6-12-2016

Characterization of HA and NA-containing VLPs produced in suspension cultures of HEK 293 cells

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Innovative Influenza vaccines, a challenging regulatory path in pandemic situations

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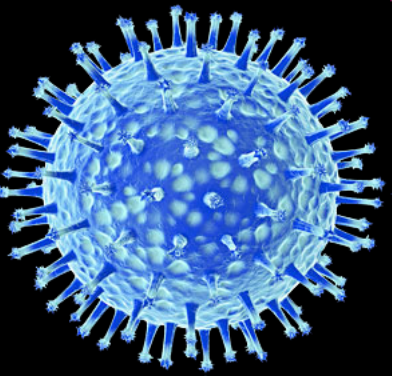
Vaccine Technology VI, Albufeira, Portugal, June 12-17, 2016



Influenza

- Influenza viruses :
  - ✓ Annual epidemics
  - ✓ high probability of new influenza pandemics
- Up to 500,000 deaths due to influenza infection occur annually, with 3-5 million cases of severe illness
- Pandemic Influenza preparedness plan requires rapid responses and capacity for vaccine manufacturing
- Approximately 70% to 90% in healthy adults <65 years of age when the vaccine and circulating viruses are well-matched (CDC)

Health Canada Santé Canada

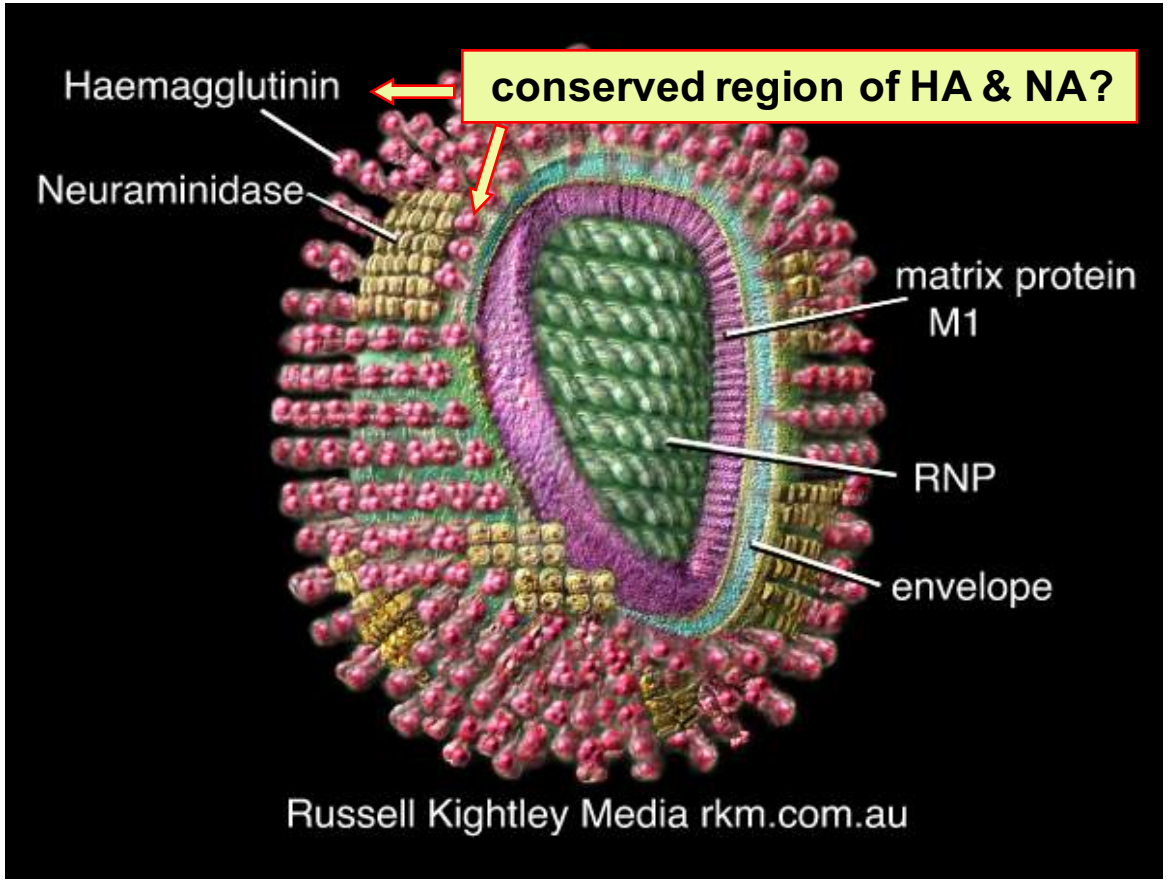
Influenza A Viruses

17 Hemagglutinin
X
9 neuraminidase

↓

153 different virus subtypes,

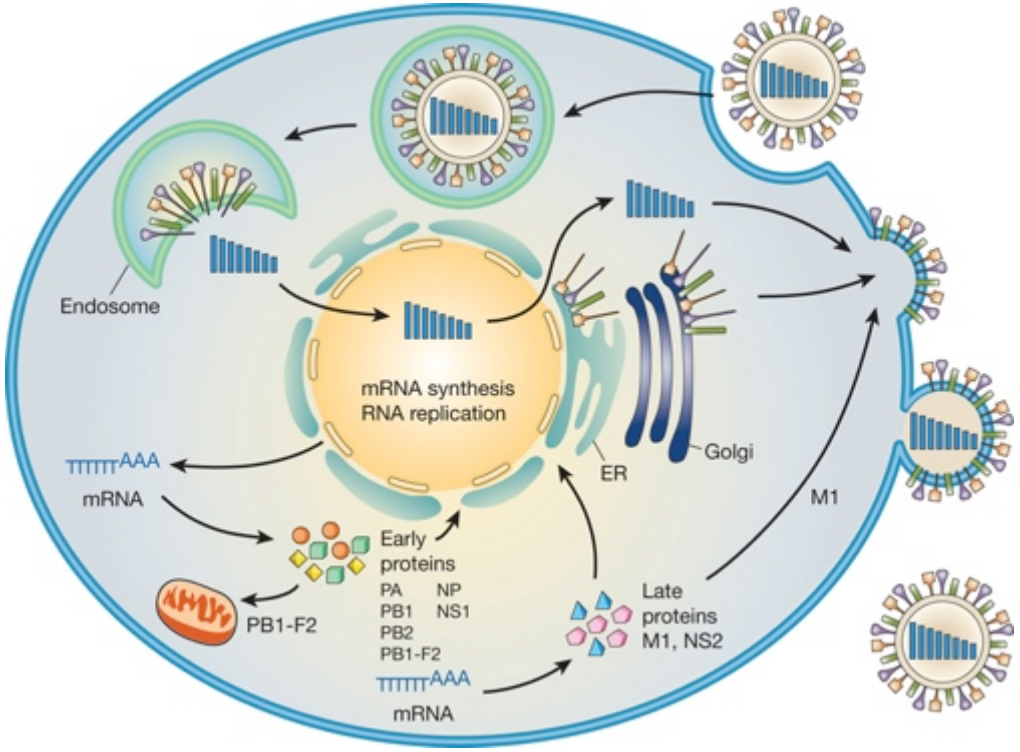
over 11900 different strains (A, human host IRD data base)



http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html

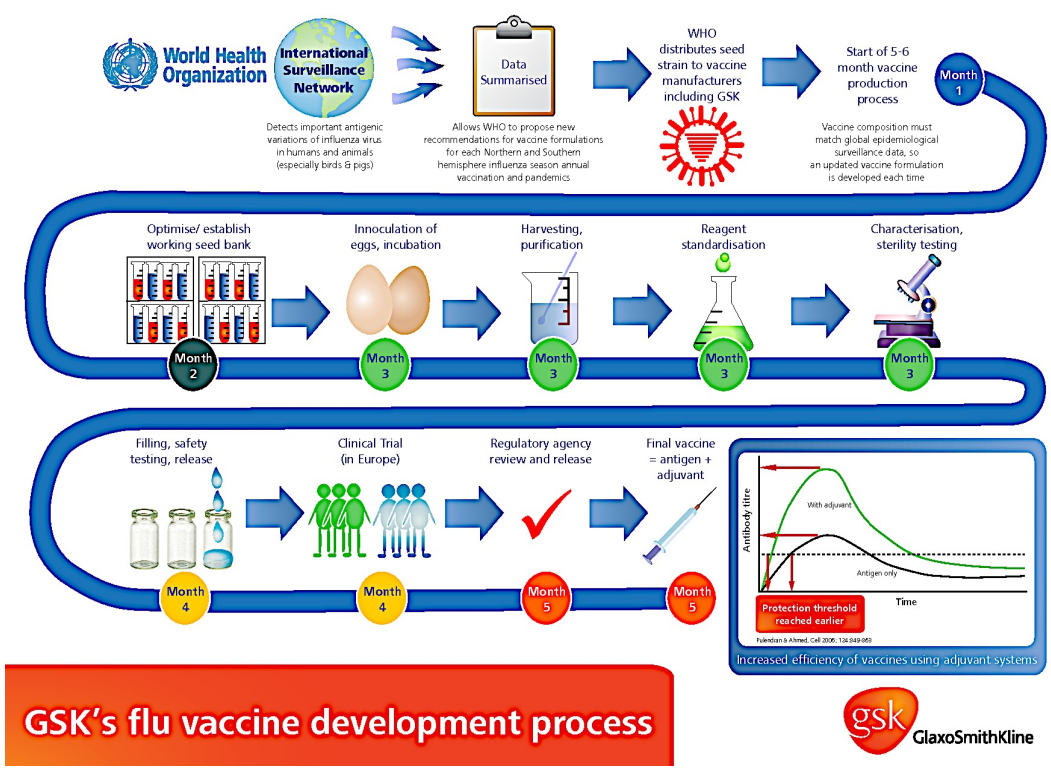
Influenza Life Cycle

- Attach to cell via binding HA1 to sialic acid receptors
- HA, NA, M2 produced and sent to cell membrane
- M1 joins via binding to HA NA or M2
- Released from cell via budding, NA cleaves sialic acid which HA1 could bind back to



Neumann, G., Noda, T., & Kawaoka, Y. (2009). Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature*, *459*(7249), 931–9.

Current Influenza Vaccine Manufacturing

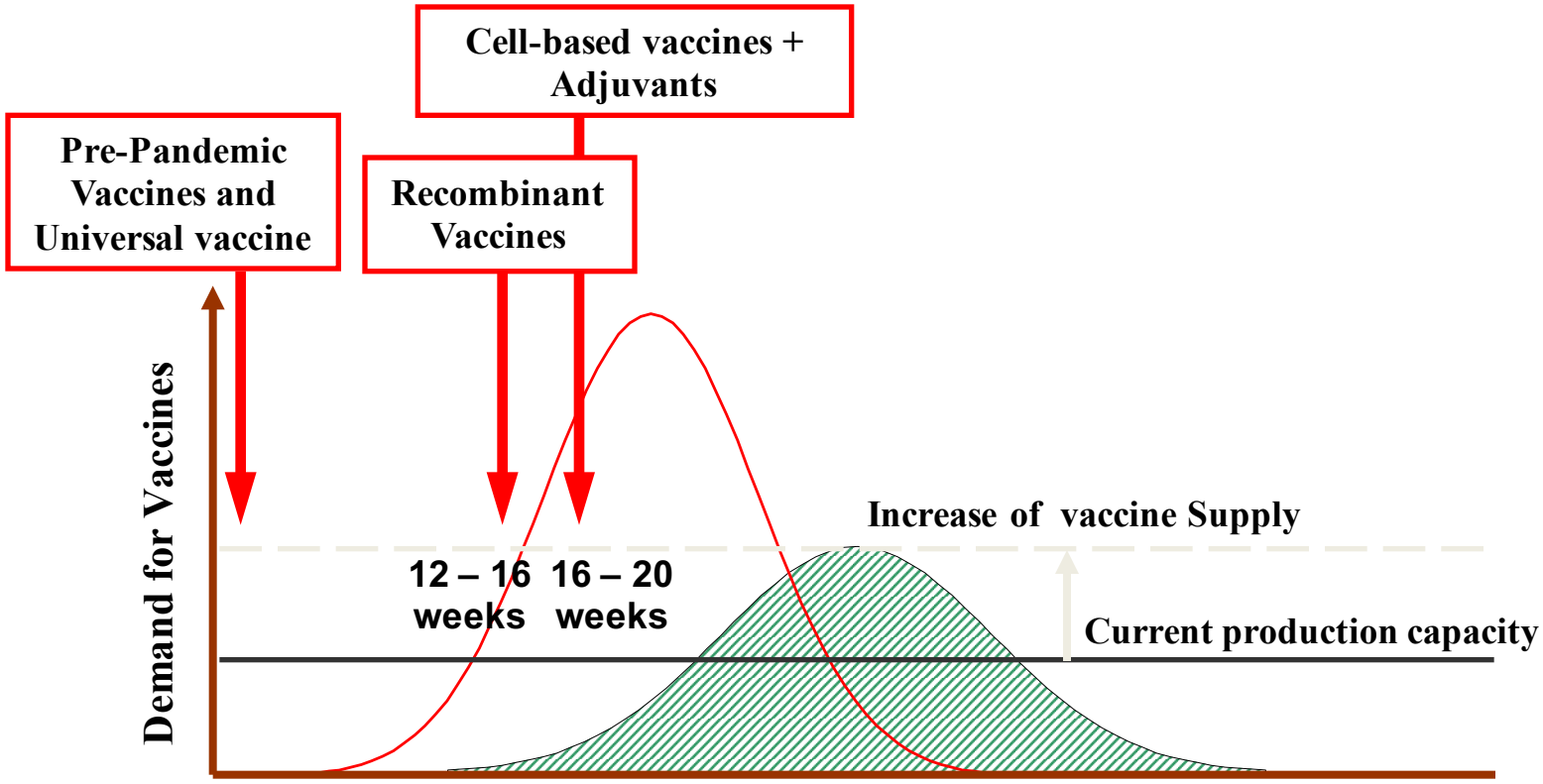


Inactivated or split/subunit	Live-attenuated
Fluzone Fluviral Agriful *etc…*	Flumist

Important to complement with <u>new flu vaccine production method more rapid, more efficient and more flexible</u>

trivalent: 2 influenza A & 1 influenza B virus strains currently: H1N1 & H3N2 or quadrivalent: additional B strain

Opportunity: "More and better influenza vaccine, sooner"



Slide adapted from US department of Health Services Influenza vaccine action plan

Cell Culture Influenza vaccine manufacturing

- FDA approved Flucelvax, the first seasonal influenza vaccine manufactured using cell culture technology Nov. 20, 2012

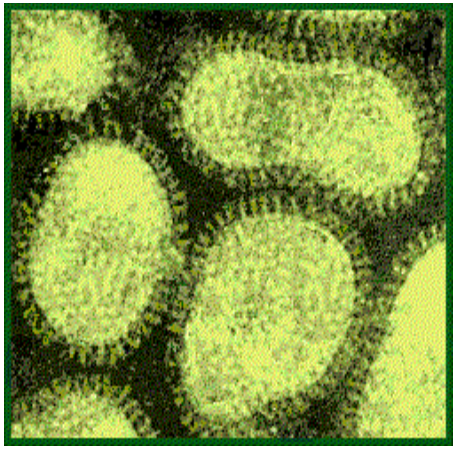
http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm328982.htm

- FDA approved FluBlok, the first trivalent influenza vaccine made using an insect virus (baculovirus) expression system and recombinant DNA technology. Jan 16, 2013

http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm335891.htm

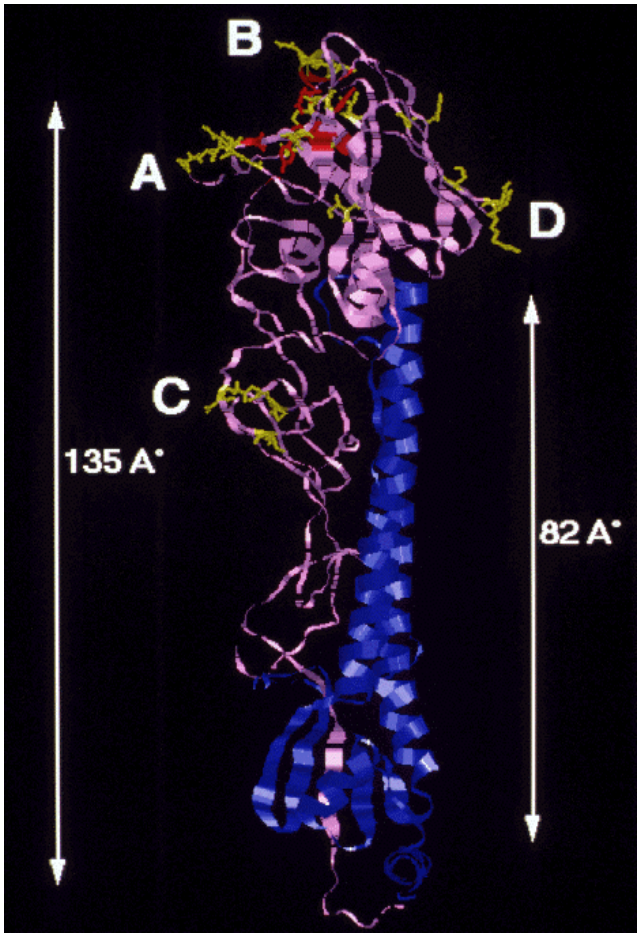
Improving Production of an influenza vaccine

Human Influenza



HA & NA Glycoprotein Spikes

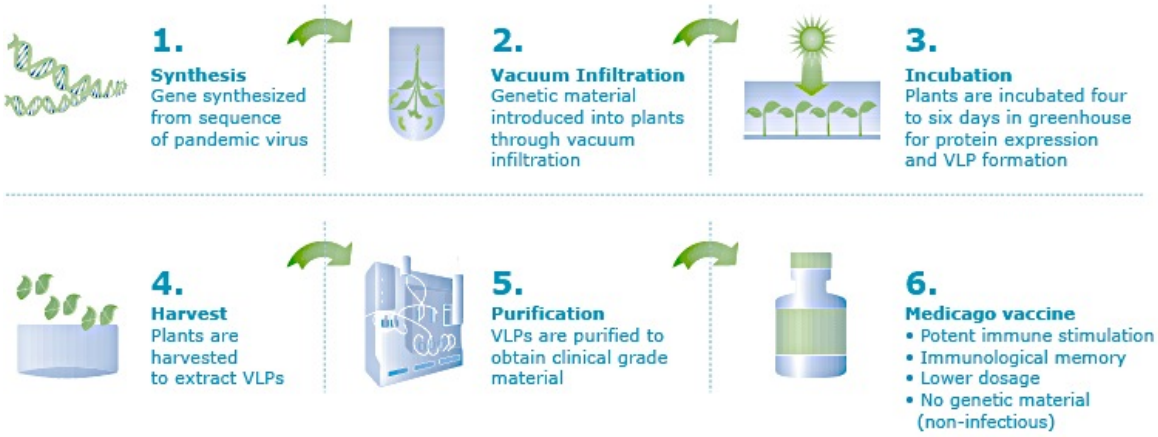
HA = active ingredient in licensed vaccine



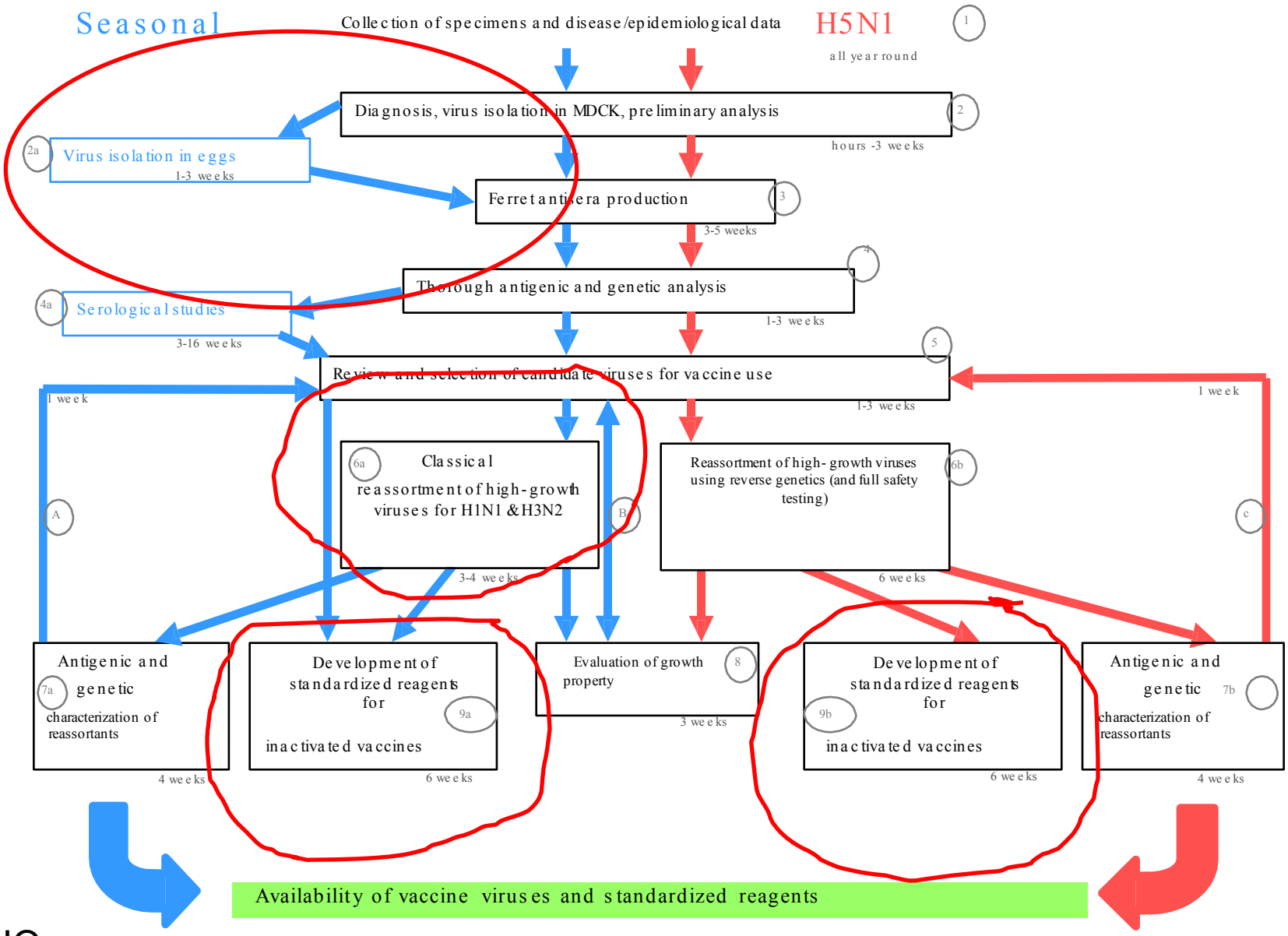
Meghrous et al. (2010) Vaccine

Influenza vaccine manufacturing: other technologies

- Insect cell VLPs: Novavax
- Mammalian cell VLPs (poster # 10)
- Plant VLPs: Medicago

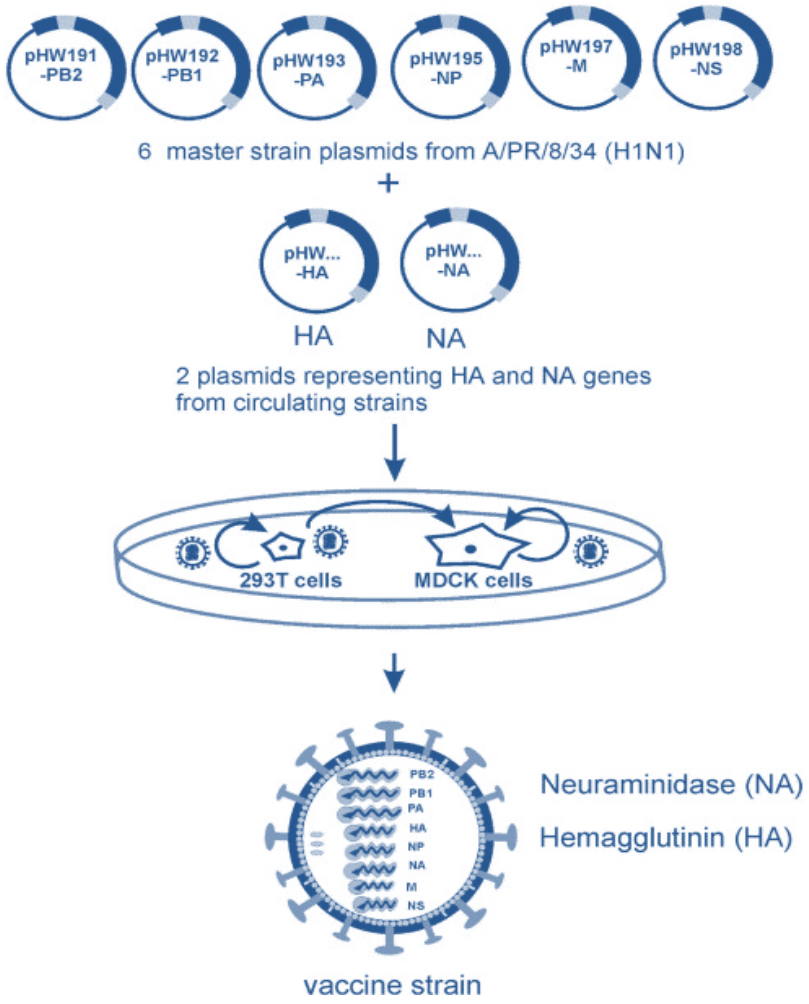


Process of influenza vaccine virus selection and development



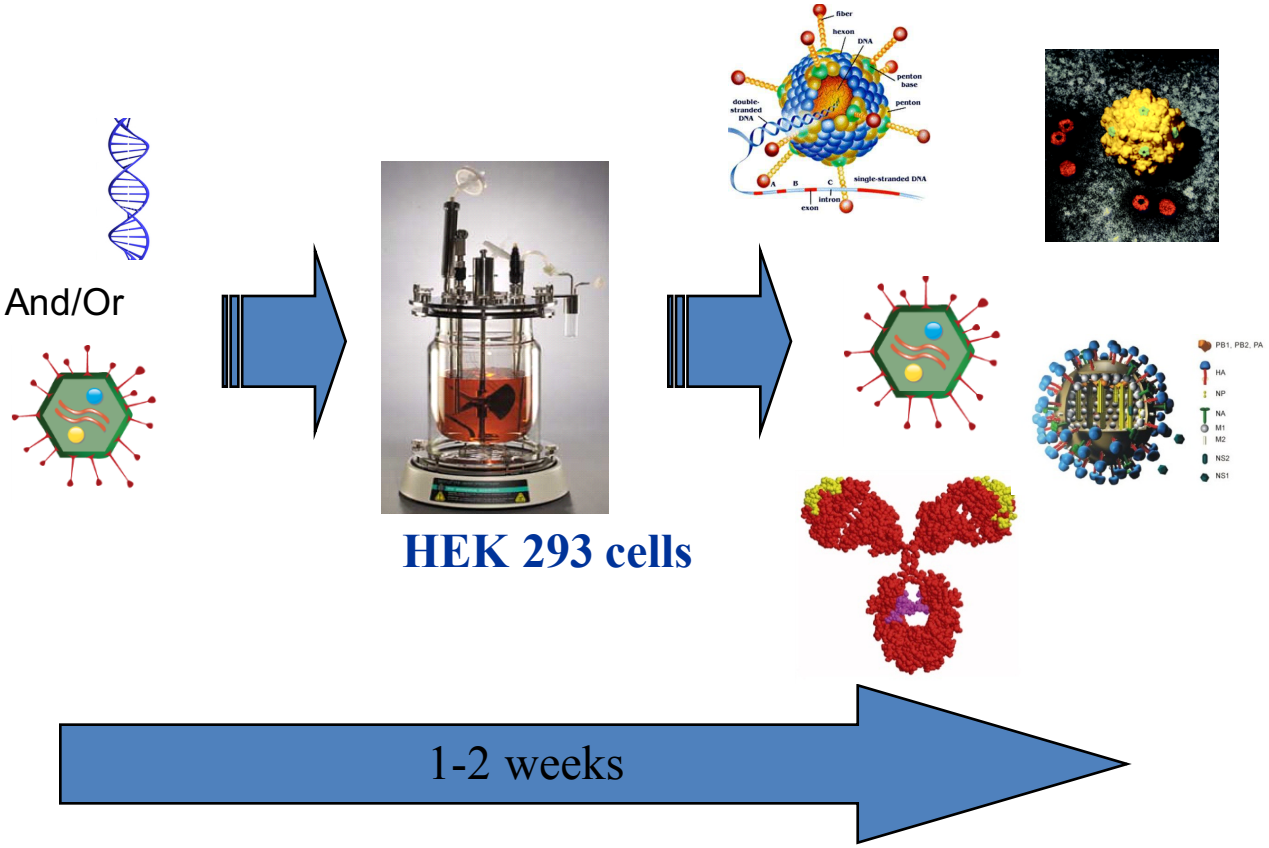
WHO,

Reverse genetics in mammalian cells



Erich Hoffmann, Scott Krauss, Daniel Perez, Richard Webby, Robert G Webster, **Eight-plasmid system for rapid generation of influenza virus vaccines**, Vaccine (2002) 20:3165-3170.

Viral Vector Production: Infection/Transfection Technology

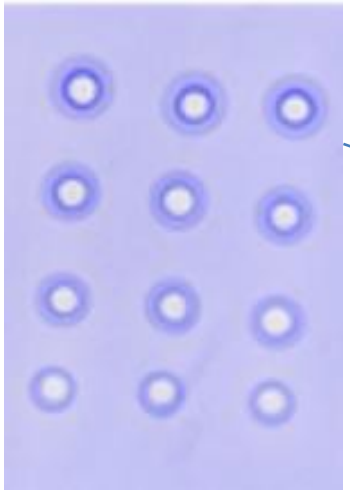


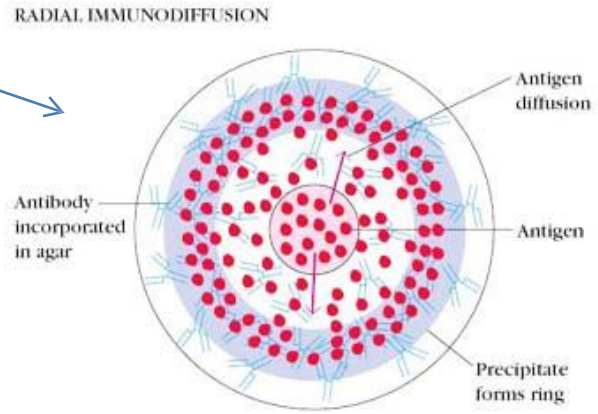
Pham PL, Kamen A, Durocher Y. (2006) Mol Biotechnol., 34:225-37.

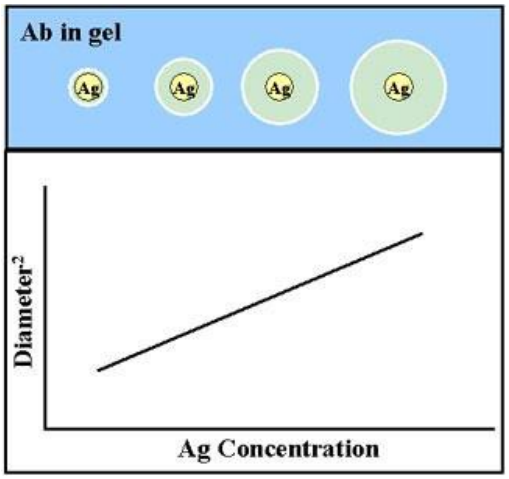
Virus production quantification
Potency assays

Single-Radial Immunodiffusion assay (SRID) - Antigen concentration is determined by the degree of antigen/antibody precipitate around a well into which detergent solubilized virus is placed. **A standardized antigen preparation and a specific polyclonal antibody are required.**

- An agarose solution containing a predetermined amount of polyclonal antibody is plated in a thin gel layer which is punched with 3 to 4mm holes.
- Dilutions of standard antigen are placed into the wells alongside samples with unknown antigen concentrations. The gel is incubated and resulting precipitin rings are visiualized with Coomassie blue stain.
- Ring diameters are measured and compared to a standard curve in order to calculate vaccine potency.







Alternative methods to SRID

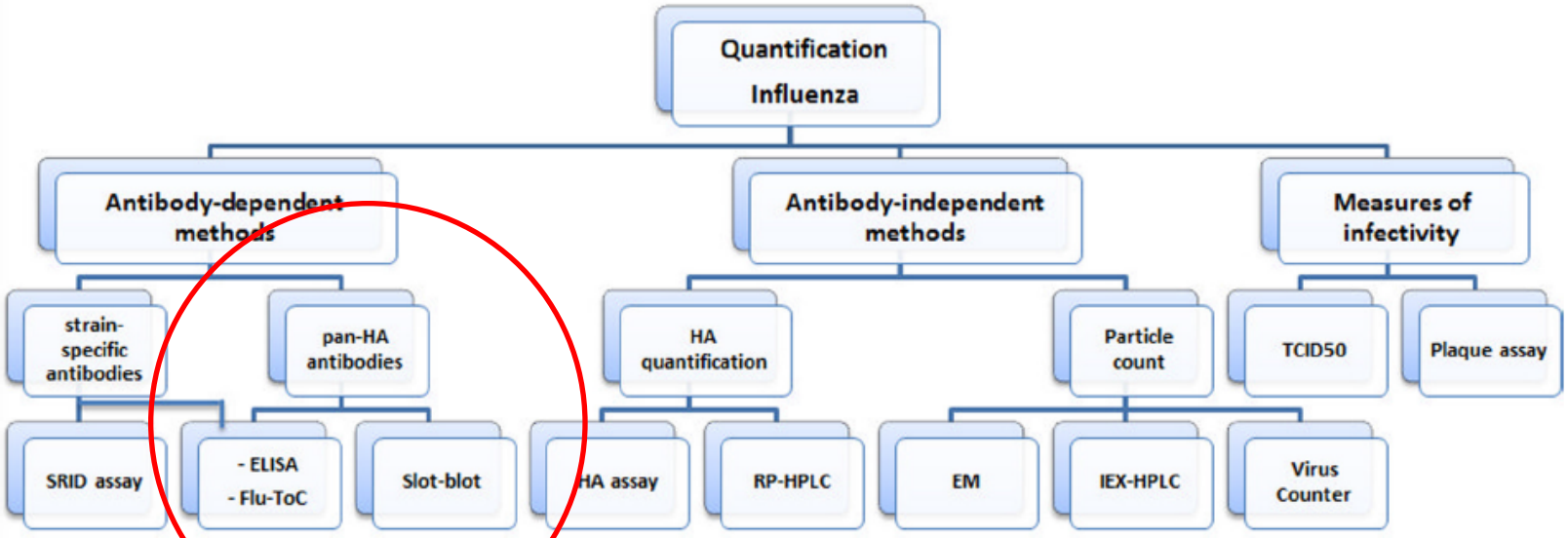


Fig. 3. Overview of current assays and emerging strategies available to quantify influenza. Legend: EM, electron microscopy; HA assay, hemagglutination assay; RP-HPLC, reverse-phase HPLC; IEX-HPLC, ion-exchange HPLC.

Poster # 8 by Manceur A. et al

WHO, Influenza Vaccine Response during the Start of a Pandemic – WHO Informal Consultation REPORT 2015

- Vaccine manufacturers should ensure that they have import permits in place for receipt of CVVs. This should include wild-type viruses, conventional reassortants and reassortants generated by RG, which in some countries are judged to be genetically modified organisms (GMOs).
- It is clear that the availability of pandemic vaccine reagents may be problematic. In 2009, alternative potency assays were used during the early stages of pandemic vaccine production. This is an area for considerable research and WHO has provided guidance on the key characteristics for improved potency assays

Challenges/Solutions

- Coordination between WHO-CC, ERL, and National Authorities
- 1) Cell culture production still depends on viral seed stocks (CVV) made in eggs
  - Reverse genetics in cell culture to meet pandemic timelines
- 2) SRID: delays in generation of specific antibodies
  - Alternative potency assay (adapted to the new influenza antigen design)

"“Science will fulfill its promises when the benefits are equally shared by the REALLY poor of the world”

- César Milstein –