1280	NIB 53/14
		E4 clone 126	16										K (-CHO)				P		I					N							
		E4 clone 128	8										K (-CHO)				P		I					N							

* Numbered from the start of the HA1 glycoprotein (signal peptide removed).

X indicates amino acid polymorphism at the HA position indicated.

Insertion (+CHO) or removal (-CHO) of a N-linked glycosylation site.

Antigenic analyses (HI/TRBC) of influenza B/Victoria-lineage viruses

Viruses	Genetic group	Collection date	Passage History	Haemagglutination inhibition titre: Post infection ferret sera								
				B/Bris[1,3] 60/08 Sh 522	B/Mal[2] 2506/04 F37/11	B/Bris[2] 60/08 F22/12	B/Paris[2] 1762/09 F07/11	B/Malta[2] 636714/11 F29/13	B/Jhb[2] 3964/12 F01/13	B/Sth Aus[2] 81/12 F41/13	B/HK[2] 514/09 F9/13	B/Odessa[2] 3886/10 F19/11
	Genetic group			1A		1A	1A	1A	1A	1A	1B	1B
REFERENCE VIRUSES												
B/Malaysia/2506/2004		2004-12-06	E3/E6	1280	640	80	<	80	160	160	20	<
B/Brisbane/60/2008	1A	2008-08-04	E4/E3	1280	160	320	80	640	640	1280	80	40
B/Paris/1762/2009	1A	2009-02-09	C2/MDCK2	2560	10	20	80	40	40	80	80	80
B/Malta/636714/2011	1A	2011-03-07	E4/E1	1280	80	160	40	320	320	640	40	20
B/Johannesburg/3964/2012	1A	2012-08-03	E1/E2	5120	320	640	80	1280	1280	1280	160	80
B/South Australia/81/2012		2012-11-28	E4/E1	1280	160	320	80	320	320	1280	80	40
B/Hong Kong/514/2009	1B	2009-10-11	MDCK1/MDCK2	2560	10	80	160	160	160	320	160	160
B/Odessa/3886/2010	1B	2010-03-19	MDCK2/MDCK4	2560	<	40	80	40	80	160	160	160
TEST VIRUSES												
B/Cameroon/743/2014		2014-02-05	MDCK1	2560	10	40	80	20	40	160	80	160
B/Norway/970/2014	1A	2014-03-07	MDCK1	5120	10	40	80	40	40	160	80	160
B/Kumamoto/46/2014	1A	2014-03-14	MDCK1/MDCK1/MDCK1	5120	20	80	160	80	160	320	160	80
B/Cameroon/2080/2014		2014-03-24	MDCK2/MDCK1	5120	10	40	40	10	<	40	80	40
B/Cameroon/2053/2014		2014-03-26	MDCK2/MDCK1	5120	<	40	160	40	80	160	80	160
B/Cameroon/2052/2014	1A	2014-03-26	MDCK2/MDCK1	5120	<	20	80	40	10	80	80	160
B/Cameroon/2315/2014	1A	2014-03-31	MDCK2/MDCK1	5120	20	40	160	160	160	160	160	160
B/Cameroon/2293/2014	1A	2014-04-03	MDCK2/MDCK1	5120	<	20	80	40	10	80	80	160
B/Ghana/DILI-0434/2014	1A	2014-04-28	C1/MDCK1	2560	<	20	160	80	80	80	80	40
B/Ghana/DILI-0487/2014	1A	2014-05-20	C1/MDCK1	2560	20	20	160	40	40	160	80	80
B/Ghana/DILI-0506/2014	1A	2014-05-27	C1/MDCK1	2560	10	40	80	80	40	160	80	80
B/Ghana/DILI-0531/2014	1A	2014-06-03	MDCK1	5120	10	20	80	80	80	160	80	80
B/Ghana/DILI-0572/2014	1A	2014-06-16	C1/MDCK1	2560	10	20	80	80	80	160	80	80
B/Cameroon/4641/2014		2014-06-25	MDCK1/MDCK1	5120	10	20	20	40	10	40	80	40
B/Cameroon/4314/2014	1A	2014-06-25	MDCK1/MDCK1	5120	10	20	20	40	10	40	80	40
B/Cameroon/4736/2014	1A	2014-07-04	MDCK1/MDCK1	5120	10	20	20	20	10	40	80	20
B/Cameroon/4737/2014		2014-07-05	MDCK1/MDCK1	5120	10	20	20	20	10	40	80	80
B/Cameroon/4681/2014	1A	2014-07-09	MDCK1/MDCK1	2560	<	20	20	20	10	20	80	40

1. < = <40; 2. < = <10; 3. hyperimmune sheep serum

* B/Victoria-lineage virus recommended for use in quadravalent vaccines

Vaccine* N197S (-CHO)

Cell propagated surrogate for B/Bris/60/08

Common feature of egg isolates is the loss of glycosylation at position 197

Test viruses show good reactivity with sera raised against cell-propagated viruses but low reactivity with sera raised against egg-propagated viruses.

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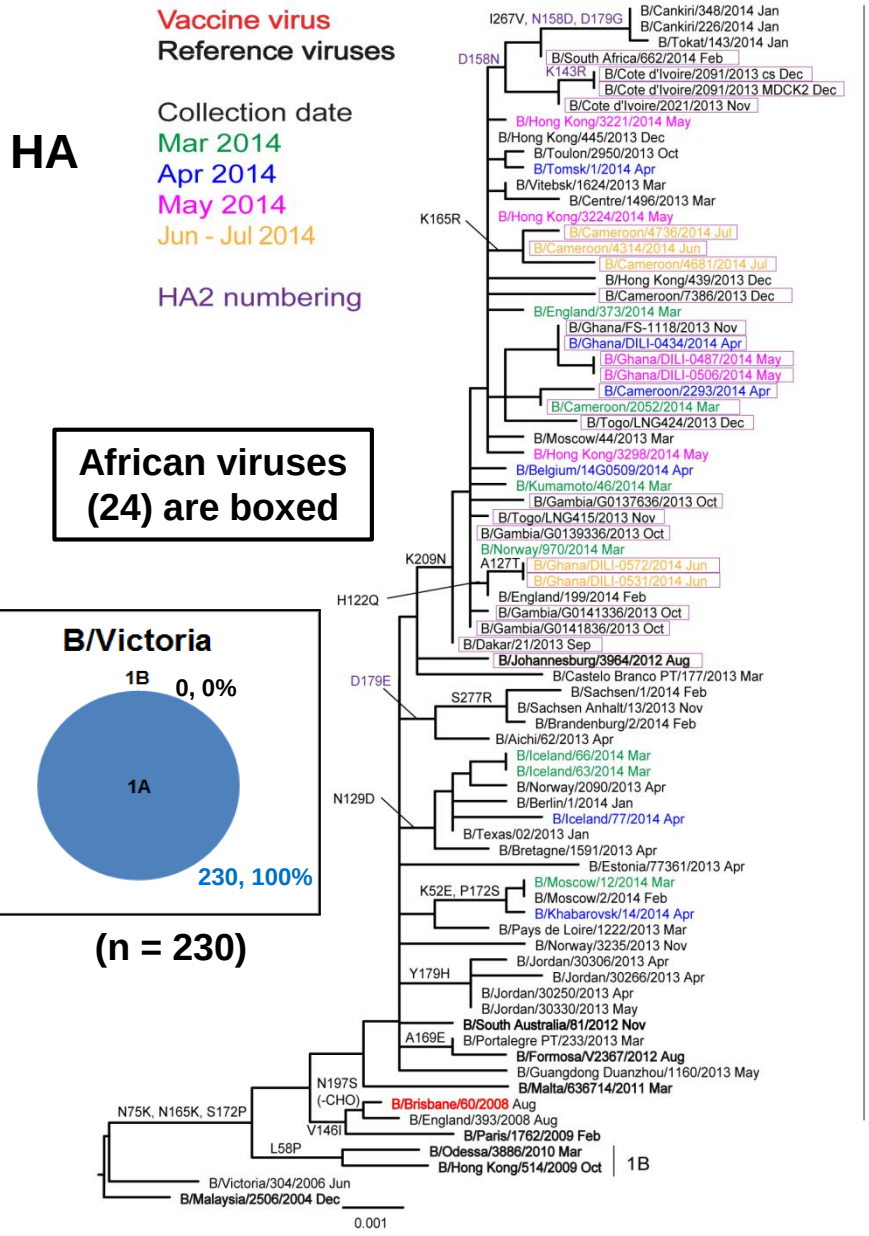
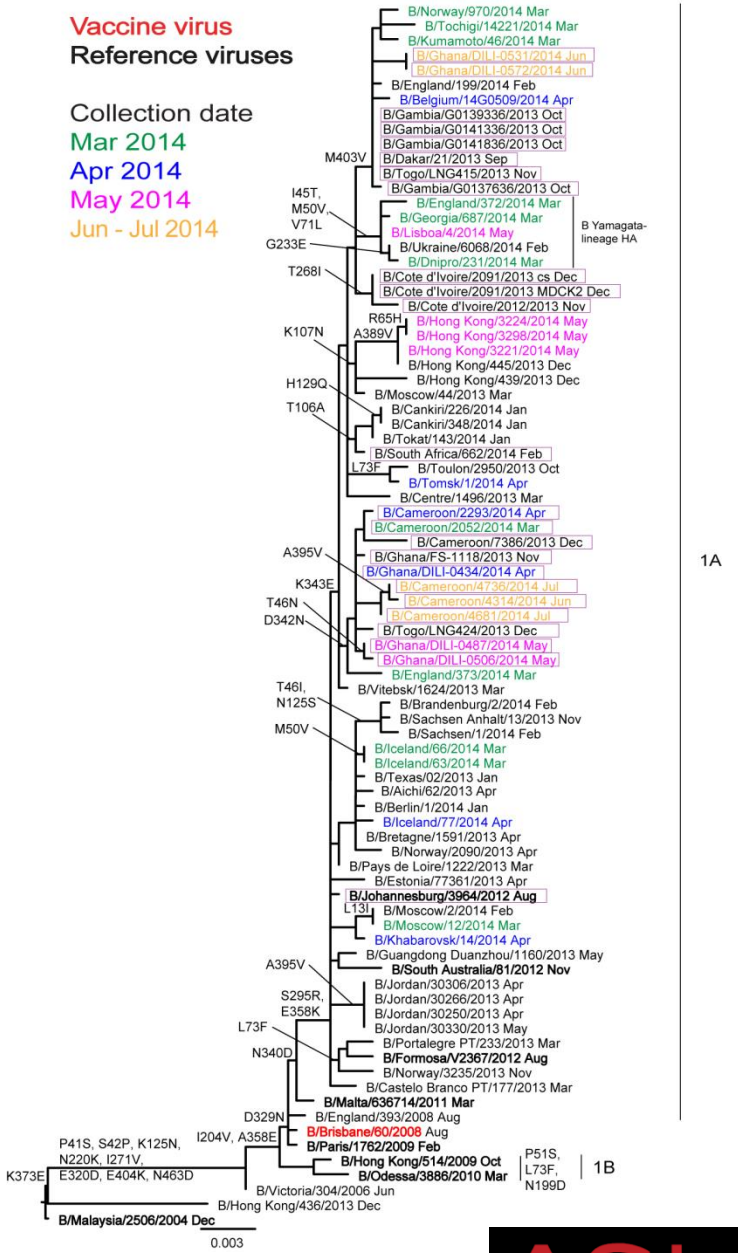
Influenza B/Vic low reactors in HI assays in WHO CC (September 2014 VCM)

WHO CC	Victoria (Bris/60/2008)
CDC	223 (98%)
Low Reactors	4 (2%)
CNIC	30 (63%)
Low Reactors	18 (37%)
NIID	10 (100%)
Low Reactors	0 (0%)
NIMR	23 (77%)
Low Reactors	7 (23%)
VIDRL	26 (96%)
Low Reactors	1 (4%)
Total	312 (91%)
Low Reactors	30 (9%)

Influenza B/Vic Phylogenetic Analyses – September 2014 VCM





http://www.nimr.mrc.ac.uk/documents/about/NIMR-VCM-report-Sep-14-web.pdf

Antigenic analyses (HI/TRBC) of influenza B/Yamagata-lineage viruses

Viruses	Genetic Group	Collection date	Passage History	Haemagglution Inhibition Titre: Post infection ferret antisera						
				B/Fl[1,3] 4/06 SH479	B/Estonia[2] 55669/11 F26/11	B/Mass[2] 02/12 Egg F2/13	B/Mass[2] 02/12 T/C F15/13	B/Wis[2] 1/10 F10/13	B/Stock[2] 12/11 F12/12	B/Phuket[2] 3073/13 AUS F3064-21D Egg
	Genetic Group			1	2	2	2	3	3	3
REFERENCE VIRUSES										
B/Florida/4/2006	1	2006-12-15	E7/E1	2560	160	640	160	320	640	640
B/Estonia/55669/2011	2	2011-03-14	MDCK1/MDCK1	1280	640	320	640	80	80	160
B/Massachusetts/02/2012	2	2012-03-13	E3/E4	5120	160	1280	320	320	1280	1280
B/Massachusetts/02/2012	2	2012-03-13	MDCK1/C2/MDCK3	5120	640	1280	640	320	640	1280
B/Wisconsin/1/2010	3	2010-02-20	E3/E2	1280	<	320	40	320	640	640
B/Stockholm/12/2011	3	2011-03-28	E4/E1	1280	<	320	40	80	320	320
B/Phuket/3073/2013	3	2013-11-21	E4/E1	1280	<	320	40	160	320	640
TEST VIRUSES										
B/Phuket/3073/2013	3	2013-11-21	MDCK2/MDCK1	1280	80	320	160	20	320	640
B/Norway/1877/2014	3	2014-05-21	MDCK1	1280	80	320	160	20	320	320
B/Norway/2011/2014	3	2014-06-19	MDCK1	640	40	320	80	20	320	320
B/Brisbane/9/2014	3	D/M unknown	E4/E1	640	<	160	40	160	320	320
B/Norway/2045/2014		2014-05-28	MDCK2	1280	80	160	160	160	320	ND
B/Cameroon/1640/2014	2	2014-03-10	MDCK1/MDCK1	2560	640	320	640	10	160	320
B/Cameroon/2082/2014	2	2014-03-20	MDCK1/MDCK1	2560	640	160	640	10	160	160

1. < = <40; 2. < = <10; 3. hyperimmune sheep serum; ND = Not done

Vaccine

Previous vaccine

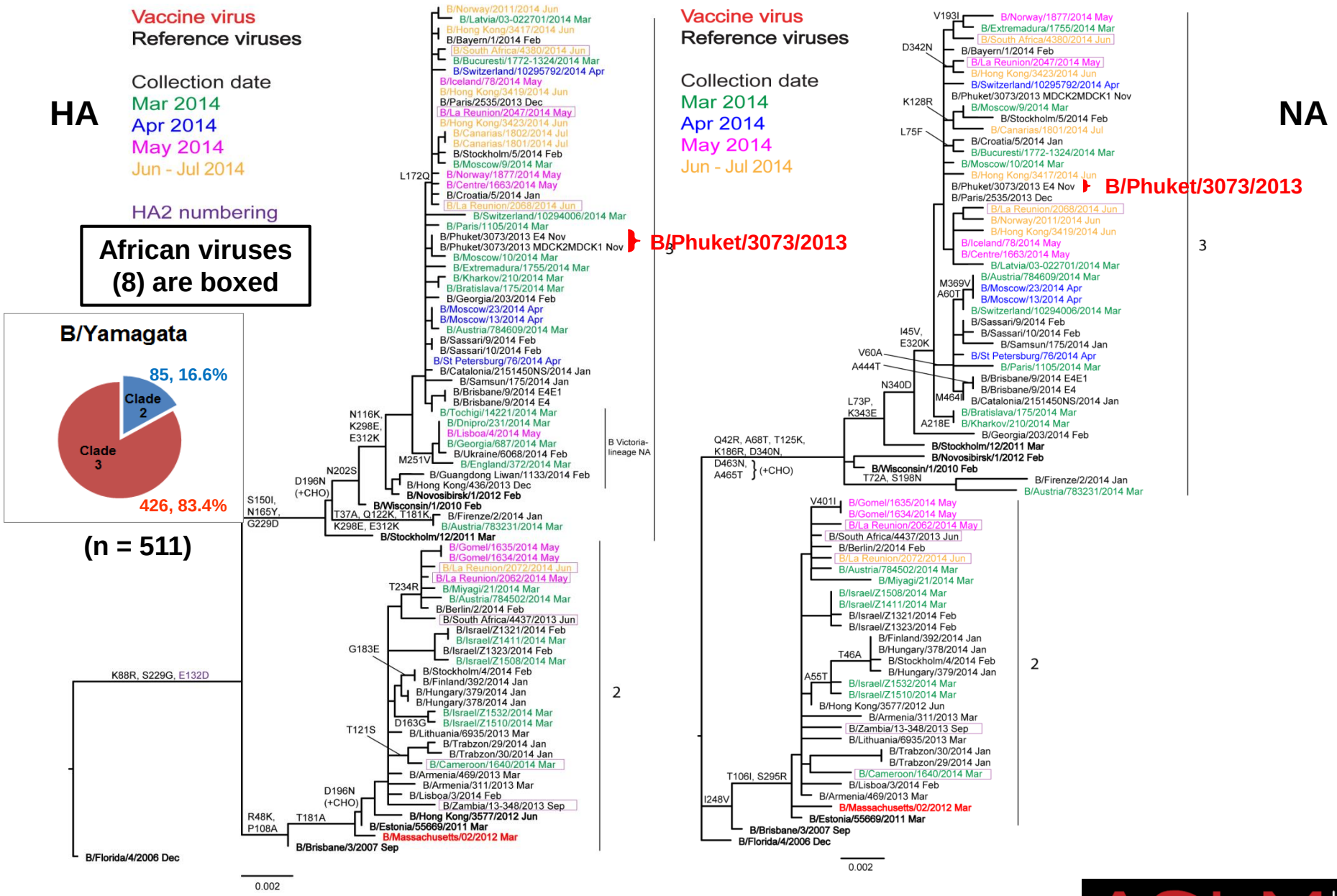
≥8-fold reduction in HI titre compared to the homologous titre

Good reactivity with antisera raised against more recent clade 3 viruses

Influenza B/Yam low reactors in HI assays in WHO CC (September 2014 VCM)

WHO CC	Yamagata (Mass/2/2012)
CDC	541 (99%)
Low Reactors	3 (1%)
CNIC	821 (94%)
Low Reactors	55 (6%)
NIID	73 (100%)
Low Reactors	0 (0%)
NIMR	38 (67%)
Low Reactors	19 (33%)
VIDRL	65 (30%)
Low Reactors	153 (70%)
Total	1538 (87%)
Low Reactors	230 (13%)

Influenza B/Yam Phylogenetic Analyses – September 2014 VCM



http://www.nimr.mrc.ac.uk/documents/about/NIMR-VCM-report-Sep-14-web.pdf

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WHO: Availability and Provision of Candidate Vaccine Viruses

Seasonal Influenza

http://www.who.int/influenza/vaccines/virus/candidates_reagents/summary_a_h1n1_cvv_sh15.pdf?ua=1

http://www.who.int/influenza/vaccines/virus/candidates_reagents/summary_a_h3n2_cvv_sh15.pdf?ua=1

http://www.who.int/influenza/vaccines/virus/candidates_reagents/summary_b_yam_cvv_sh15.pdf?ua=1

http://www.who.int/influenza/vaccines/virus/candidates_reagents/summary_b_vic_cvv_sh15.pdf?ua=1

Sites are updated after each VCM and as additional High Growth Reassortants (HGRs) become available

Zoonotic Influenza (Pandemic Potential)

http://www.who.int/influenza/vaccines/virus/201409_zoonotic_vaccinevirusupdate.pdf?ua=1

The latest update following the September 2014 VCM, giving indication of what viruses have been selected for production of candidate vaccine viruses (attenuated, using reverse genetics) and those that are already available.

The Team: WHO CC London

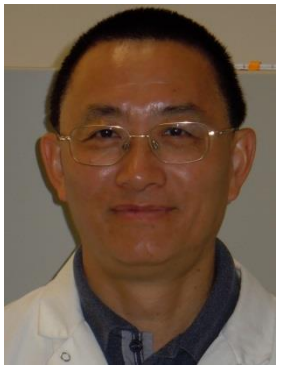
John McCauley

Rod Daniels

YiPu Lin

Victoria Gregory

Lynne Whittaker



Zheng (John) Xiang

Karen Cross

Chandi Halai

Aine Rattigan

Burcu Emetal