

Universal EpitoGen® SARS-CoV-2 ELISA assay

At a population level, the humoral response to SARS-CoV-2 is heterogeneous. In the course of our development of EpitoGen SARS-CoV-2 assays, it was observed that significant number of cases (5%) react only to the spike or nucleocapsid antigens, but not both. Also, genomic variability of SARS-CoV-2 as a result of mutations in the spike and nucleocapsid proteins will further reduce sensitivity of the diagnostic tests. Therefore, traditional antibody assays using either the spike or nucleocapsid as antigens are less sensitive.



A novel approach is required to overcome inherent technical problems in existing assays to enhance specificity and sensitivity.

Principles of Universal EpitoGen® COVID-19 test

1. Appreciation of the B-cell immunodominance phenomenon. This will enrich for the positive signal and improve sensitivity. Another advantage is that cross-reactive epitopes, especially those with homology with seasonal coronaviruses, can be eliminated, subsequently improving specificity.
2. A platform to overcome population heterogeneity (i.e. HLA genetic variation, co-morbidities, age, ethnicity, etc).
3. Universal EpitoGen is Differential EpitoGen amenable to combining multiple SARS-CoV-2 proteins (5 antigens) in one test, subsequently improving sensitivity of the assay.
4. Universal EpitoGen overcomes SARS-CoV-2 genetic variability (i.e. mutations) by inclusion of prevalent mutations.

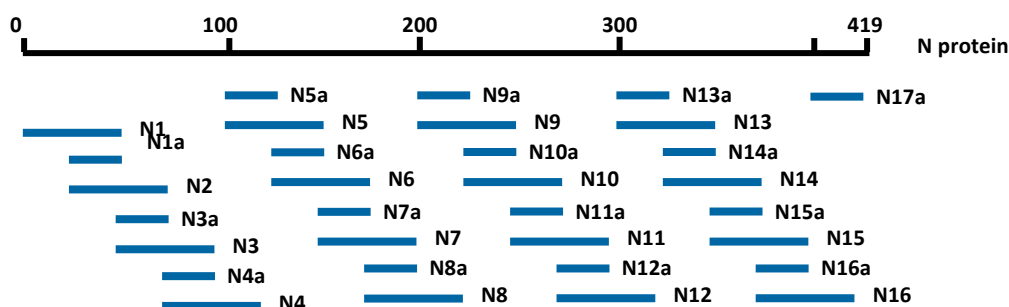
Using [EpitoGen Technology](#), a set of 15 immunodominant epitopes, along with their corresponding major circulating mutants (**45 prevalent mutations**), from five (N, S, M, ORF3a and ORF7a) SARS-CoV-2 proteins were complexed to create a universal antigen. *The mutations are present in the UK, South African, Brazilian and Indian variants.*

Intended Use of EpitoGen® Universal

Detection of antibody response to SARS-CoV-2 resulting from vaccination and/or infection

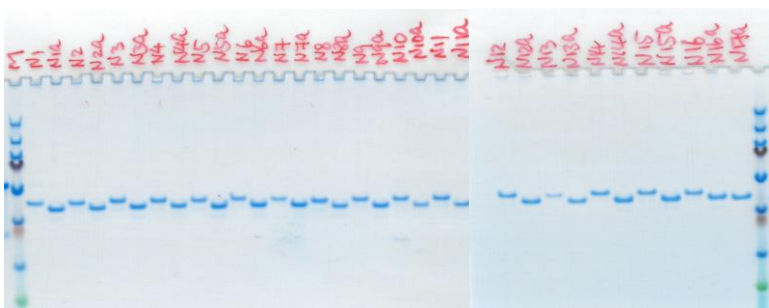
1 – B-cell immunodominance

a) SARS-CoV-2 immunogenicity mapping using [EpitopePredikt](#)



Immunogenicity profiling of immunodominant epitopes corresponding to SARS-CoV-2 nucleocapsid protein.

b) Expression of individual epitopes on EpitoGen platform



Recombinant expression of 33 epitopes

Developers

AIBIOLOGICS
EPIPOPE
PREDIKT

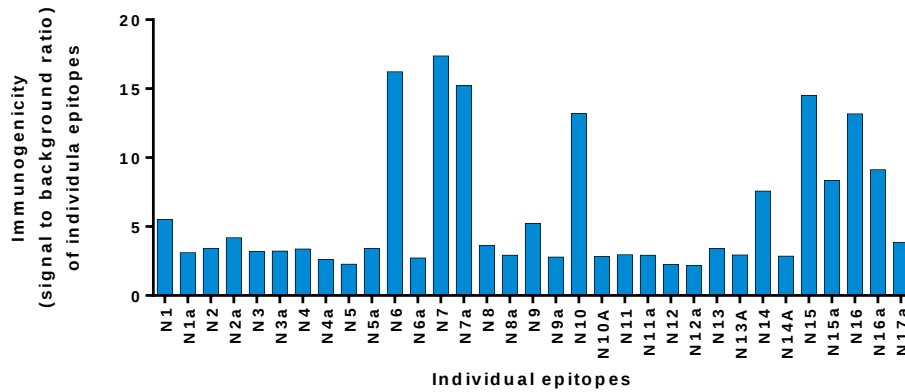
VERTEBRATE
ANTIBODIES

UNIVERSITY
OF ABERDEEN

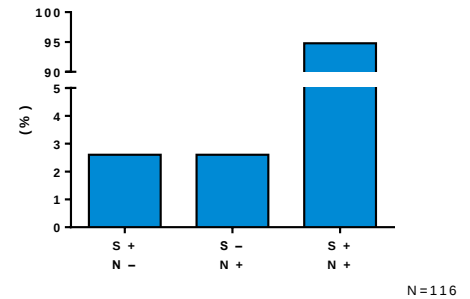
NHS
Grampian

2 – Population heterogeneity

a) Identify population-prevalent immunodominant epitopes by screening a large population

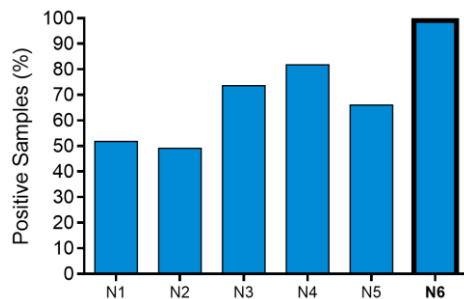
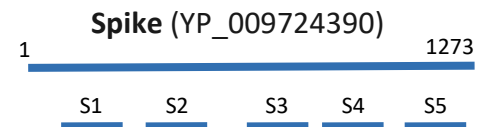
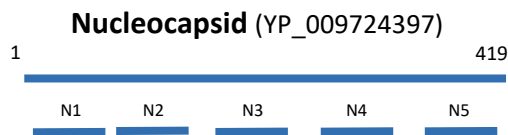


Individual epitopes were screened across a heterogeneous population to identify immunodominant epitopes

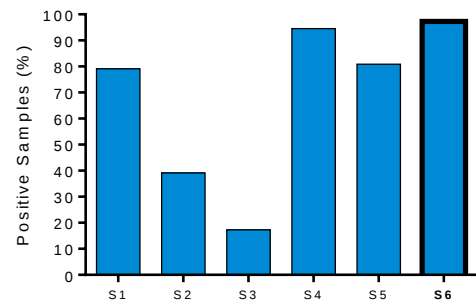


Antibody response varies among individuals towards SARS-CoV-2 spike (S) and nucleocapsid (N) antigens.

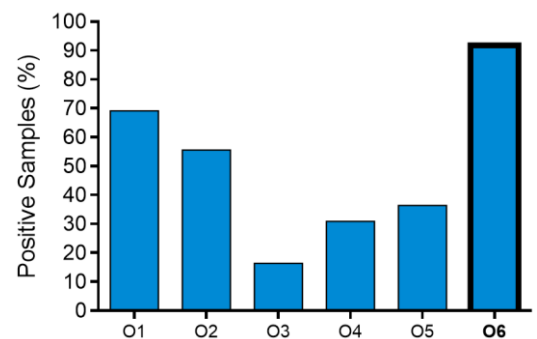
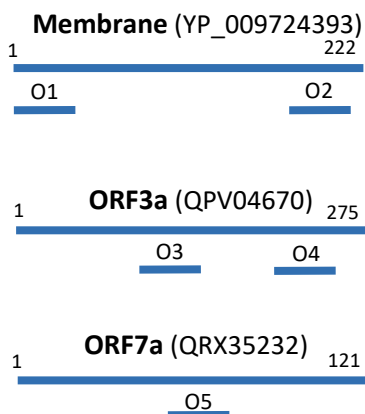
b) Epitopes were fused in one antigen-complex to enhance sensitivity



Percentage of COVID-19 samples which tested positive for SARS-CoV-2 specific antibodies by each epitope. The composite epitope N6 showed **99.1%** sensitivity (n=110).



Percentage of COVID-19 samples which tested positive for SARS-CoV-2 specific antibodies by each epitope. The composite epitope S6 showed **97.3%** sensitivity (n=110).



Percentage of COVID-19 samples which tested positive for SARS-CoV-2 specific antibodies by each epitope. The composite epitope O6 showed **91.8%** sensitivity (n=110).

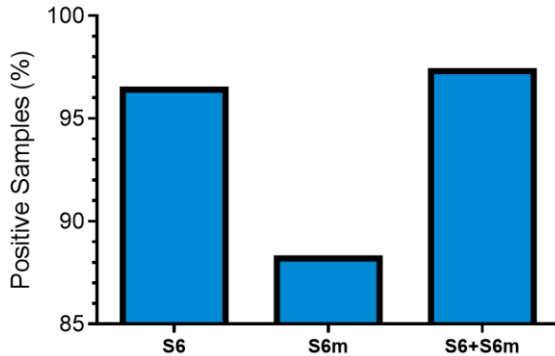
3 – SARS-Cov-2 genetic variation

Using EpitoGen Technology, major circulating mutants (**45 prevalent mutations**), from five (N, S, M, ORF3a and ORF7a) SARS-CoV-2 proteins were complexed to create an universal **mutant** antigen. *The logic behind including mutants is that often mutations will create additional epitopes that will elicit antibody responses.*

Composite Spike S6



Composite Spike S6m
16 mutations

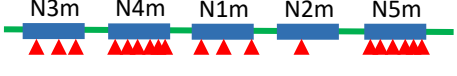
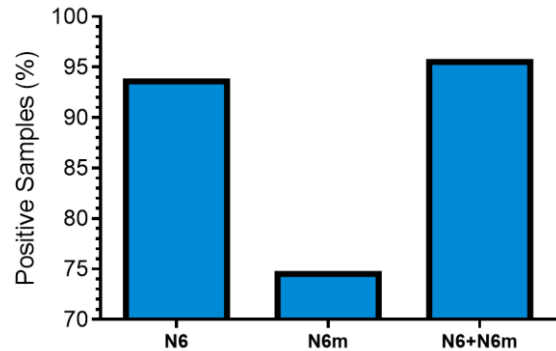



Percentage of COVID-19 samples tested positive when using composite spike epitome S6, spike mutant epitope-complex S6m, and S6+S6m combined. **The inclusion of mutants-complex S6m increased the assay sensitivity to 97.3% (n=110).**

Composite N6



Composite N6m
19 mutations

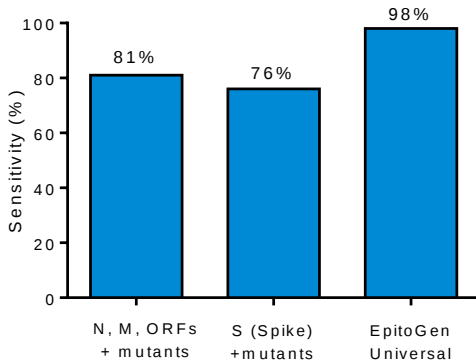



Percentage of COVID-19 samples which tested positive when using composite epitome P6, P6m, and N6+N6m combined. **The inclusion of mutants-complex N6m increased the assay sensitivity to 96% (n=110).**

4 – Utilise five SARS-CoV-2 viral antigens in one test to boost sensitivity (S, N, M, ORF3a and ORF7a)

Universal EpitoGen

EpitoGen Complexes

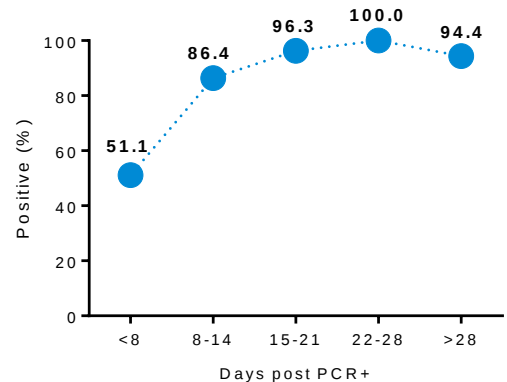


Combining epitopes from 5 viral SARS-CoV-2 proteins and the prevalent circulating mutations **enhances the assay sensitivity.**

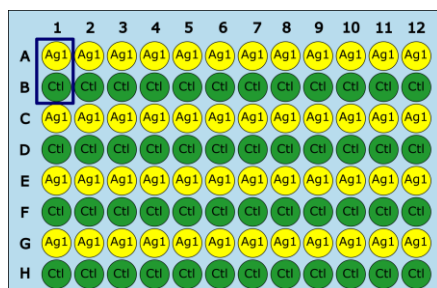
N=204

Combines the 30 epitopes: (N6+N6m+S6+S6m+O6+O6m)

EpitoGen Clinical Sensitivity



ELISA EpitoGen® Universal layout



Ag1 **Antigen 1 (Ag1)** = A set of composite EpitoGens comprising 30 epitopes from five viral proteins (S, N, M, ORF3a and ORF7a.)

Ctl **Control (Ctl)** = The EpitoGen scaffold protein.

It is designed for the detection of **IgG* antibody response to SARS-CoV-2** infection and immunisation.

This is **the first Epitope-based SARS-CoV-2 test** to utilise epitopes from **five SARS-CoV-2 proteins in one assay**, and to include prevalent **mutations**.

* The epitopes selected are also **IgM and IgA rich** meaning the assay is suitable for the detection of IgA and IgM antibody responses to evaluate active SARS-CoV-2 infection or **mucosal immunity**.