Unconjugated Peptide Vaccine Comprising Conserved Epitopes from Both SARS-CoV-2 and Influenza Virus Generates Durable and Broadly Reactive Antibodies to Multiple Coronavirus and Influenza Virus Strains

Nimisha Rikhi[1]*, Clara J. Sei[1], Kevin Muema[1], Kellie A. Kroscher[1], Richard F. Schuman[2], Aba Assiaw-Dufu[1], and Gerald W. Fischer[1]

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[1]Longhorn Vaccines and Diagnostics, Gaithersburg, Maryland, USA , [2]Antibody and Immunoassay Consultants, Rockville, Maryland, USA

*Corresponding author: **Nimisha Rikhi**, PhD, Senior Research Scientist, Longhorn Vaccines and Diagnostics. Email: nr@lhnvd.com, Phone: +1.718.710.2415

Background: Seasonal infections with influenza and SARS-CoV-2 along with co-infections are a new threat in the post-pandemic world. Co-vaccination may provide an effective preventative strategy. Unconjugated peptides targeting conserved epitopes of multiple pathogens provide a cost-effective, easily scalable vaccine approach for preventing these infections. In this study, we demonstrate that an unconjugated composite peptide vaccine comprising highly conserved influenza neuraminidase (NA) and Matrix (M1/M2) epitopes, and SARS-CoV-2 spike protein (SP) and RNA polymerase (POL) epitopes generated broad and durable neutralizing antibodies to coronavirus and influenza virus.

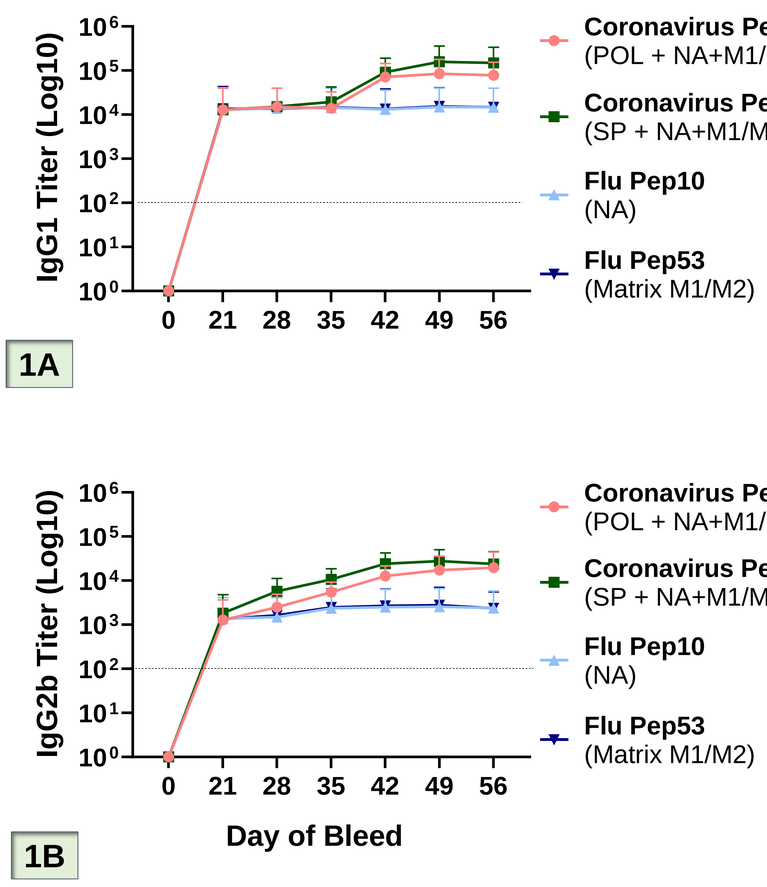
Methods:

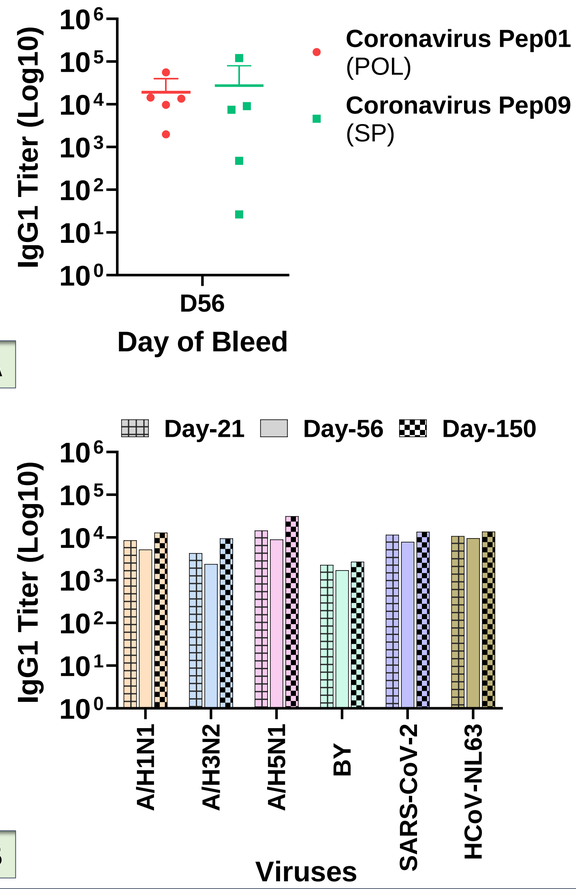
- ICR (outbred) mice were immunized subcutaneously (SQ) with 20 µg of Coronavirus Pep05 (Cor Pep05) and Coronavirus Pep11 (Cor Pep11) adjuvanted with AddaVax™ and screened for antibodies to coronavirus and influenza peptides and whole viruses using ELISA.
- Neutralizing activity against influenza virus was tested using microneutralization assay (MNA) and against coronavirus was tested using plaque reduction neutralization test (PRNT).

Peptide ID	Epitopes
Coronavirus Pep05	**SARS-CoV-2** RNA polymerase **(POL)** + **Influenza Virus (IV)** neuraminidase **(NA)** + matrix **(M1/M2)** + tetanus **T cell epitope**
Coronavirus Pep11	**SARS-CoV-2** spike protein **(SP)** + **IV (NA)** + **(M1/M2)** + **T cell epitope**

Mouse	Immunogen	Number of mice	Adjuvant	Dose	Immunization days	Route
ICR (outbred)	**Coronavirus Pep 05 + Coronavirus Pep 11** (Unconjugated)	**5**	**AddaVax**	**20µg**	**0, 21, 35**	**SQ**

Table 1. Top: SARS-CoV-2 and Influenza epitope composition of the two composite peptides included in the vaccine. **Bottom:** Immunization schedule for vaccination of outbred mice with combination of Coronavirus Pep05 and Coronavirus Pep11, both unconjugated.





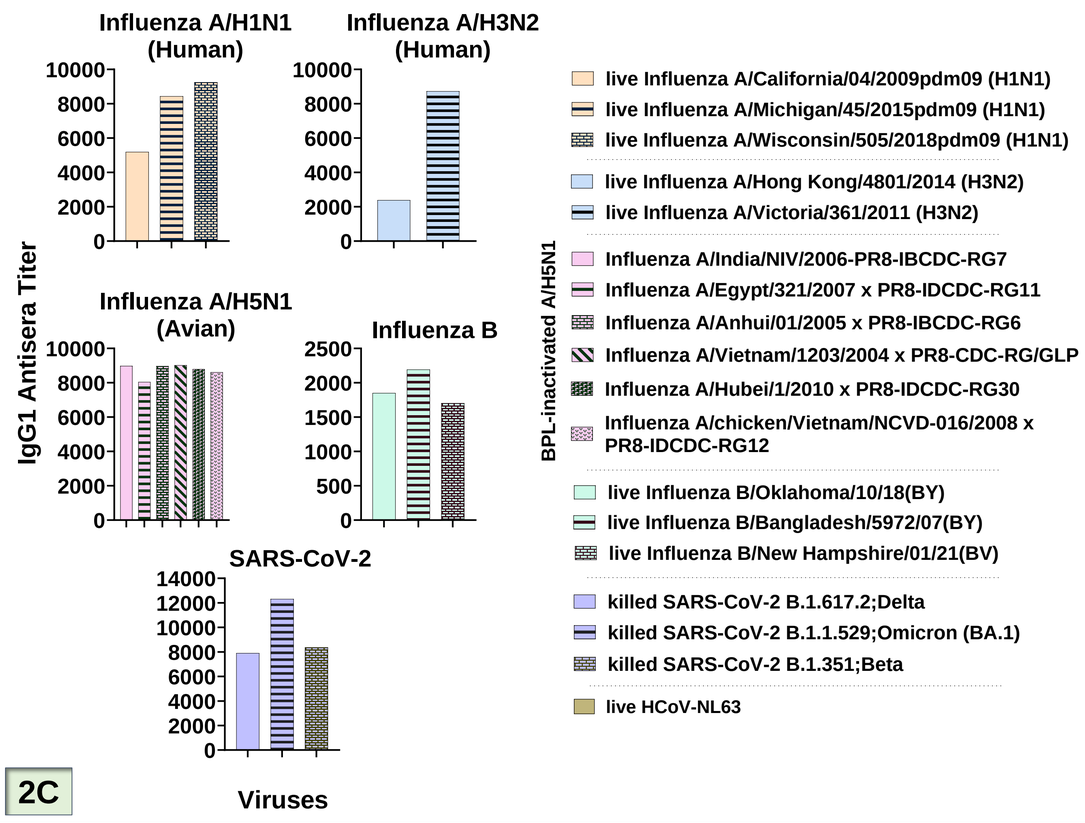
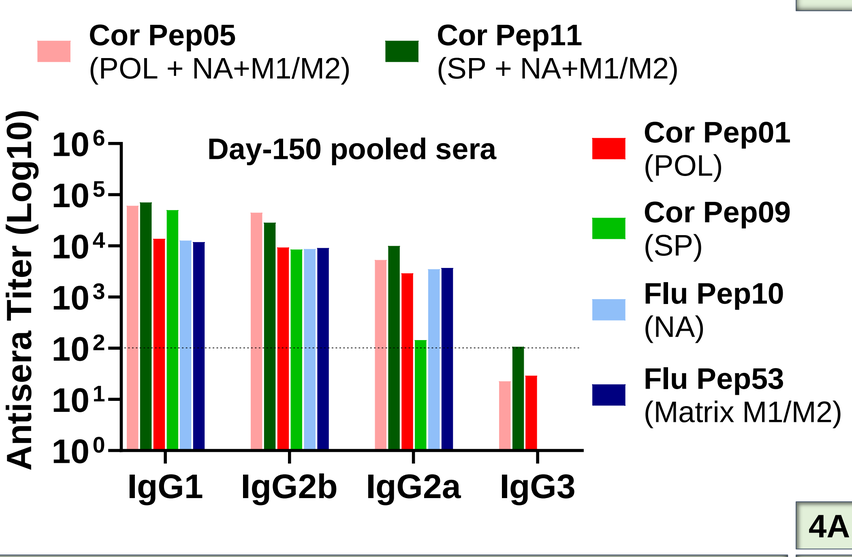
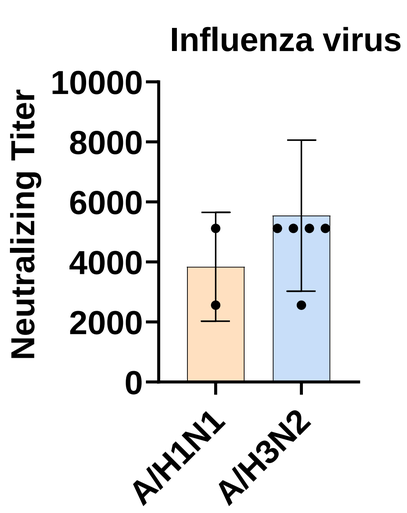


Figure 1. IgG1 **(A)** and IgG2b **(B)** antisera profile on composite coronavirus peptides and individual influenza peptides.

Figure 2. (A) Day-56 IgG1 titers to individual coronavirus peptides. **(B)** IgG1 antisera titers from day-21, day-56 and day-150 pooled sera to live influenza virus A/H1N1 and A/H3N2, inactivated A/H5N1, live influenza B/Yamagata, and killed SARS-CoV-2, live hCoV-NL63 coronaviruses. **(C)** IgG1 titers from day-56 pooled sera to multiple strains/variants of live influenza A/H1N1 and A/H3N2, inactivated A/H5N1, live influenza B and killed SARS-CoV-2 viruses.





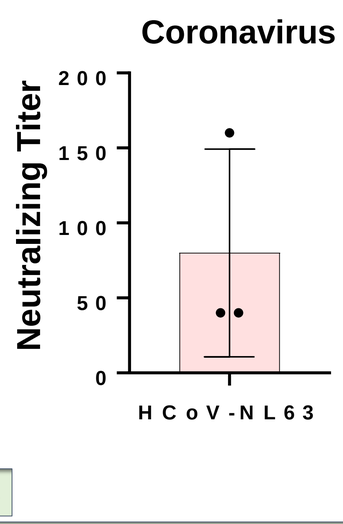


Figure 3. IgG1, IgG2b, IgG2a & IgG3 binding titers to coronavirus and influenza peptides from day-150 pooled sera.

Figure 4. Neutralizing titers from day-63 pooled sera against **(A)** human influenza virus A/H1N1 and A/H3N2 (MNA); **(B)** coronavirus hCoV-NL63 (PRNT). **(C)** Neutralizing titers from day-35, day-70 and day-150 pooled sera against SARS-CoV-2 (Omicron BA.5) (PRNT).

Conclusions:

- An unconjugated composite peptide vaccine comprising highly conserved coronavirus and influenza epitopes generated broad and durable (up to day-150 post primary immunization) antibodies to hCoV-NL63, SARS-CoV-2 (Beta, Delta, Omicron), human (A/H1N1, A/H3N2) and avian (A/H5N1) influenza A, and influenza B (BV, BY) viruses.
- IgG isotypes representing both Th1 and Th2 responses were observed.
- Generation of functional antibodies was demonstrated with cross-neutralizing activity against influenza virus A/H1N1, A/H3N2 and, coronavirus hCoV-NL63 and SARS-CoV-2 Omicron BA.5.
- Unconjugated composite peptide vaccines with highly conserved epitopes of multiple pathogens may provide an important strategy to combat seasonal and pandemic viruses as well as co-infections with different respiratory viruses.

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