# Peptide Tools to Support the Fight against COVID-19

Aude-Marie Alem[1], Michael Drosch[1], Ulf Reimer[1], Pavlo Holenya[1], Maren Eckey[1], Florian Kern[1,2], Holger Wenschuh[1], Karsten Schnatbaum[1]

[1] JPT Peptide Technologies GmbH, Volmerstrasse 5, 12489 Berlin, Germany. [2] Brighton and Sussex Medical School, Medical School Research Building, Rm 109, Brighton BN1 9PS, East Sussex, UK.

To date, the understanding of the immune response to COVID-19 is still incomplete. In this application note we describe innovative peptide-based tools for the assessment of humoral and cellular immunity to SARS-CoV-2. These tools are suitable for immune response target identification and clinical immune monitoring. They can also be used for T-cell and antibody response profiling and will be useful for the development of effective diagnostics, treatments, and vaccines.

## Introduction

As the COVID-19 pandemic is still ongoing,[1] the development of effective vaccines is a pressing need. As a result of an unprecedented effort across the scientific community, two vaccine candidates are already approved in the US and EU based on positive phase III data[2,3].

SARS-CoV-2, which is the cause of COVID-19, is a single-stranded RNA virus containing several proteins, including the structural proteins, Spike (S), Nucleocapsid (N), Membrane (M), and Envelope small membrane protein (E), as well as a number of non-structural proteins (Figure 1). The S protein is the target of the vast majority of vaccine candidates. However, other proteins are increasingly attracting attention, *e.g.* for the assessment of SARS-CoV-2-specific immunity or diagnostic test development.

Peptides have proven to be powerful tools for COVID-19 research. Here, we provide a systematic summary on the use of JPT's peptide-based products for the development of potential SARS-CoV-2 diagnostic tests, treatments, and vaccines.

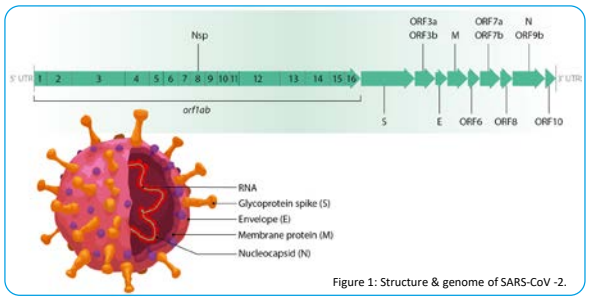


Figure 1: Structure & genome of SARS-CoV -2.

## Materials & Methods

Peptide libraries spanning the entire viral proteome were created and are provided in different peptide qualities and quantities as well as in various formats (Figure 2). Additionally, libraries covering the mutational landscape of SARS-CoV-2 and the ubiquitous human common cold corona-viruses were generated.

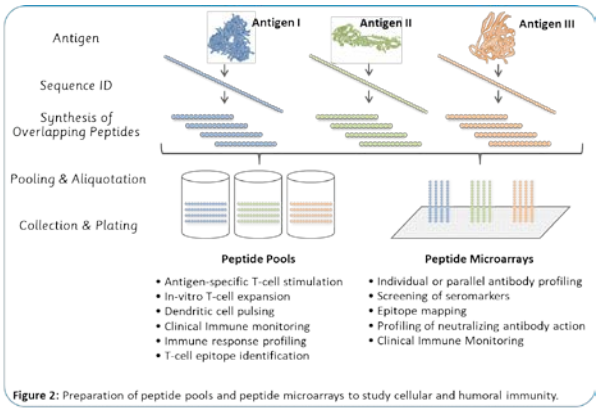


**Figure 2:** Preparation of peptide pools and peptide microarrays to study cellular and humoral immunity.

Peptide tools for studying cellular immunity, e.g. for T cell epitope discovery and immune monitoring, comprise individual peptides, matrix pools or antigen spanning pools for direct use in T cell assays such as ELISpot and ICS by flow-cytometry (Figure 2, left).

For B cell epitope discovery and humoral immune monitoring, peptide microarrays[4] represent an efficient technology using overlapping peptides to 'display' the entire virus proteome (Figure 2, right). Following incubation with samples, fluorescently labeled antibodies are used for detecting antibody binding.

Table 1: Overview on JPT's peptide based tools for SARS-CoV-2 and related corona viruses. *Epitope Mapping Peptide Set.

| Corona Virus Species | Protein | Cellular Immunity | | | Humoral Immunity | |
|---|---|---|---|---|---|---|
| | | PepMIx | EMPS* | Antigen Peptides | High density Microarray | Multiwell Microarray |
| SARS-CoV-2 | Spike glycoprotein (S) | x | x | x | x | x |
| | Spike glycoprotein (S) RBD | x | - | x | x | x |
| | Membrane protein (M) | x | x | x | x | x |
| | Envelope small membrane protein (E) | x | x | - | x | x |
| | Nucleocapsid protein (N) | x | x | x | x | x |
| | Non-structural / ORF proteins /Replicase polyprotein | x | - | - | x | - |
| | Spike/N mutations - Updated regularly | x | | | | |
| Common Cold Viruses NL63, HKU1, 229E, OC43 | Spike glycoprotein (S) | x | x | - | x | - |
| | Nucleocapsid protein (N) | x | - | - | x | - |
| | Membrane (M), Envelope (E) | - | - | - | x | - |
| SARS-CoV-1 & MERS | Spike glycoprotein (S) | x | x | - | x | - |
| | Membrane (M), Envelope (E), Nucl. (N) | - | - | - | x | - |

It has been shown that SARS-CoV-2 elicits strong cellular and humoral immune responses and both B and T cell immunity appear critically important for the control of the virus as well as for the development of effective SARS-CoV-2 vaccines.[5] Therefore, peptide based tools addressing both types of adaptive immunity have been developed (Table 1).

Emphasis was placed on providing complete coverage of all SARS-CoV-2 proteins. This is because of increasing evidence that not only the S protein, but also the other structural proteins (N, M, E, M), and even the non-structural / ORF proteins might substantially contribute to an immune response against SARS-CoV-2. For example, cellular immune responses against 12 different SARS-CoV-2 proteins have been observed in recovered COVID-19 patients.[6] Other studies detected T cells against 21 and 6 different proteins..[7,8] Humoral immune responses to SARS-CoV-2 were found to be mainly focused on the S and N protein.[9] However, significant IgG reactivity has also been observed to peptides derived from the M, AP3A and R1AB proteins. [10]

**Clinical Immune Monitoring**

Several recent studies describe the use of JPT's PepMix™ peptide pools for immune monitoring of COVID-19 vaccine candidates (Table 2). These include clinical studies with some of the most advanced vaccine candidates to date.

Table 2: Recent publications on the use of JPT's PepMix™ peptide tools for immune monitoring of vaccines against SARS-CoV-2. ICS = Intracellular cytokine staining. CBA = Cytometric bead array.

| Study | T Cell Assay | Reference |
|---|---|---|
| Phase I/II | ELISpot, ICS | Sahin et al., medRxiv preprint https://doi.org/10.1101/2020.07.17.20140533 |
| Phase I | ICS | Jackson et al., N Engl J Med. 2020 doi: 10.1056/NEJMoa2022483 |
| Rhesus macaque | ELISpot, ICS | Vogel et al., bioRxiv preprint https://doi.org/10.1101/2020.09.08.280818 |
| Rhesus macaque | ICS | Corbett et al., N Engl J Med. 2020 doi: 10.1056/NEJMoa2024671 |
| Rhesus macaque | ELISpot, ICS | Mercado et al., Nature 2020 doi: 10.1038/s41586-020-2607-z |
| Non-human primate | ELISpot | Kalnin et al., bioRxiv preprint https://doi.org/10.1101/2020.10.14.337535 |
| Hamster | ICS, CBA | Rauch et al., bioRxiv preprint https://www.biorxiv.org/content/10.1101/2020.10.23.351775v1 |

**Cell Therapy Development**

With the recent success of novel immunotherapies, including cell-based therapies, the need for clinical grade peptides & peptide pools has grown rapidly. Building on an ISO 9001:2015 certified quality management system we have established an enhanced peptide production environment that adds critical quality measures to the standard production process used for research use only (RuO). The resulting quality levels, referred to as ISO PLUS Peptides and Clinical Grade Peptides (CGP), focus on the more stringent requirements of immunotherapy: for example, cell therapy, clinical immune monitoring, as well as vaccine development. These peptides have been approved for a variety of clinical applications.

Cell-based therapies using peptide pools for preparing expanded antigen specific T-cell populations have been developed for a number of infectious diseases. Examples include pools for the *ex vivo* generation of T cells for HIV[11,12], CMV[13,14], broad-spectrum antiviral (AdV, EBV, CMV, BKV, HHV6)[15] and, very recently, COVID-19 treatment.[16] Several investigational new drug (IND) applications for cell therapies using clinical grade peptides have been submitted, including a program against SARS-CoV-2.

**Diagnostic Test Development**

Many diagnostic tests for prior SARS-CoV-2 infection, based on the detection of antibodies, have been developed using full-length viral proteins as capture antigens.[17] However, epitope defined analysis of B cell immunity of COVID-19 patients using peptide microarrays showed that significant cross reactivity existed against other corona viruses, i.e. SARS, MERS, and the common cold viruses OC43, HKU1, NL63 and 229E.[10] The discovery of immunodominant epitopes that are specific for SARS-CoV-2[10] will accelerate the development of a sensitive and highly specific test for previous SARS-CoV-2 infection.

Development of T cell based diagnostics for SARS-CoV-2 will have to consider cross reactivity to common cold viruses, as pre-existing SARS-CoV-2 (cross-)reactive T cells have been found in donors not previously exposed to this novel virus.[18] Several T cell based tests are in development, and a discovery assay is already available[19] that builds on a technology that is already in clinical use.[20]

## Discussion & Conclusions

To support the fight against the COVID-19 pandemic, we established peptide based tools that combine high throughput peptide synthesis, innovative peptide presentation approaches, and synergistic assay formats. Examples include COVID-19 immunity assessment, target identification, clinical immune monitoring, cell therapy development and diagnostic test development.

## References

1. WHO. Coronavirus disease (COVID-2019) situation reports. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports.
2. Walsh et al., N. Engl. J. Med. (2020) – PMID: 33053279
3. Baden et al., N. Engl. J. Med. (2020) – PMID: 33378609
4. Masch et al., Methods Mol Biol (2010) – PMID: 20857365.
5. Cox et al., Nat Rev Immunol. (2020) – PMID: 32839569.
6. Bonifacius et al., Immunity Sneak Peek (2020), Available at SSRN: https://ssrn.com/abstract=3661946.
7. Grifoni et al., Cell (2020) – PMID: 32473127.
8. Peng et al., Nat Immunol. (2020) – PMID: 32887977.
9. Amrun et al., EBioMedicine (2020) – https://doi.org/10.1016/j.ebiom.2020.102911.
10. Holenya et al., Preprint, medRxiv 2020.11.24.20216663.
11. Lam et al., Mol Ther. (2015) – PMID: 25366030.
12. Patel et al., Mol Ther Methods Clin Dev. (2019) – PMID: 31720305.
13. Smith et al., Clin Infect Dis. (2019) – PMID: 29982441.
14. Smith et al., J Clin Invest. (2020) – PMID: 32750039.
15. Papadopoulou et al., Sci Transl Med. (2014) – PMID: 24964991.
16. Vasileiou et al., 62nd ASH Annual Meeting, Abstract no. 612.
17. La Marca et al., Reprod Biomed Online (2020) – PMID: 32651106.
18. Braun et al., Nature (2020), https://doi.org/10.1038/s41586-020-2598-9.
19. Oxford Immunotec, Press release, 2020.
20. Rego et al., Tuberculosis (Edinb). (2018) – PMID: 29523321.

## The Author

**Aude-Marie Alem**
aude.alem@jpt.com
Business Development Associate
JPT Peptide Technologies GmbH, Berlin, Germany

## The Company

**JPT Peptide Technologies** is a DIN ISO 9001:2015 certified integrated provider of innovative peptide solutions for immunotherapy development, cellular and humoral immune monitoring, epitope & target discovery, targeted proteomics, and enzyme profiling.

**Contact us for further information!**
Email: peptide@jpt.com
Phone: +49 30 6392 7878

**Please visit us online at:**
- > http://jpt.com