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Leveraging Innovative Platforms for Novel Vaccines

Leveraging Innovative platforms to investigate novel vaccines

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Full Professor in Public Health – University of Siena

Chief Scientific Officer – VisMederi srl

1996 - EMA guideliness

FLU

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 simultaneously.

The titre assigned to each sample shall be the geometric mean of two independent determinations;

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2017 - EMA guideliness

6.1.1. Immunological assays and parameters to be assessed

The assessment of the immunogenicity of influenza vaccines i
haemagglutination inhibition assay [HI] that detect antibody
single radial haemolysis assay [SRH]. Neither the HI nor the
shown that they are both subject to considerable inter-labora

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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

Guideline on Influenza Vaccines
Non-clinical and Clinical Module

FLU

The Virus Neutralisation (VN) assay quantifies functional a
detecting the ability of human serum at various dilutions t
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.

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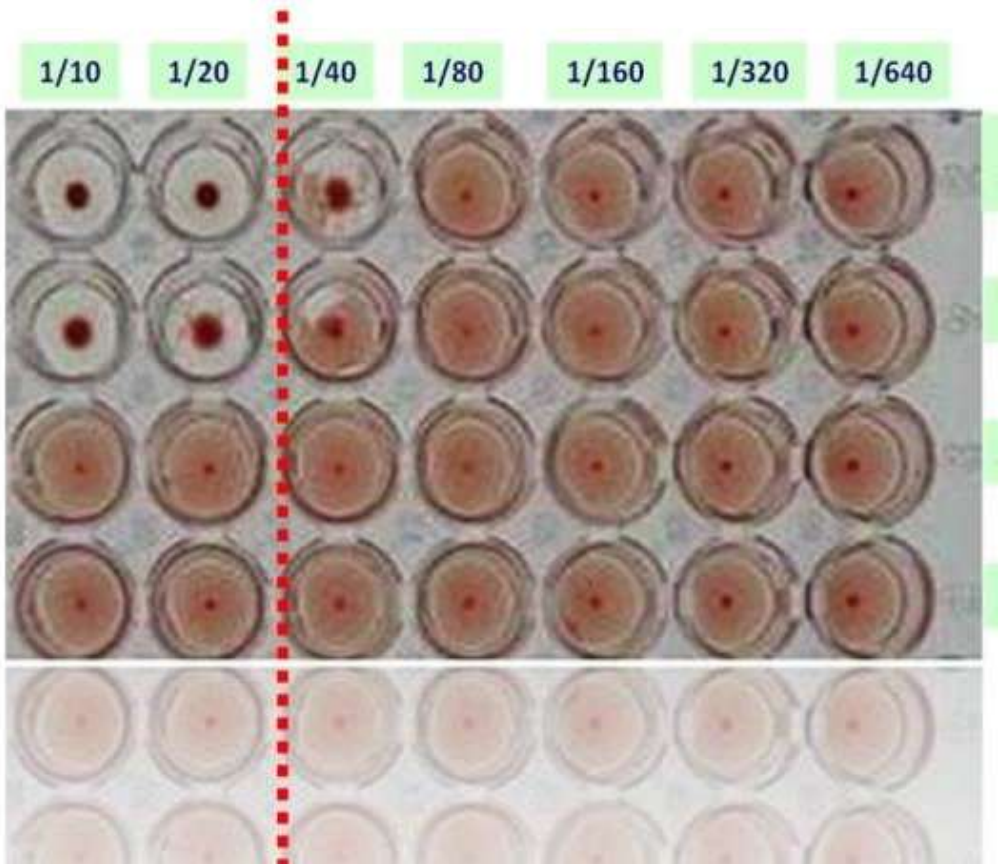
Actual Assays for FLU Ab detection

HAI – Haemagglutination Inhibition

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



MN – Virus Neutralization

- Titration of functional antibody only
- Gold Standard for confirmation
- Not easy for screening a large number of samples
- High containment (BSL3plus) needed in case of pandemic strains
- Detects Ab that bind around globular head and block virus attachment
- Detects cross-reactive Ab that bind to stem region
- No correlate of protection established

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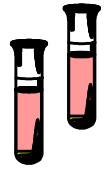
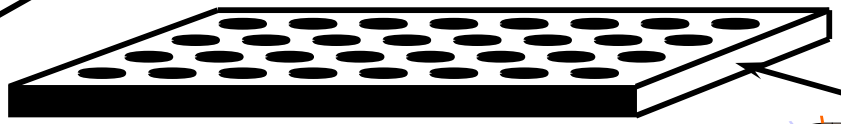
Neutralization (CDC protocol)

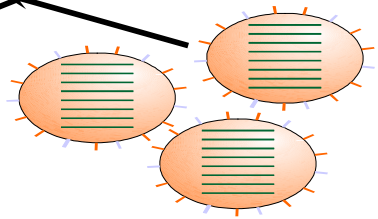


1. Sera

2. Add Sera

3. Dilute sera



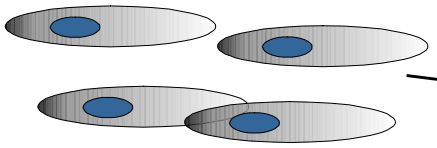


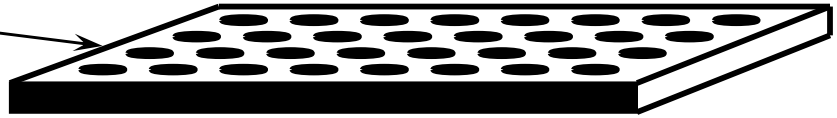
4. Add Virus

m.o.i has to be optimized for every strain

1 h @37°C

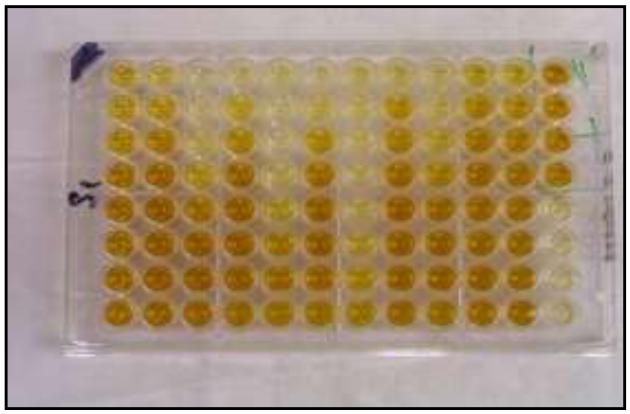
5. MDCK Cells





16-22 hrs no trypsin @37°C

6. Fix cells and run NP ELISA



From: Rowe T. J Clin Microb 1999

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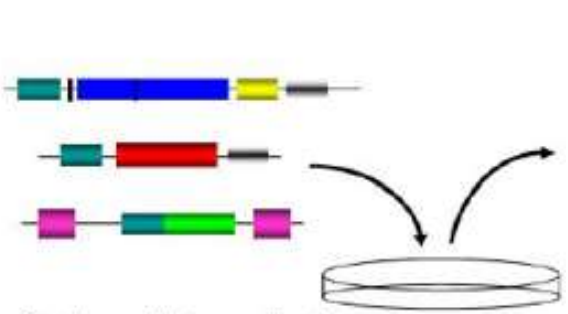
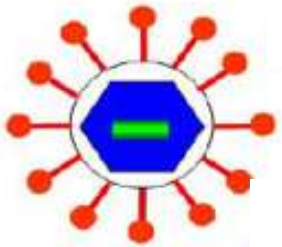
MN for Pandemic Assay *using PP*



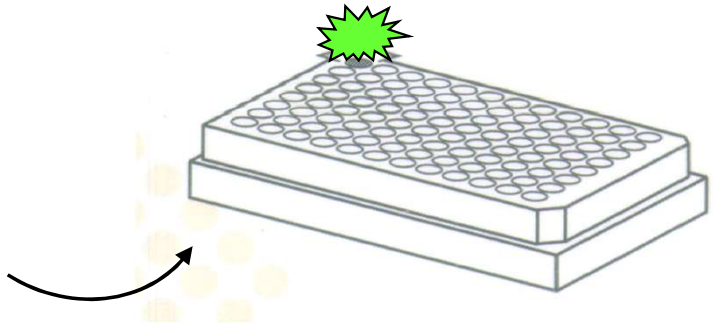
3 plasmid transient transfection into 293T cells



Haemagglutin (HA)

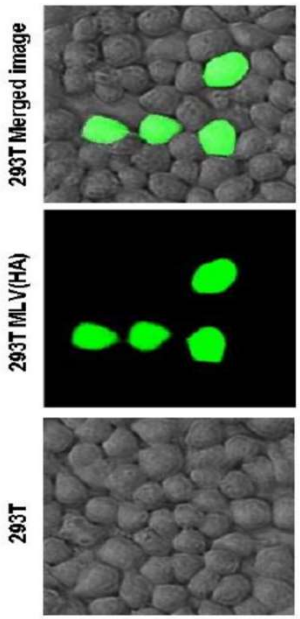
Lentiviral Gag-Pol

Vector encoding luciferase



Measure N-Ab titre of serum using a luciferase readout

Vaccine 27 (2009) 5998–6003



Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Pseudoparticle neutralization is a reliable assay to measure immunity and cross-reactivity to H5N1 influenza viruses

Isabella Alberini[a], Elena Del Tordello[a], Alba Fasolo[a], Nigel J. Temperton[b], Grazia Galli[a], 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]

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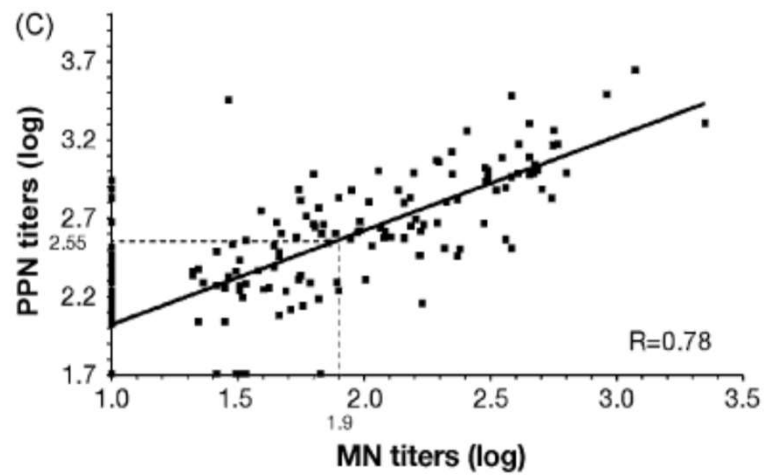
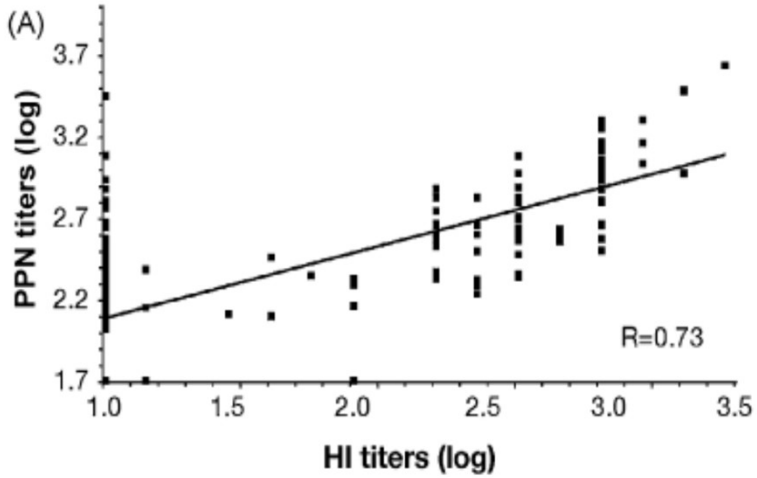
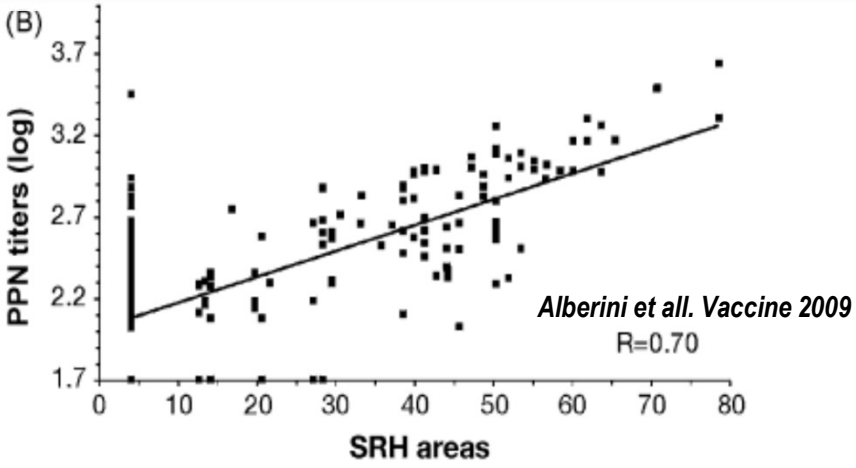
H5N1/VIET MN PP-based correlation

http://www.ClinicalTrials.gov NCT00382187

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







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).

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CEPI

CEPI expands global footprint of its COVID-19 vaccine lab network, and opens testing against Variants of Concern

The clinical sample testing laboratories part of this collaborative vaccine network are: Nexelis (Canada), Public Health England (PHE; UK), VisMederi Srl (Italy), Viroclinics-DDL (The Netherlands), icddr,b (formerly International Centre for Diarrhoeal Disease Research Bangladesh), and Translational Health Sciences and Technological Institute (THSTI, India), the National Institute for Biological Standards and Control (NIBSC; UK), Q2 Solutions (USA), and Universidad Nacional Autónoma de México (UNAM; Mexico).

Sars Cov2

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Sars Cov2

ELISAs

ELISAs for detection antibody (IgG; IgM; IgA) vs:

- S protein
- N protein
- RBD
- ……. Other (ACE2, avidity, etc….)

ELSEVIER

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Comparative analyses of SARS-CoV-2 binding (IgG, IgM, IgA) and neutralizing antibodies from human serum samples

Livia Mazzini [a], Donata Martinuzzi [b], Inesa Hyseni [b], Linda Benincasa [b], Eleonora Molesti [b,*], Elisa Casa [b], Giulia Lapini [a], Pietro Piu [a], Claudia Maria Trombetta [c], Serena Marchi [c], Ilaria Razzano [b], Alessandro Manenti [a,b], Emanuele Montomoli [a,b,c]

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NEUTRALIZATION

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DOI: 10.1002/jmv.25986

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Evaluation of SARS-CoV-2 neutralizing antibodies using a CPE-based colorimetric live virus micro-neutralization assay in human serum samples

Alessandro Manenti[1,2] | Marta Maggetti[2] | Elisa Casa[1,2] | Donata Martinuzzi[1] | Alessandro Torelli[1] | Claudia M. Trombetta[3] | Serena Marchi[3] | Emanuele Montomoli[1,2,3]

Sars Cov2

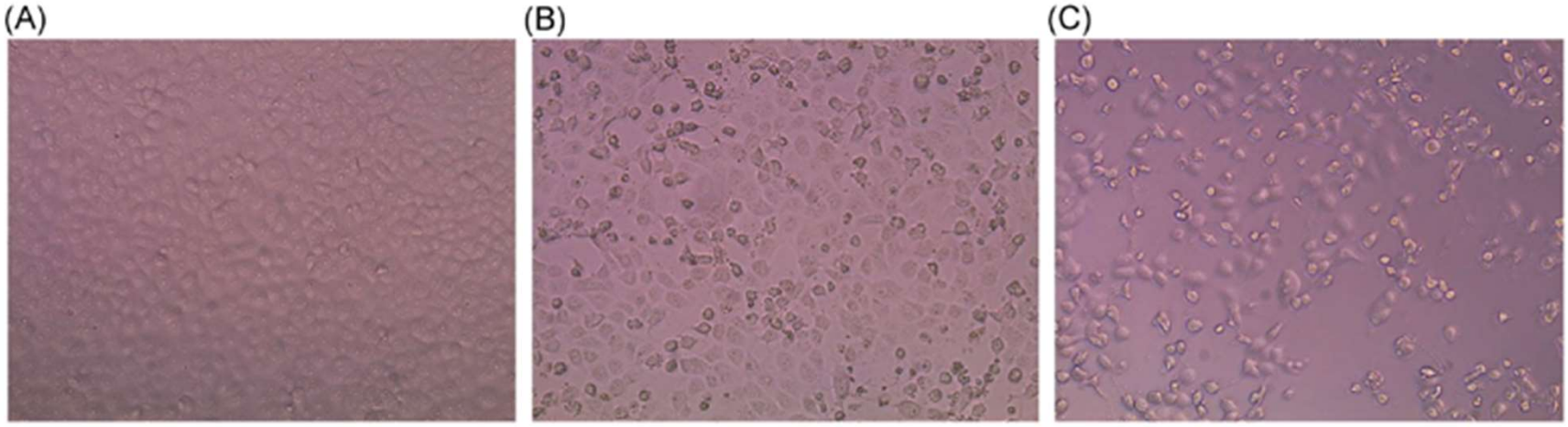

FIGURE 1 Vero E6 cells at different stage of infection. A, Not infected VERO E6 cell monolayer after 72 hours, complete absence of CPE. B, SARS-CoV-2 infected VERO E6 cell monolayer after 36 hours postinfection, 20%-30% of CPE recovered. C, SARS-CoV-2 infected VERO E6 after 52 hours postinfection, 80% of CPE recovered. CPE, cytopathic effect; SARS-CoV-2, Severe Acute Respiratory Syndrome-Coronavirus-2

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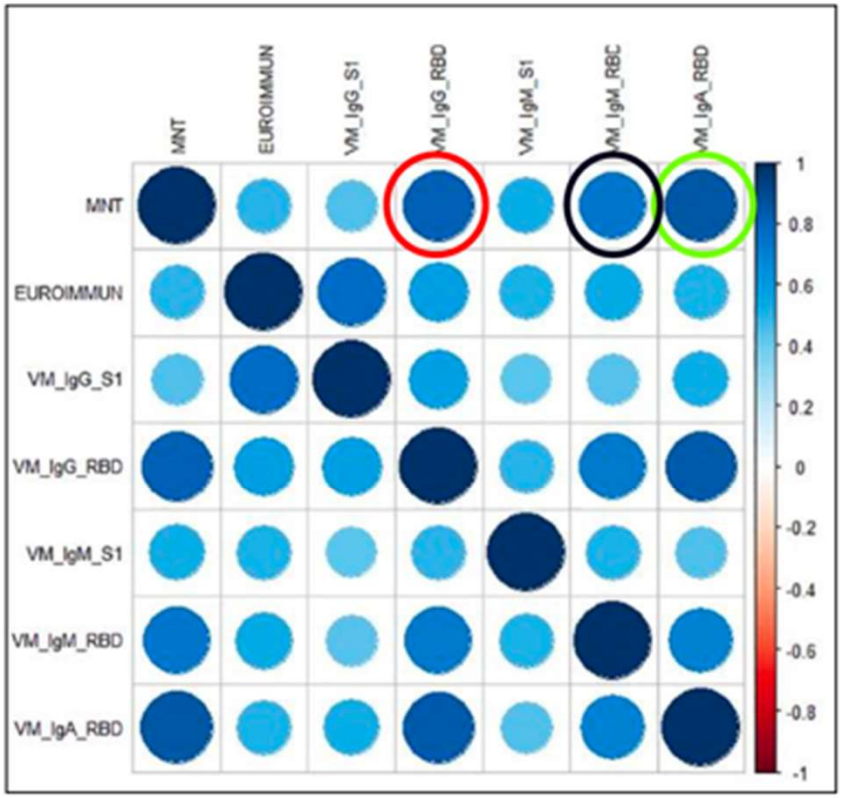
ELISAs vs MNT

Table 4
Statistical significance of Spearman's rank correlation coefficients.

	MNT	EUROIMMUN	VM_IgG_S1	VM_IgG_RBD	VM_IgM_S1	VM_IgM_RBD	VM_IgA_RBD
MNT	0.0E+00	3.8E-07	3.3E-10	8.1E-47	3.5E-14	1.5E-31	7.0E-53
EUROIMMUN	3.8E-07	0.0E+00	2.8E-20	1.9E-10	1.8E-07	1.5E-08	1.3E-07
VM_IgG_S1	3.3E-10	2.8E-20	0.0E+00	3.4E-18	2.1E-09	5.8E-10	2.1E-14
VM_IgG_RBD	8.1E-47	1.9E-10	3.4E-18	0.0E+00	1.5E-12	3.1E-31	2.5E-50
VM_IgM_S1	3.5E-14	1.8E-07	2.1E-09	1.5E-12	0.0E+00	5.5E-13	3.1E-10
VM_IgM_RBD	1.5E-31	1.5E-08	5.8E-10	3.1E-31	5.5E-13	0.0E+00	2.9E-27
VM_IgA_RBD	7.0E-53	1.3E-07	2.1E-14	2.5E-50	3.1E-10	2.9E-27	0.0E+00

Fig. 1. The correlation plot associated to the measured coefficients of Spearman's rank correlation. The magnitude of the coefficient is represented by circles and a color gradient: the larger the area of the circle and the more intense the tone of the color, the greater the correlation. The direction of the correlation is indicated by the color scale: blue tones for positive correlations and red tones for negative correlations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



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MULTI-ARRAY PLATFORMS

- need qualification of reagents
- need validation
- need *"correlates of protection"*

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HAI / MN / SRH different Ab detection