Leveraging Innovative Platforms for Novel Vaccines

Leveraging Innovative platforms to investigate novel vaccines

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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:
6.1.1. Immunological assays and parameters to be assessed

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

The **Virus Neutralisation (VN)** assay quantifies functional antibodies by detecting the ability of human serum at various dilutions to neutralise influenza virus 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 a non-overlapping subset.

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.
**HAI – Haemagglutination Inhibition**

- Suitable for screening a large number of samples
- Detects Ab that bind around receptor-binding site
- 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
1. Sera

2. Add Sera

3. Dilute sera

4. Add Virus

5. MDCK Cells

6. Fix cells and run NP ELISA

1 h @37°C

16-22 hrs no trypsin @37°C

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

From: Rowe T. J Clin Microb 1999
Pseudoparticle neutralization is a reliable assay to measure immunity and cross-reactivity to H5N1 influenza viruses

Isabella Alberini\textsuperscript{a}, Elena Del Tordello\textsuperscript{a}, Alba Fasolo\textsuperscript{a}, Nigel J. Temperton\textsuperscript{b}, Grazia Galli\textsuperscript{a}, Chiara Gentile\textsuperscript{c}, Emanuele Montomoli\textsuperscript{c}, Anne K. Hilbert\textsuperscript{d}, Angelika Banzhoff\textsuperscript{d}, Giuseppe Del Giudice\textsuperscript{a}, John J. Donnelly\textsuperscript{a}, Rino Rappuoli\textsuperscript{a,*}, Barbara Capecchi\textsuperscript{a}
The administered vaccine was a monovalent H5N1 subunit influenza vaccine derived from the /Vietnam/1194/2004 strain.

In the picture, vertical dashed line indicate the value of MN log10 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).

Alberini et al. Vaccine 2009
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).
ELISAs

ELISAs for detection antibody (IgG; IgM; IgA) vs:
- S protein
- N protein
- RBD
- .......... Other (ACE2, avidity, etc....)
Evaluation of SARS-CoV-2 neutralizing antibodies using a CPE-based colorimetric live virus micro-neutralization assay in human serum samples

Alessandro Manenti\textsuperscript{1,2} \textsuperscript{,} Marta Maggetti\textsuperscript{2} \textsuperscript{,} Elisa Casa\textsuperscript{1,2} \textsuperscript{,} Donata Martinuzzi\textsuperscript{1} \textsuperscript{,} Alessandro Torelli\textsuperscript{1} \textsuperscript{,} Claudia M. Trombeta\textsuperscript{3} \textsuperscript{,} Serena Marchi\textsuperscript{3} \textsuperscript{,} Emanuele Montomoli\textsuperscript{1,2,3}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1}
\caption{Vero E6 cells at different stages 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}
\end{figure}
Table 4
Statistical significance of Spearman’s rank correlation coefficients.

<table>
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<tr>
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<th>MNT</th>
<th>EUROIMMUN</th>
<th>VM_IgG_S1</th>
<th>VM_IgG_RBD</th>
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<th>VM_IgM_RBD</th>
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</tbody>
</table>

**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.)
- need qualification of reagents
- need validation
- need “correlates of protection”
The group
HAI / MN / SRH different Ab detection

not in H1

functional, only in MN

IgG1  IgG3  IgG4

IgG1  IgG2 not in SRH  IgG4

IgG1  IgG2 not in SRH  IgG4

IgG3