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AN OVERVIEW ON INFLUENZA VACCINES: LABORATORY ASSAYS FOR THEIR IMMUNOGENICITY EVALUATION

Elena Gianchecchi, Project Director

Siena, October 30 2023

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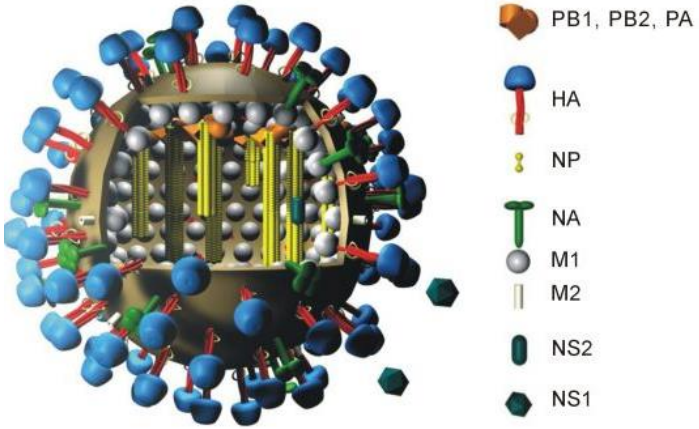
INFLUENZA

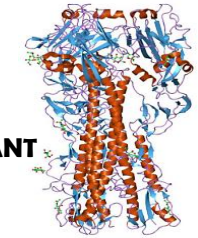


- **Negative sRNA Virus**
- **Composed of 8 segments**
- **Causes 3 to 5 million cases of severe illness, and about 250.000 to 500.000 deaths each year**

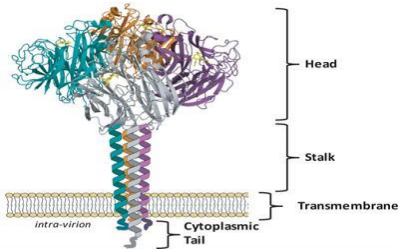
Four types of influenza viruses: A, B, C, and D.

TARGET INFLUENZA ANTIGENS FOR VACCINE PRODUCTION

IMMUNODOMINANT



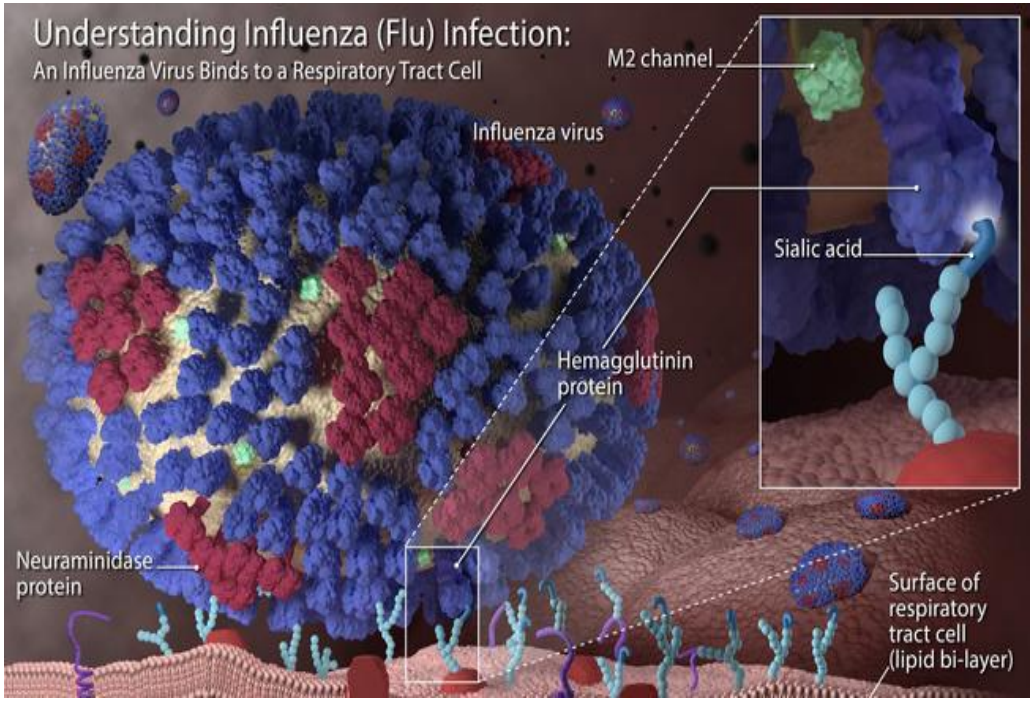
HA



NA

IMMU Sub-DOMINANT

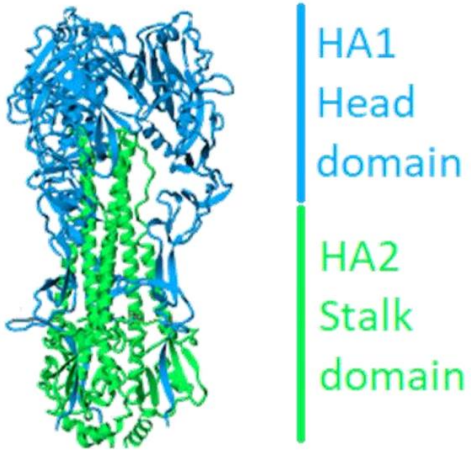
INFLUENZA HA



- Major surface glycoprotein (80%)
- Responsible for the attachment to sialic acid and internalization of virus into cells
- Responsible for the membrane fusion which allows vRNP release into the cell
- Mainly response against globular head (HA1)

Correlates of protection established (SRH and HAI)

- Laboratory marker of immune response that correlates with the protection from disease



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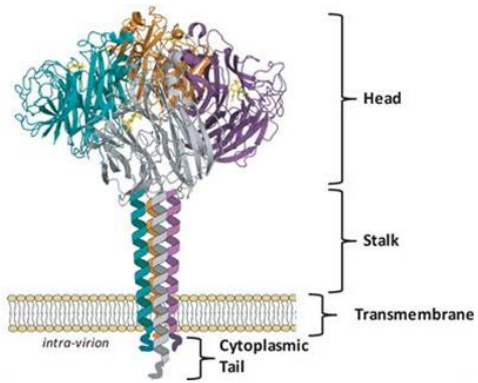
NEURAMINIDASE (NA)



NA

- The second most abundant glycoprotein on the Influenza virus surface (17% of the overal surface) after HA
- Expressed at a ratio 1:4 (40-50 NA and 160-200 HA spikes)
- Multiple roles:

✓Allow the release of newly formed virions from the surface of the infected cell

✓Enhance influenza infection by acting on glycoconjugates expressed on the cell surface

✓Form complexes with sialic acids on the host cell surface

Antibodies against NA were found to decrease virus replication in lungs and reduce disease severity upon challenge

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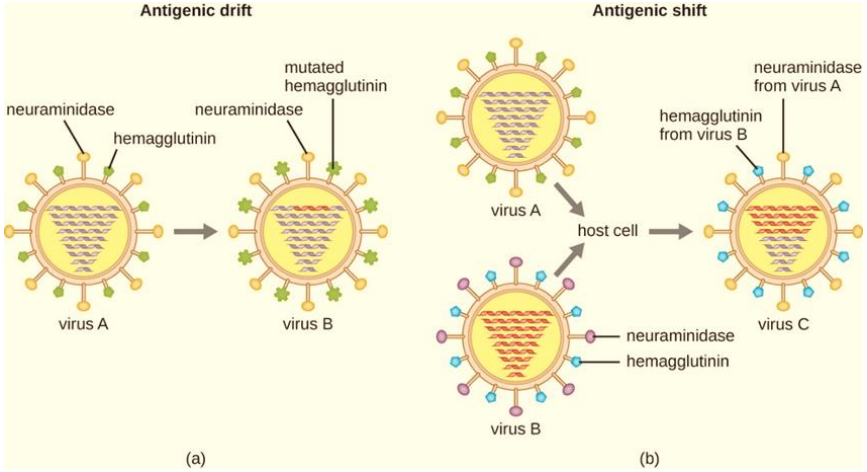
INFLUENZA ANTIGENIC VARIATIONS

ANTIGENIC DRIFT

- Minor changes in HA and NA
- Occurs in Influenza A and B
- Antibodies are not effective against new mutation
- Causes a minor epidemic about every 2 years



ANTIGENIC SHIFT

- Major changes in HA and NA
- Occurs only in Influenza A
- Two different Influenza viruses enter cell and combine causing a new influenza strain
- Causes major epidemic aboud every 10-20 years

These two mechanisms of reassortment are responsible for annual INFLUENZA EPIDEMICS and for INFLUENZA PANDEMICS that occur when the human population results completely naive towards the new influenza virus generated

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CORRELATES OF PROTECTION

- Laboratory marker of immune response that correlates with the protection from disease

- An Immune response that is responsible for and statistically correlated with protection

… a correlates reflects a statistical relation between an immune marker and protection but does not necessary imply causal agency of the marker…

Plotkin and Gilbert, 2012

Surrogate correlate of protection

- An immune response that substitutes for the true immunological correlate f protection, which may be known or not easily measured
- *i.e. non mechanistic correlate of protection, which does not cause protection but nevertheless predicts protection through its (partial) correlation with another immune response that mechanistically protects.*
- Different –vaccine type and formulation, age, health status

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CORRELATES OF PROTECTION

The European Agency for the Evaluation of Medicinal Products
Human Medicines Evaluation Unit

12 March 1997
CPMP/BWP/214/96

COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS (CPMP)

2.5 Antibody titration

All sera shall be assayed for anti-hemagglutinin antibody against the prototype strains by HI (Palmer et al., 1975) or SRH (Schild et al., 1975, Aymard et al., 1980) tests.

Positive and negative sera as well as reference preparations may be obtained from a reference laboratory.

2.6 Interpretation of results and statistics

Antibody titrations shall be done in duplicate; pre- and post-vaccination sera shall be titrated

REGULATORY REQUIREMENTS FOR SEASONAL INFLUENZA VACCINE LICENSURE (EMA, 1996)

- ***The seroconversion rate (SCR) (at least 4-fold increase in titers between pre and post vaccinated serum) (>40% or > 30%)***
- ***Geometric mean increase (ratio of pre and post vaccination) (>2.5 or >2)***
- ***Proportion of subject with HI titre ≥40 (or SRH area ≥25mm^2) (>70% or >60%)***

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NEW EMA GUIDELINES

21 July 2016
EMA/CHMP/VWP/457259/2014
Committee for Medicinal Products for Human Use

6.1.1. Immunological assays and parameters to be assessed

The assessment of the immunogenicity of influenza vaccines is traditionally based on two tests, the haemagglutination inhibition assay [HI] that detect antibody directed against the HA antigen, and the single radial haemolysis assay [SRH]. Neither the HI nor the SRH assays are standardised. It has been shown that they are both subject to considerable inter-laboratory variability. In any one clinical

The Virus Neutralisation [VN] assay quantifies functional antibody. The assay is usually based on detecting the ability of human serum at various dilutions to prevent viral replication in microplates (i.e. using a microneutralisation technique [MN]). It is essential that neutralizing antibody titres are determined in all studies, at least in a representative subset of the study population and preferably in

It is recommended that studies should monitor the quantity and quality of T-cell responses. For example, antigen-specific T-cell frequencies should be estimated (e.g. including Th1, Th2, T regulator cells, memory T cells and relevant cytokines). In addition, a thorough analysis of CD4+ and CD8+ responses, as well as the activation of memory B cells, would allow for a better characterisation of the effect of vaccination on antibody responses and clinical protection.

Applicants may consider evaluating anti-neuraminidase NA antibodies at least in randomly selected subsets. If conducted, the assay used should be validated and should be performed in appropriately experienced laboratories.

New EMA Guidelines abolished the traditional criteria

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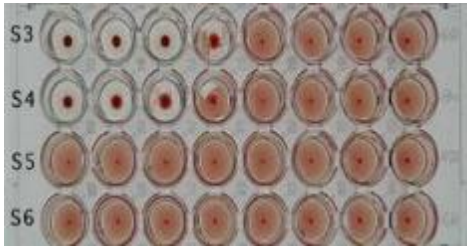
EVALUATION OF INTRAMUSCOLAR AND INTRADERMAL INFLUENZA VACCINES

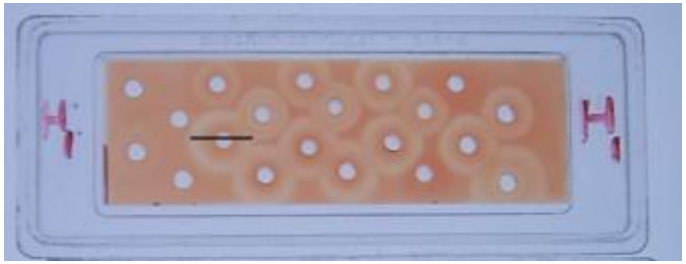
HAI – Haemagglutination Inhibition

- Suitable for screening a large number of samples
- Detects Ab that bind around receptor-binding site in globular head and block agglutination
- Good correlation with MN for seasonal strains
- BSL2 lab need also for pandemic strains
- EMA and FDA Approved



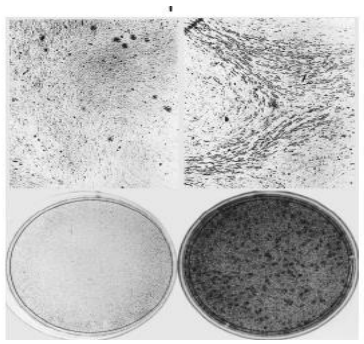
SRH – Single Radial Haemolysis

- Suitable for screening a large number of samples
- Detects Ab that bind around the virus and fix the complement (not IgG2)
- Good correlation with MN for pandemic strains
- BSL2 lab need also for pandemic strains
- EMA Approved



MN – Virus Neutralization

- Titration of functional antibody only
- Gold Standard for confirmation
- High containment (BSL3plus) needed in case of pandemic strains
- Detects Ab that bind around globular head and block virus attachment/entry
- No correlate of protection established



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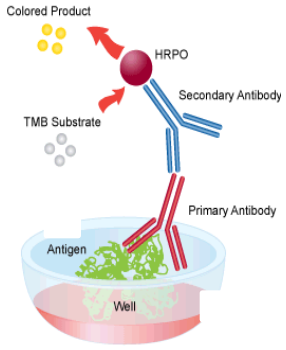
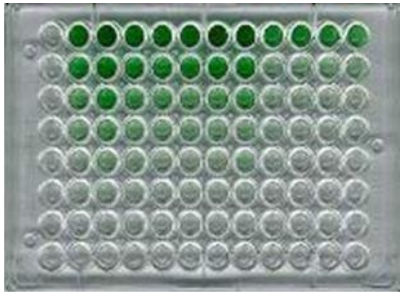
Evaluation of Intramuscolar and intradermal influenza vaccines

ELISA – Enzyme Linked ImmunoSorbent Assay

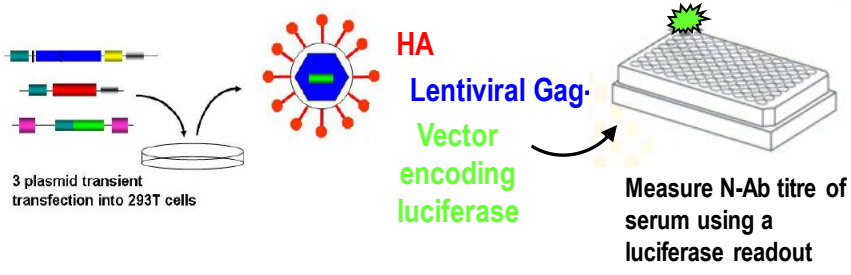
- Suitable for screening a much larger number of samples
- Automation is possible
- No correlate of protection established
- Use of HA is preferable ….. HA1 is better
- Suitable to detect IgG, IgM, IgA in serum and nasal washes





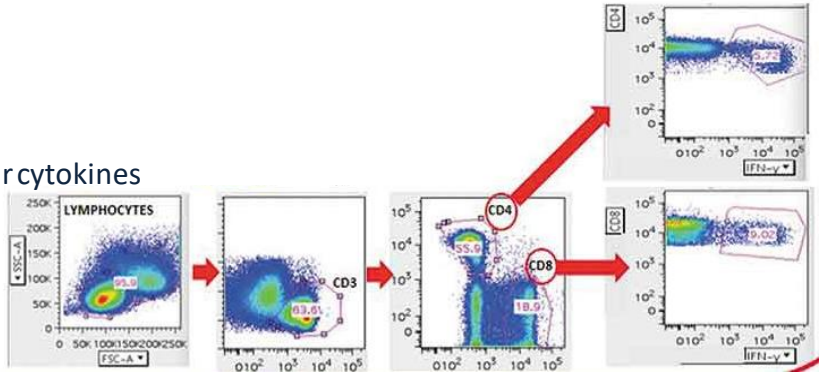
Neuraminidase Assays (for NA antibody)

- Several assays available
- Should be better define role on NA-Ab in protection
- BSL2 lab need also for pandemic strains
- No correlate of protection established

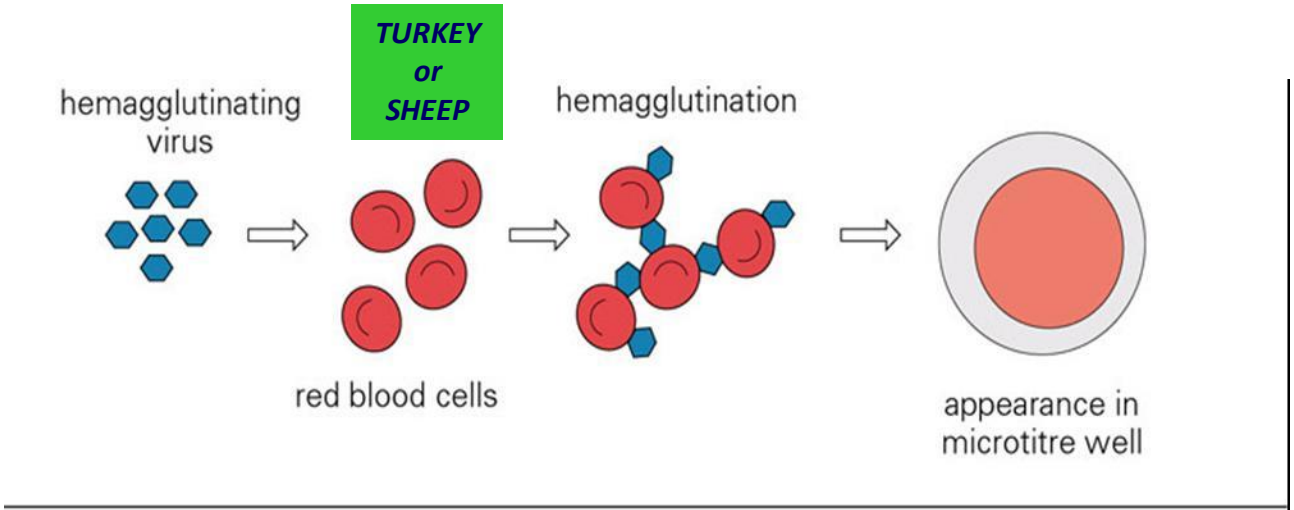


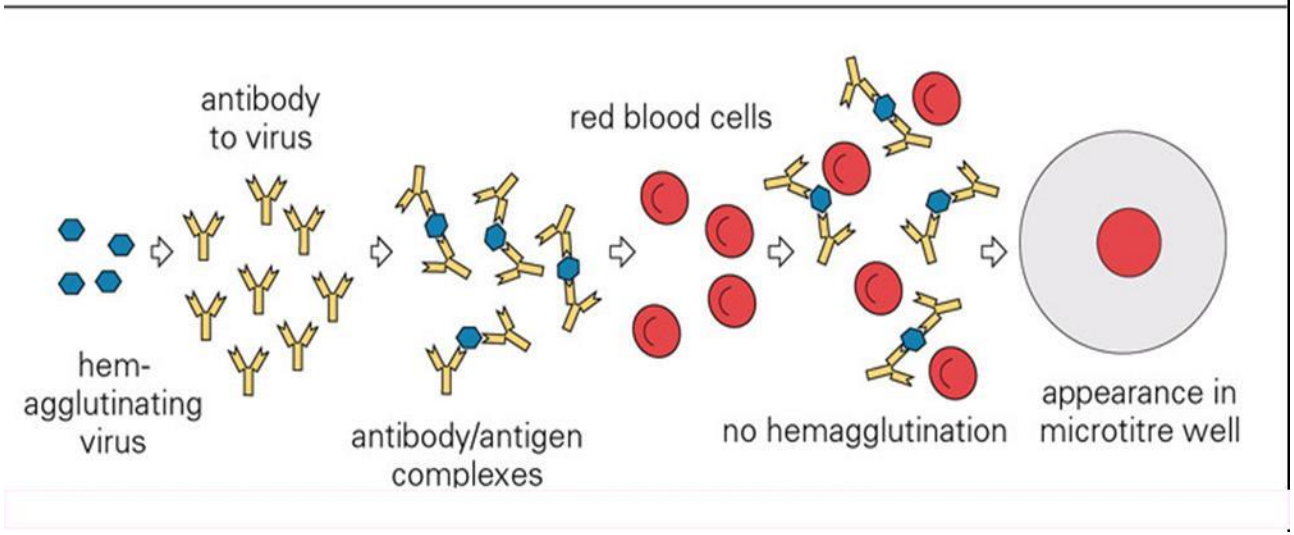
Cell-Mediated Immunity (CMI)

- Identification of subpopulations within of stimulated PBMCs
- Simultaneous analysis of several extracellular and intracellular cytokines
- No correlate of protection established
- ICS and ELISPOT available



HAI – Haemagglutinin Inhibition





The HAI titer is the serum dilution which induces 50% of hemagglutination, the reciprocal of this serum dilution is the HAI titre

-Correlate of protection:

HAI titre ≥ 40 for seasonal vaccines

HAI – as a "correlate" established in efficacy trials long time ago

Hobson et al, 1972

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SINGLE RADIAL HAEMOLYSIS (SRH)

Single Radial Haemolysis (SRH) is routinely used for the detection of influenza-specific (and rubella) IgG antibody.

- SRH has been shown to be sensitive, specific, and reliable.
- SRH plates are usually prepared in the laboratory using commercially available reagents.
- Test sera are placed in wells on a plate containing agar with influenza antigen-coated RBC and guinea-pig complement.
- The presence of influenza-specific IgG is detected by the lysis of influenza antigen-coated RBC mediated from GUINEA-PIG complement.
- The zone of lysis around the well is dependent on the level of specific antibody present.

Schild GC, Pereira MS, Chakraverty P. ***Single-radial-hemolysis: a new method for the assay of antibody to influenza haemagglutinin. Applications for diagnosis and seroepidemiologic surveillance of influenza.*** Bull World Health Organ. **1975**; 52(1):43-50.

Russell SM, McCahon D, Beare AS. ***A single radial haemolysis technique for the measurement of influenza antibody.*** J Gen Virol. **1975** Apr;27(1):1-10.

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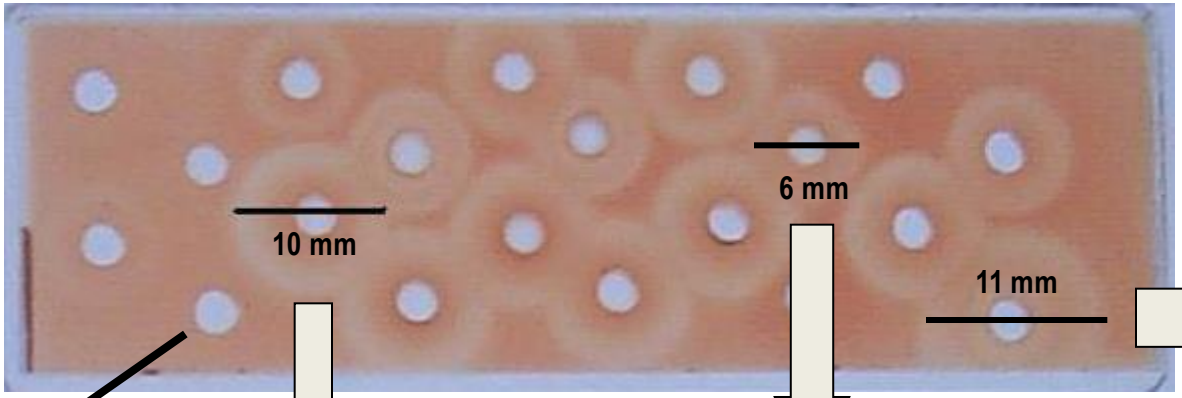
SINGLE RADIAL HAEMOLYSIS (SRH)

- The size of the haemolysis zone around a well containing serum is measured in mm. The diameter of haemolysis is then transformed in area.
- If the area size is greater than 25 mm^2, then the subject is considered to be seroprotected in accordance with EMA guidelines.
- If the area size is <= 4 mm^2, then the subject is considered negative according to EMEA guidelines.



Diam = 2.256 mm
Area = 3.997 mm^2
Negative

Area = 78,540 mm^2
High Positive - Seroprotection

Area = 28.274 mm^2
Low Positive - Seroprotection

Area = 95.033 mm^2
High Positive – Seroprotection

Positive control

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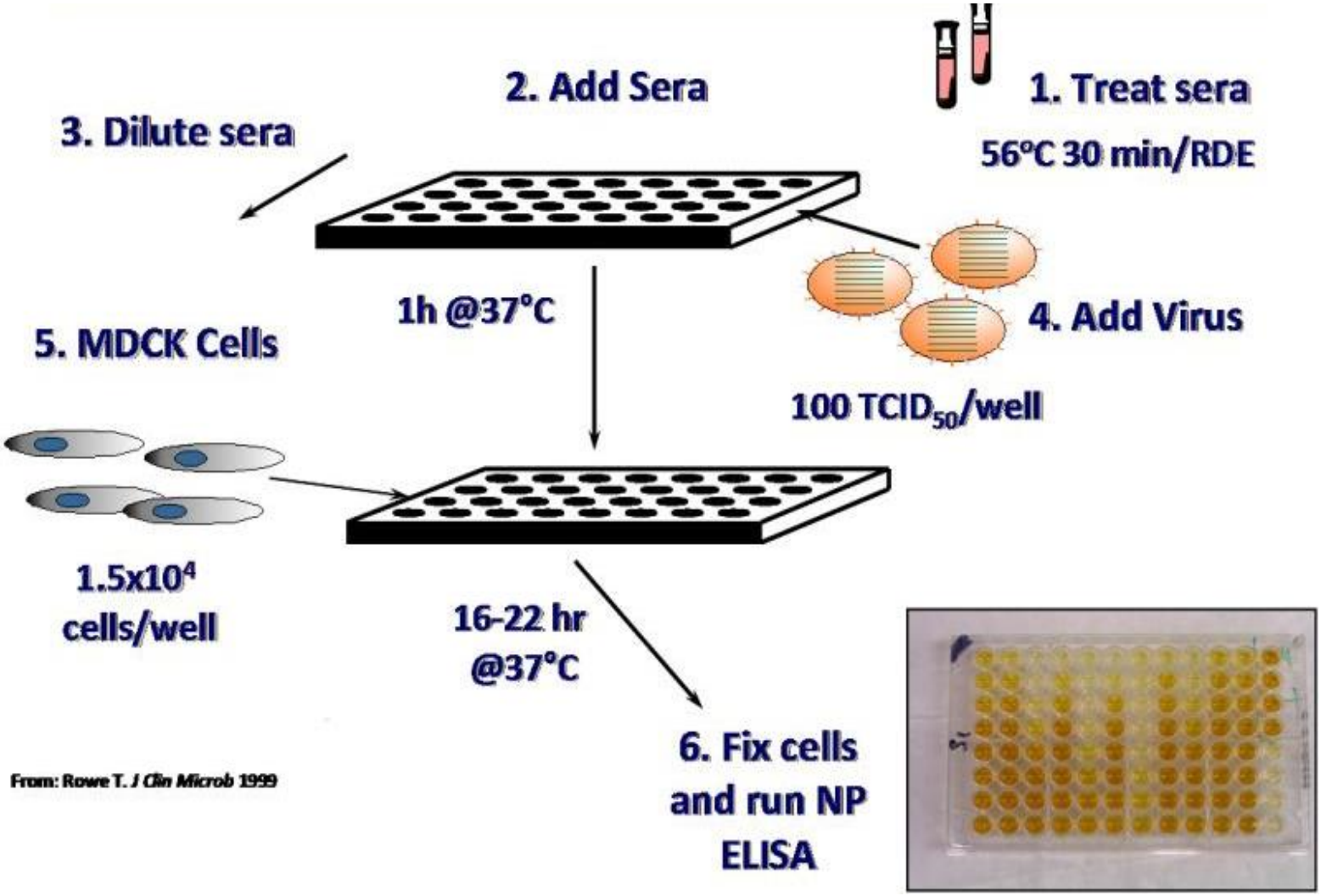
Microneutralization Assay – CDC protocol



Different read-out available

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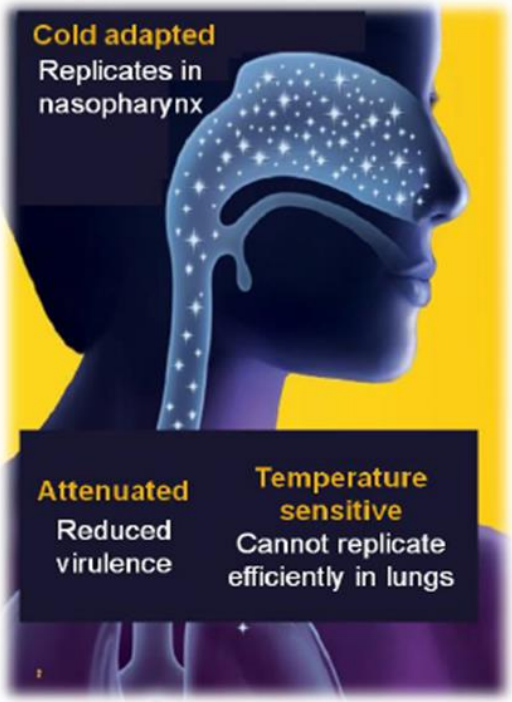
Live Attenuated Influenza Vaccine - LAIV

Trivalent intranasal spray vaccine

Made with *Live-Attenuated* viruses that are able to replicate efficiency only at temperature present in the nasal mucosa

Cold-Adapted Virus

The strains are generated by reassortment, and consist of the **six internal genes from a cold adapted, temperature sensitive master strain**, with the **HA** and **NA** genes from the circulating strains *Palese et al. 2006*



Preferred Vaccine for young children ➔ from 2 to 16 years
More immunogenic in children than in adult if compered with IIV
Mimic a natural infection – Elicit a more mucosal (local) immune response

ALTERNATIVE WAYS USED TO INDUCE MUCOSAL IMMUNITY:

-INTRANASAL VACCINATION USING INACTIVATED WHOLE OR SPLIT IV
-SUBLINGUAL ADMINISTRATION OF ADJUVANTED INFLUENZA VACCINE
-NOVEL TYPE OF LAIV – BY DEPLETING THE **NS1** GENE –

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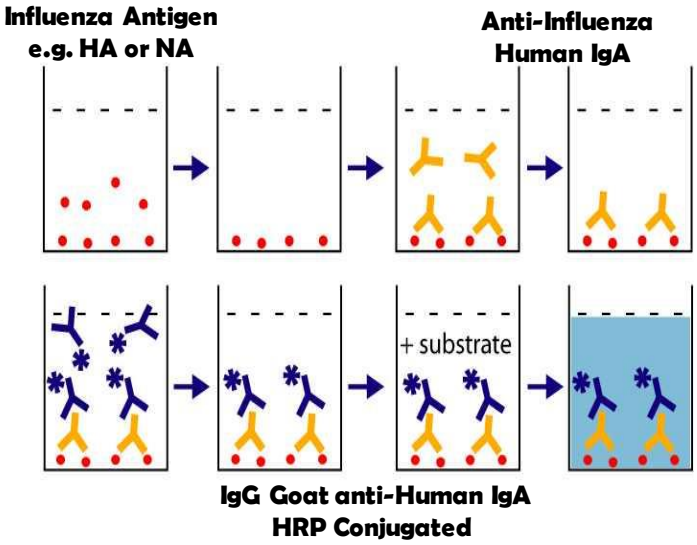
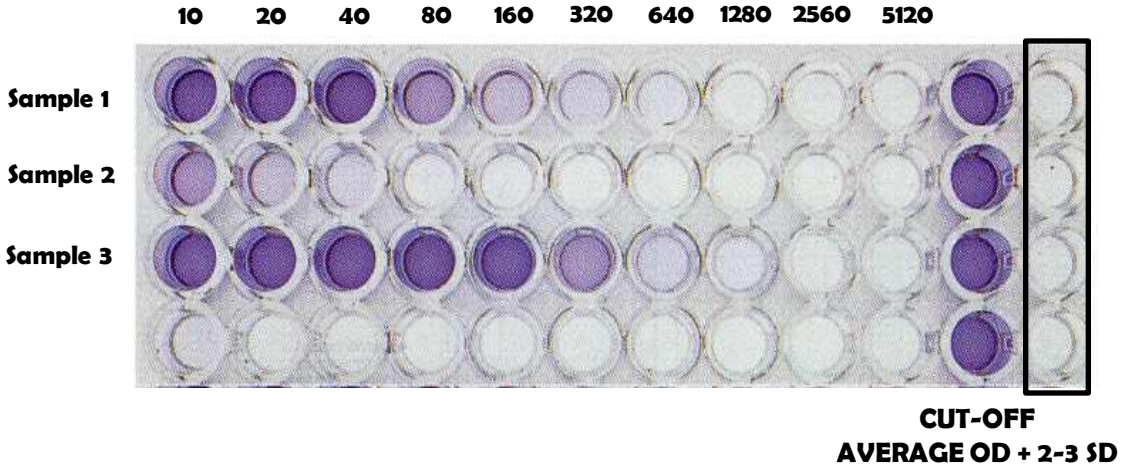
ELISA ASSAY FOR IgA DETECTION





The standardization of the mucosal sample is an important step, since the mucus and protein concentration of nasal washes varies widely between individuals (but also within the same) depending on several factors such as:

- Time of sampling
- Age : Children *VS* Young *VS* Elderly
- History or concurrance of nasal disease
- Aspiration efficacy

STANDARDIZATION USING TOTAL PROTEIN OR TOTAL IgA PRESENT IN THE SAMPLE