CONSISE

CONSORTIUM FOR THE STANDARDIZATION OF INFLUENZA SEROEPIDEMIOLOGY

ppMN standardization and comparison

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AIM

- The continuous rapid evolution of influenza viruses has major implications for the sensitivity and specificity of serological assays.
- Retroviral pseudotypes bearing influenza HA and NA envelope glycoproteins can represent a novel platform for influenza serology.
- Objective: to develop a serodiagnostic tool for highly pathogenic influenza that reproduces H5N1 biology but can be used with less biohazard.

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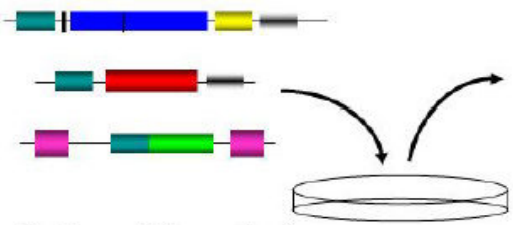
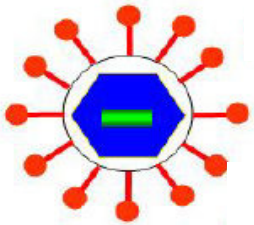
Viral Pseudotype Unit

EpidMol

MN for Pandemic Assay *using PP*



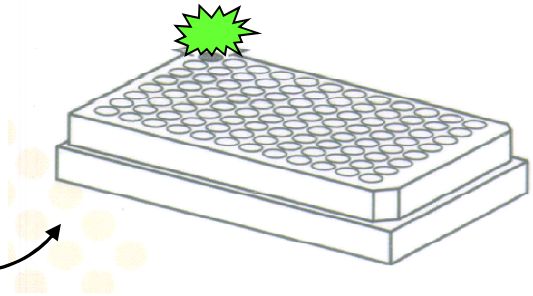
3 plasmid transient transfection into 293T cells



Haemagglutin (HA)

Lentiviral Gag-Pol

Vector encoding luciferase

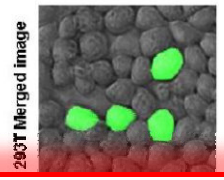


Measure N-Ab titre of serum using a luciferase readout

Vaccine 27 (2009) 5998–6003

Contents lists available at ScienceDirect

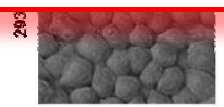
Vaccine



Following transfection of 3 plasmids, vector RNA is produced and packaged into vector virus particles that are released from the packaging cell.

These particles can be harvested and used to infect fresh target cells. Following reverse transcription and integration of the vector genome, the foreign gene (GFP/Luc) is expressed in the target cells.

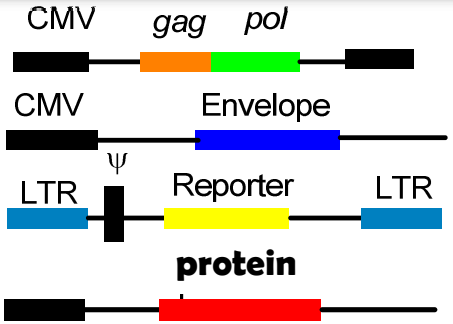
Since viral proteins are NOT produced in the target cells, further vector production and propagation does NOT occur.



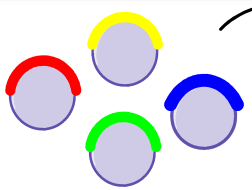
Chiara Gentile[c], Emanuele Montomoli[c], Anne K. Hilbert[d], Angelika Banzhoff[d], Giuseppe Del Giudice[a], John J. Donnelly[a], Rino Rappuoli[a,*], Barbara Capecchi[a]

Pseudotype production and cell-based assay development

Viral Pseudotype Unit



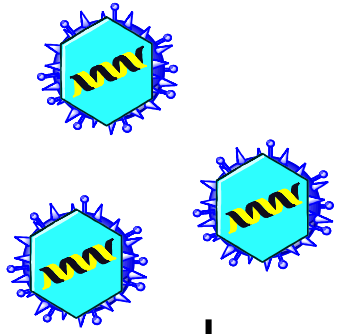
I) Plasmid co-transfection



Exogenous Neuraminidase

Suitable producer cell line

Pseudotype particles

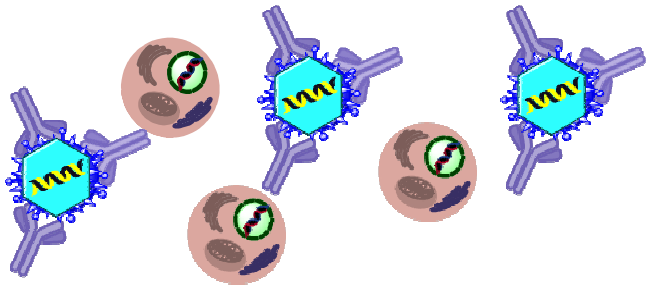


II) Harvest supernatant and pseudotype titre deterrmination

III) Fixed amount of pseudotypes are pre-incubated with titrated serum samples from different human/animal sources

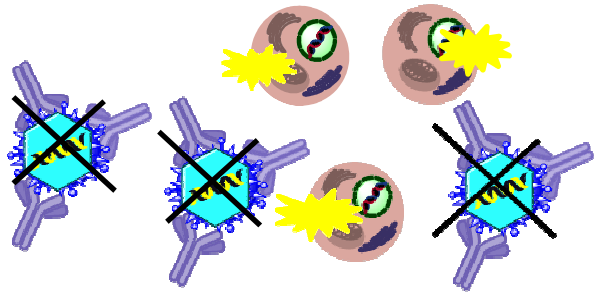
48 hrs transduction onto target cells

VNab present



IV) Measurement of reporter gene expression

VNab absent/ non neutralizing Nab



Pseudotype cell-based MN procedure

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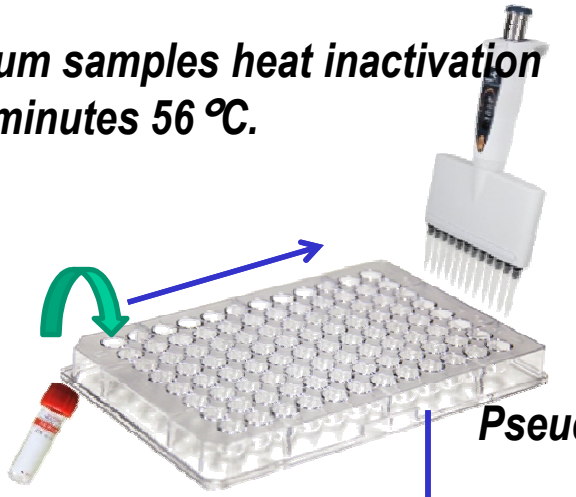
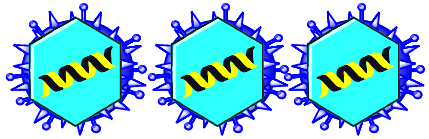
serum samples heat inactivation
30 minutes 56 °C.



Pseudotype titration

Pseudotype

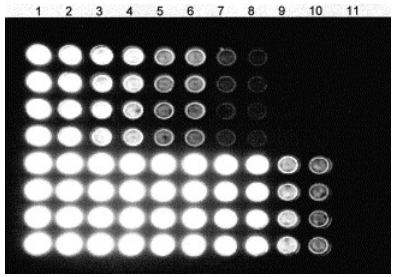


10k to 50k RLU pp *

1 hour @ 37 °C ; 5%CO2

HEK-293T cells (1x10^4 cells/50µl).

48h ; @ 37 °C ; 5%CO2



Firefly Luciferase RLU – (luminomenter)

* a pilot study with positive standard is required to optimize the amount of pp to use

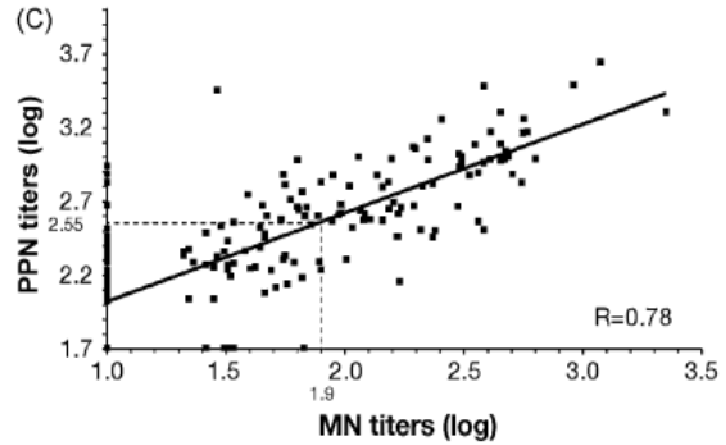
H5N1/VIET MN PP-based correlation with SRH / HI / MN-ELISA

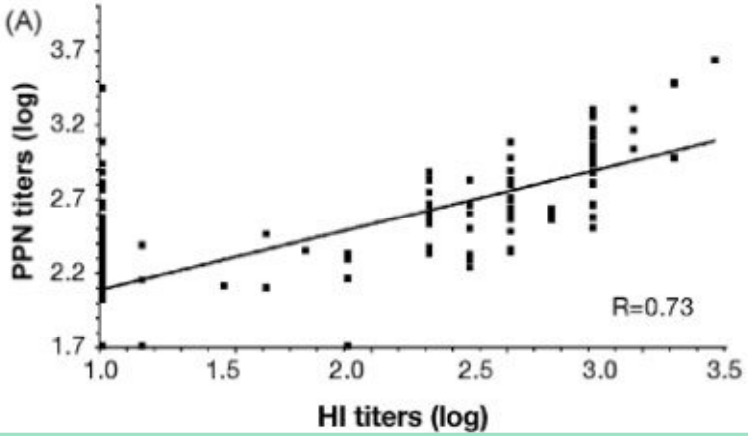
http://www.ClinicalTrials.gov NCT00382187

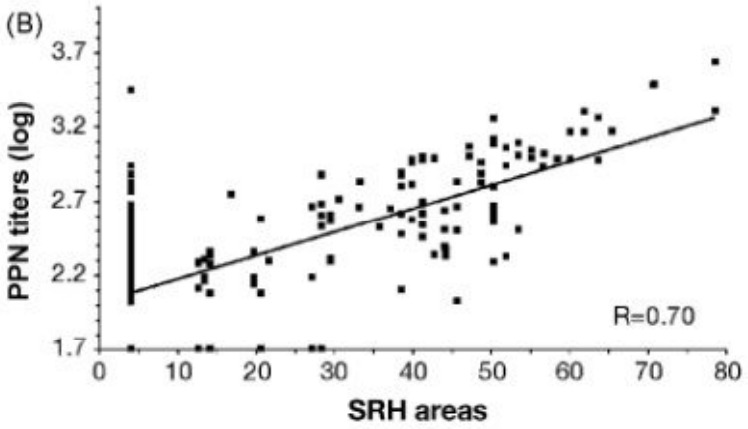
The administered vaccine was a monovalent H5N1 subunit influenza vaccine derived from the /Vietnam/1194/2004

40 adults were enrolled in the study:

- ***one group received 15g of plain H5N1(Non-Adj-15; N= 13);***
- ***second group received 7.5g of H5N1 adjuvanted with MF59 (MF59-7.5; N= 14);***
- ***third group received 15g of H5N1 adjuvanted with MF59 (MF59-15; N= 13).***





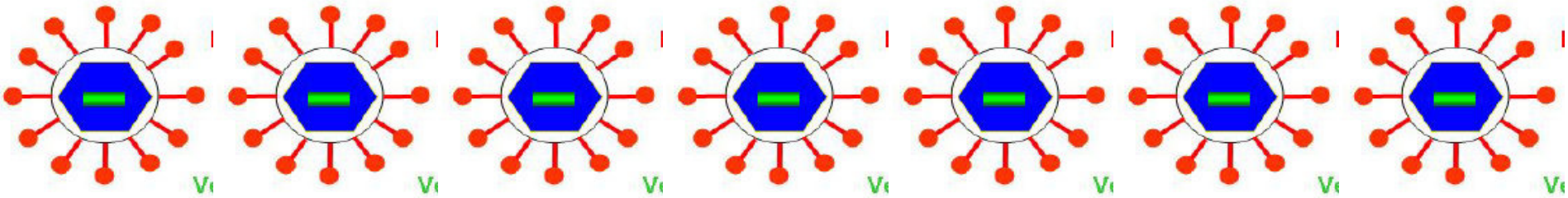


In the picture vertical dashed line indicate the value of MNlog10 titer = 1.9 (corresponding to a titer of 1:80), the proposed threshold of protective antibodies, horizontal dashed line indicate the corresponding value of PPN log10 titer = 2.55 (corresponding to a titer of 1:357).

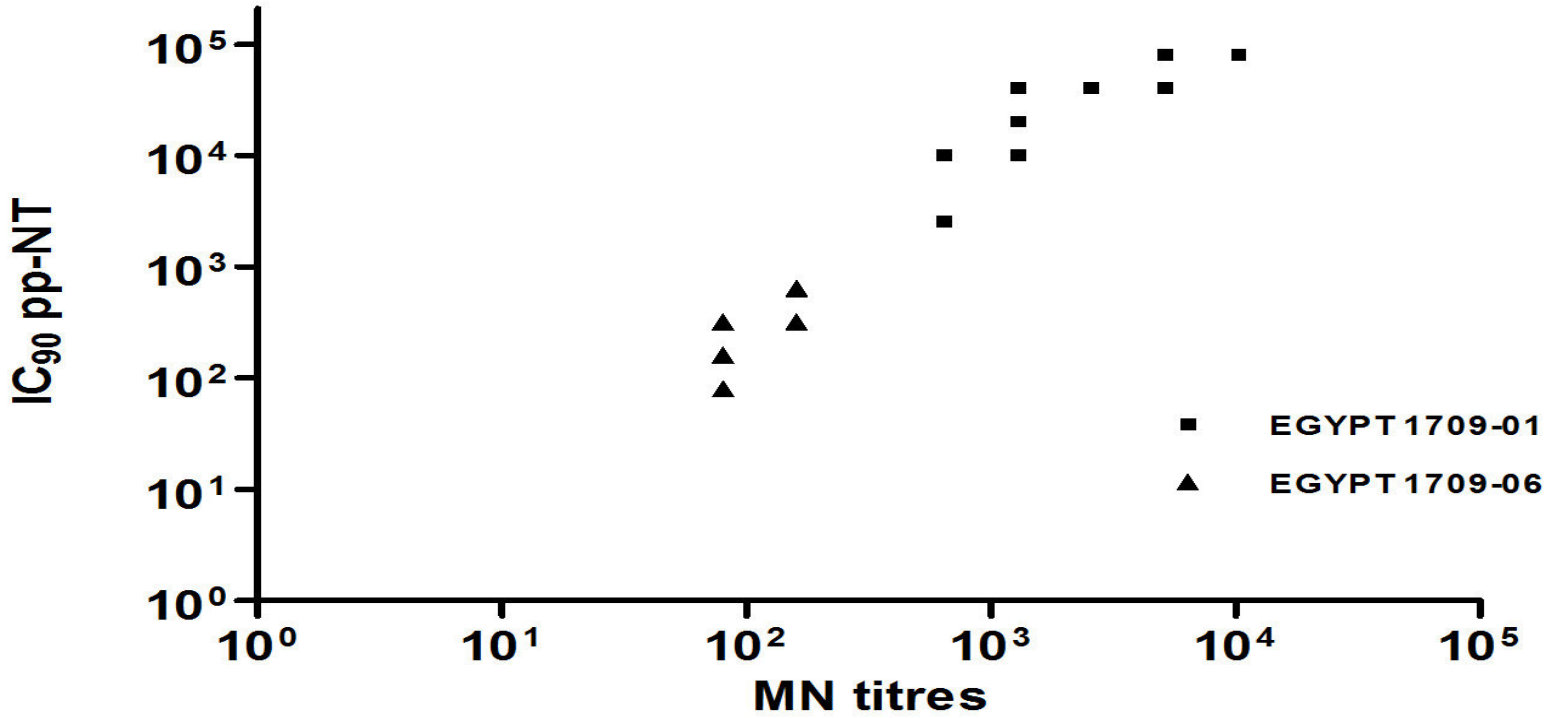
H5N1/VIET MN PP-based cut-off of seroprotection

The absolute titers obtained by PPMN assay were approximately fivefold higher than those obtained by ELISA-MN, and, based on the comparative analysis between paired PPN and MN titers on the whole data set, we extrapolated a PPMN titer of 1:357, corresponding to a MN titer of 1:80, as our seroprotection endpoint.

This difference could be due to a lower density of HA molecules on the surface of pseudoparticles compared to the wild-type virus, and/or to the absence of NA molecules on pseudoparticles that may render neutralizing epitopes on HA molecules more accessible to neutralizing antibodies



Comparison of pp-NT with MN antibody titers. Scatterplots showing the correlation of antibody logarithmic titers measured by pp-NT assay (using A/chicken/Egypt/1709-1/2007 and A/chicken/Egypt/1709-6/2008) versus MN (tested against homologous viruses)

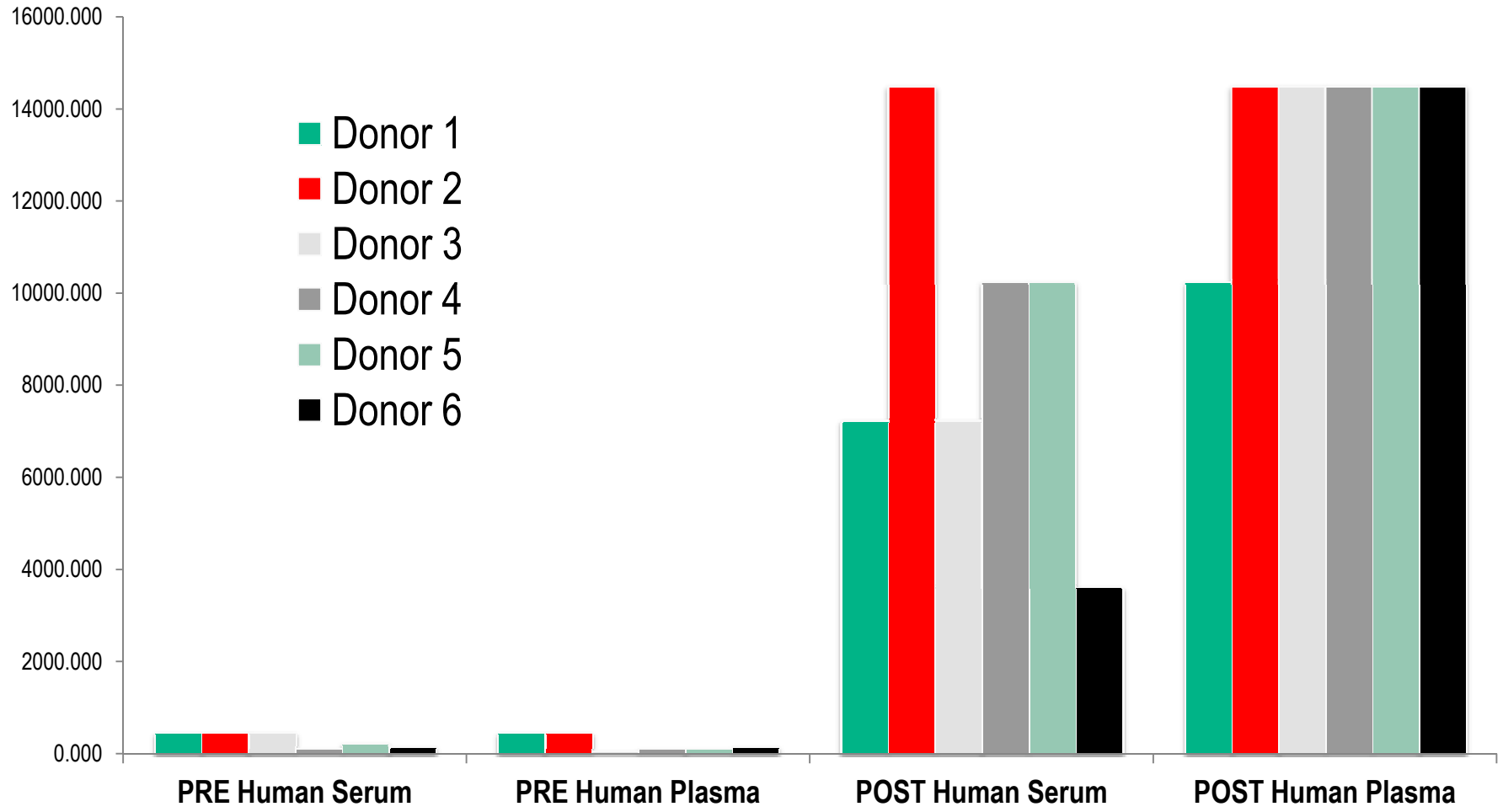


Statistical analysis:

Pearson's correlation using GraphPad Version 5. The correlation is significant, for Egypt 1709-1 r2= 0.69, for Egypt 1709-6 r2= 0. 56

Molesti E, Milani A, Terregino C, Cattoli G, Temperton N. Influenza Research and Treatment 2013. (In press)

ppMN H5N1/Viet serum/plasma comparison



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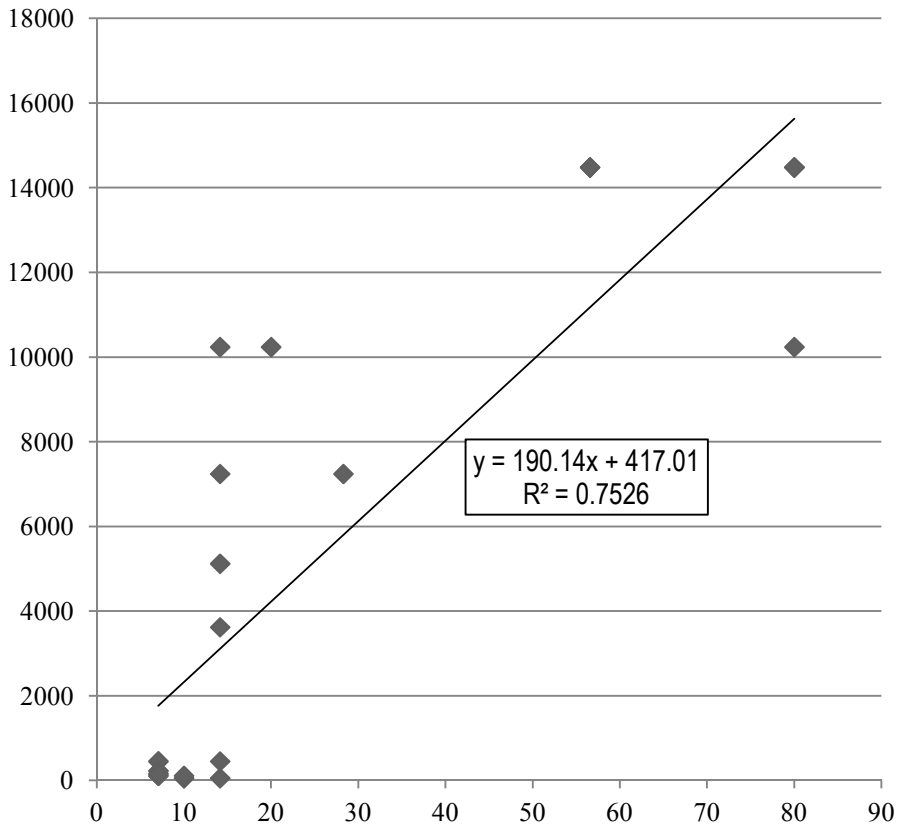
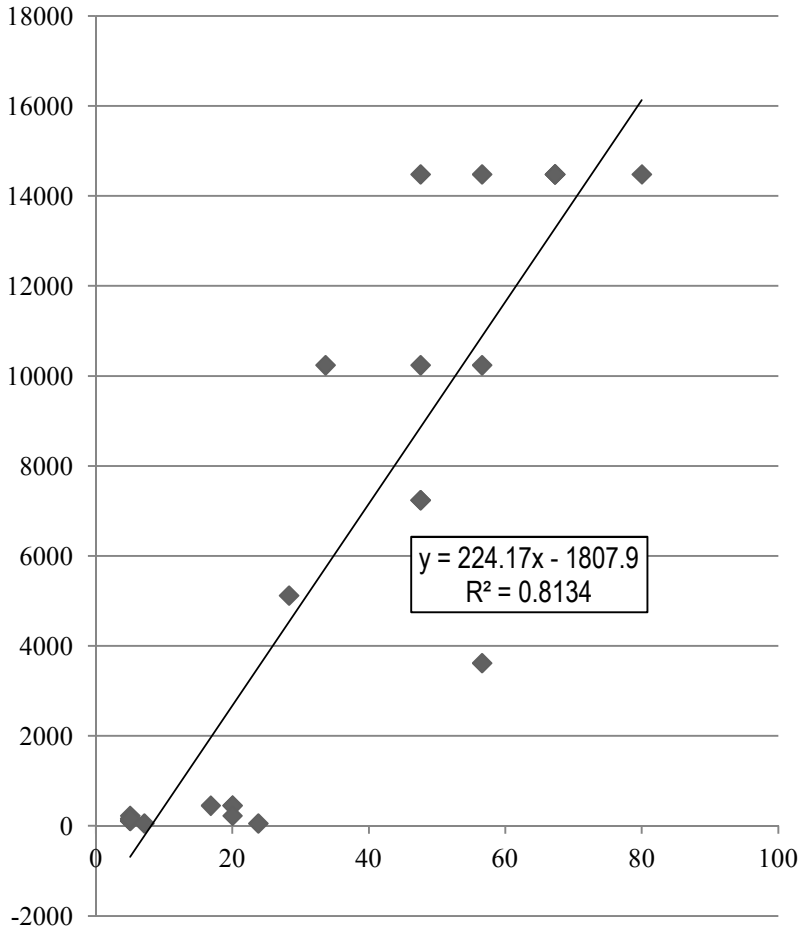
H5N1/Viet ppMN vs MN comparison

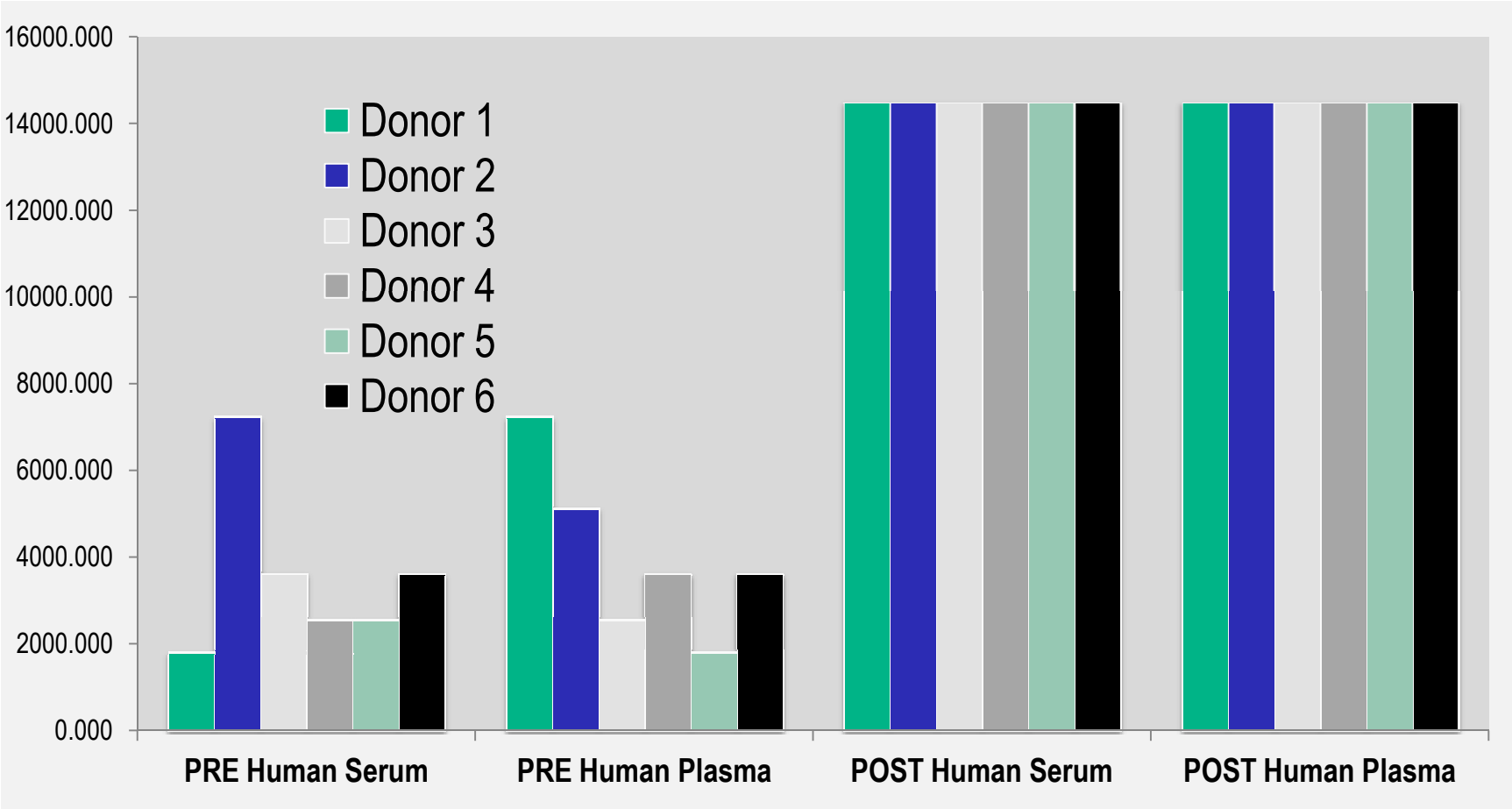
PP vs MNELISA



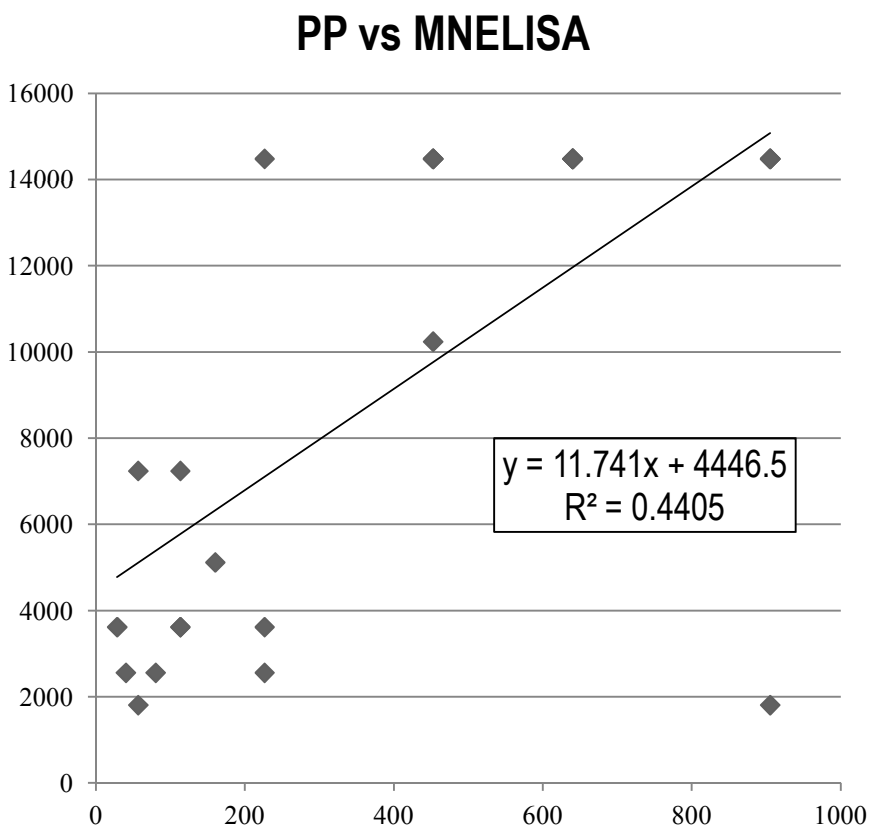
PP vs MN CPE

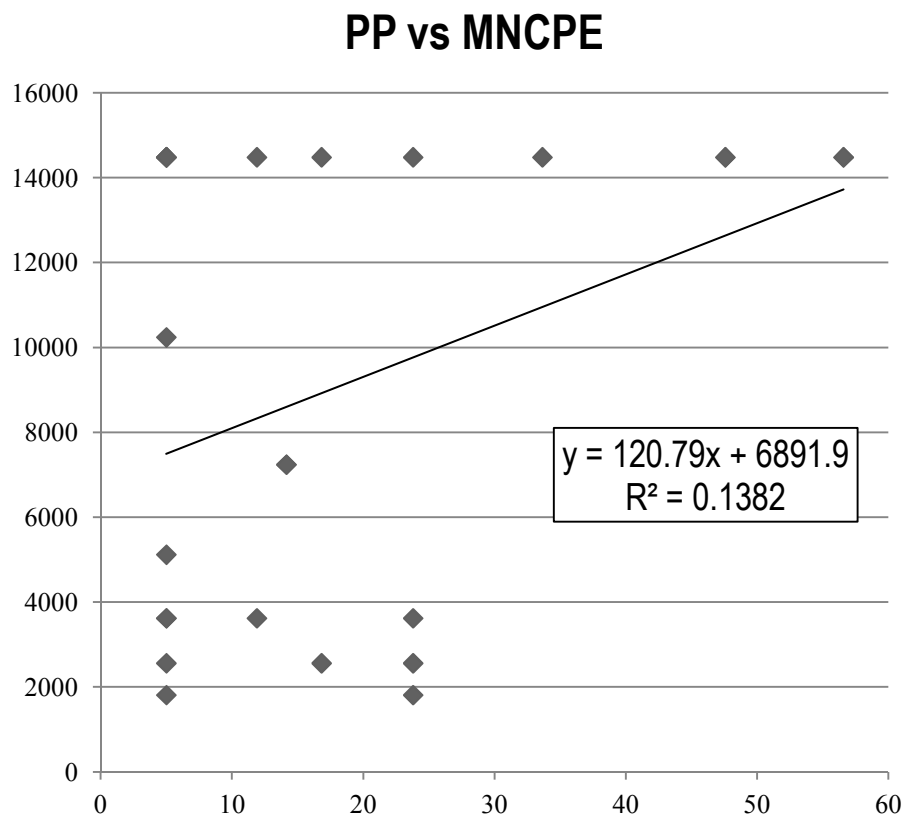


ppMN H1N1/Brisb serum/plasma comparison



H1N1/Brisb ppMN vs MN comparison





NA pseudoparticles

Flu PP expressing pandemic HA alone or in combination with homologous NA have been successfully used in the recent past as virus surrogate in Flu serological assays.

Ability to produce PP expressing NA only would represent a great improvement in this field, allowing to easily generate a set of no infectious reagents to be used as alternative NA source to detect functional antibodies vs all known NAs.

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NOVARTIS VACCINES

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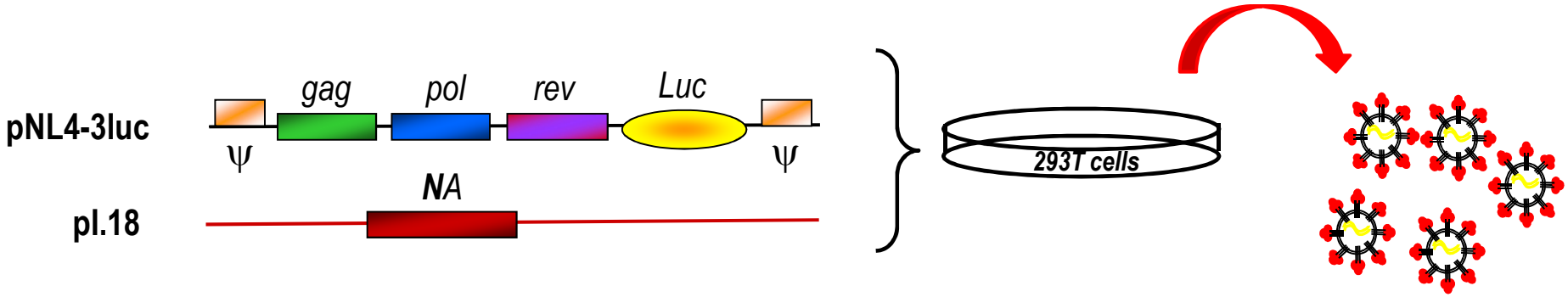
NA PseudoParticles as Mismatch Virus Surrogate

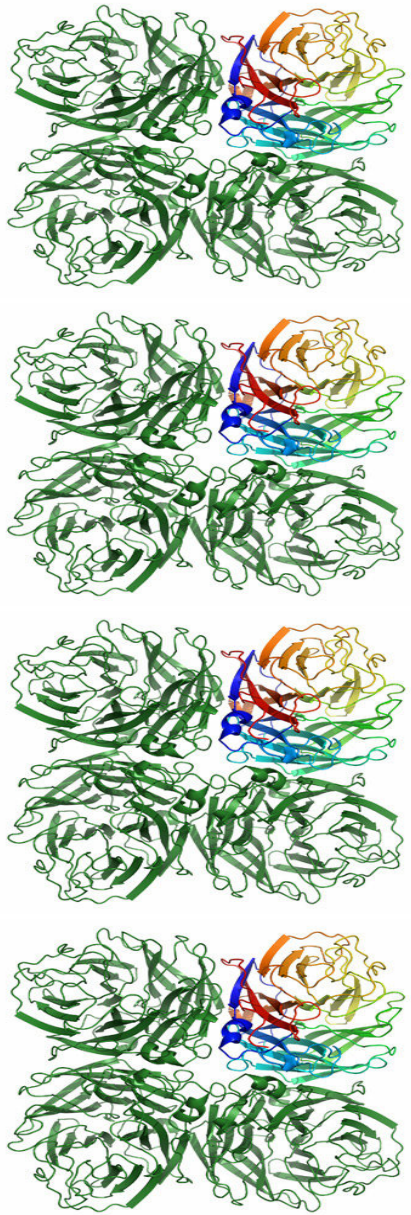
Quick & easy to generate and to scale up tool

Plasmids encoding the N1 neuraminidase of A/H1N1/California and the HA of H5N1 A/Vietnam/1194/04 strain were used to transfect human 293T cells in order to produce PPs expressing NA alone or in combination with the mismatched HA. In both cases particles were released in the culture supernatant of transfected cells; both preparations showed NA activity and gave comparable NI titers in the ELLA assay vs a set of selected sera.

1. *Seed 293T cells in p100 dishes and incubate for 24h @ 37°C, 5% CO_2*
2. *Add transfection mix in fresh culture medium and incubate for 48h @ 37°C, 5% CO_2*
3. *Filter supernatant with a 0,45 µm filter and store at -80°C in aliquots*



CONCLUSIONS



The PPN assay presents several advantages:

- *since pseudoparticles are unable to replicate, it can be carried out at BSL2, and therefore is compatible with the containment level available in most laboratories;*
- *it is promptly adaptable to high-throughput formats to evaluate immunogenicity of several pandemic vaccine formulations, and, in principle, it can be easily standardized among different laboratories;*
- *it allows a rapid and easy assessment of cross-neutralizing response by using pseudotypes bearing hemagglutinins (HA) from different clades without the need to have access to multiple clinical isolates which are often difficult to obtain and handle;*
- *can be used for detection Abs against NA as a source of Ag in the ELLA test.*

Acknowledgment

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