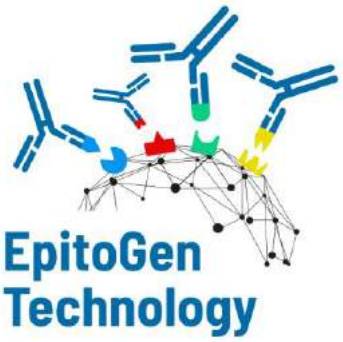
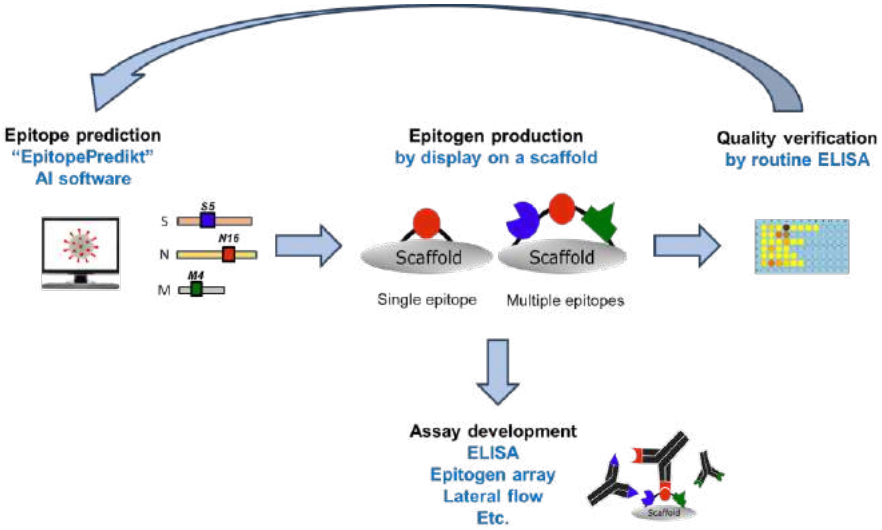
EpitoGen Technology

EpitoGen Technology is a novel epitope/peptide expression platform. The epitope antigen (Epitogen) is fused in the middle of an **inert** scaffold protein and displayed on the top of the scaffold ideally suited to a number of antigen/antibody-based assays such as ELISA and lateral flow. The technology was developed by Vertebrate Antibodies Ltd in collaboration with researchers from the University of Aberdeen.



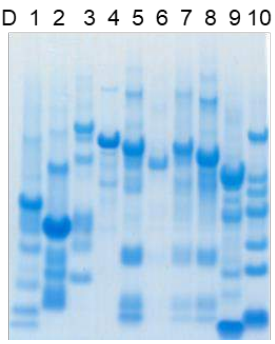
The EpitoGen Technology consists of a bioengineered scaffold protein to which a single epitope or multiple epitopes of varying sequences and length can be fused. The hydrophobic tail contained at the N and C terminals will bind preferentially to hydrophobic surfaces and exposing the candidate epitopes to the surface. This arrangement allows for the epitopes to assemble in their native conformation for specific binding to their cognate antibodies.



The scaffold exhibits a compact and structurally rigid core that is monomeric, highly stable, soluble, non-toxic, and capable of to supporting complex peptides. The scaffold is capable of high production levels in prokaryotic cells and contains a purification tag.

EpitoGen Technology efficiently expresses short epitopes to vastly improve antibody tests

Expression of short epitopes in E. coli, conventional approach

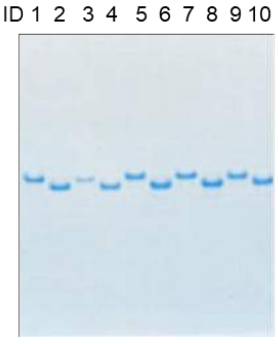


– Poor expression

– Poor stability (degradation)

– Aggregation issues

Expression of short epitopes in E. coli, Epitogen approach

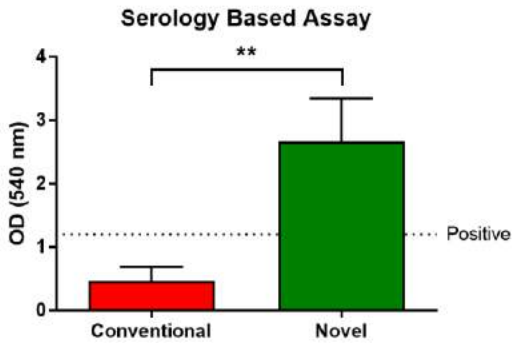


– High expression

– Increased stability

– Excellent solubility

Comparisons between epitopes expressed using conventional approach *versus* novel Epitogen Technology approach



***P*=0.007

Bioengineered scaffold ensures accurate folding of epitopes

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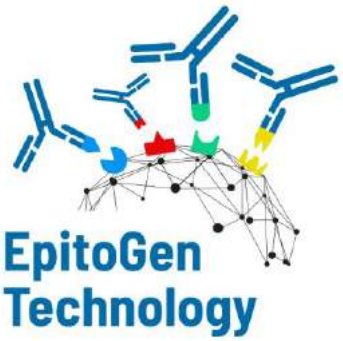
EpitoGen SARS-CoV-2 Antibody Assays

EpitoGen Technology and AiBiologics machine learning software (EpitopePredikt) were employed to develop a set of unique, highly sensitive SARS-Cov-2 ELISA assays. EpitopePredikt was used to scan SARS-CoV-2 antigen and identify immunodominant epitopes. The epitopes were then expressed in combination using **EpitoGen Technology** and validated for detection of SARS-CoV-2 antibodies in collaboration with researchers from the University of Aberdeen and the NHS (Grampian). The project was funded by **Scottish Government's Chief Scientist Office**.



Detection and characterisation of SARS-CoV-2 specific antibodies is crucial to accurately determining antibody responses across the population which demonstrates a substantial degree of heterogeneity in the humoral response to SARS-CoV-2 infection. This information is crucial to ongoing design of diagnostics and vaccines particularly with respect to emerging variant forms of SARS-CoV-2.

Using EpitoGen Technology, we created a variety of ELISA-based tests which can detect a population level spectrum of SARS-CoV-2 IgG antibodies specific to the **spike**, **nucleocapsid** and other SARS-CoV-2 viral proteins (**Membrane, ORF3a, ORF7a**). The high sensitivity of the assay is ideally suited to the study of immune response to both natural infection and vaccination for SARS-CoV-2.

Benefits of the SARS-CoV-2 EpitoGen Assays include:

- ✓ **95-100% sensitivity** and **99% specificity**
- ✓ **Differentiation** between **vaccination-induced** and **infection-induced** protection
- ✓ **Specific** detection of **SARS-CoV-2 mutants**
- ✓ **Integration into** existing ELISA assay **protocols**
- ✓ Highly **stable** components, **easy** to ship and store

Vertebrate Antibodies has produced 4 EpitoGen assays

1. **EpitoGen Universal** - Detection of antibody response to *15 epitopes* from five SARS-CoV-2 viral proteins including corresponding mutants (*45 prevalent mutations*)
2. **EpitoGen Differential -** to differentiate antibody response from vaccination-induced and infection-induced protection
3. **EpitoGen Immunity** – Evaluation of antibody response following vaccination or natural infection, and assess the antibody response against the original and mutant SARS-CoV-2
4. **EpitoGen Spike Mutant** - Detection of antibodies specific to variant forms of SARS-CoV-2 spike protein and evaluate their potential impact on vaccine performance and immunity

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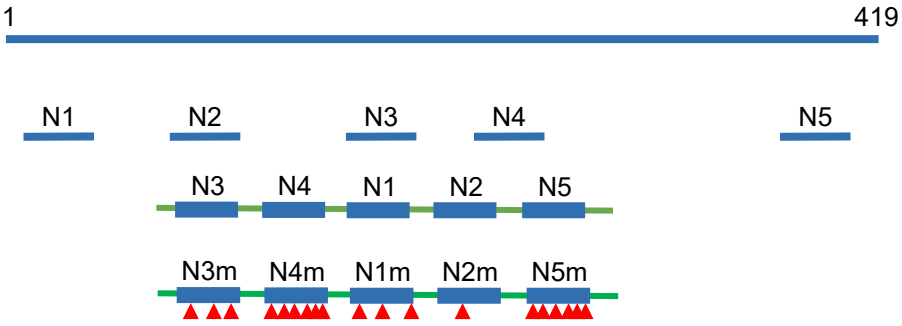
Composite EpitoGen boosts sensitivity of SARS-CoV-2 ELISA assay

EpitoGen Technology

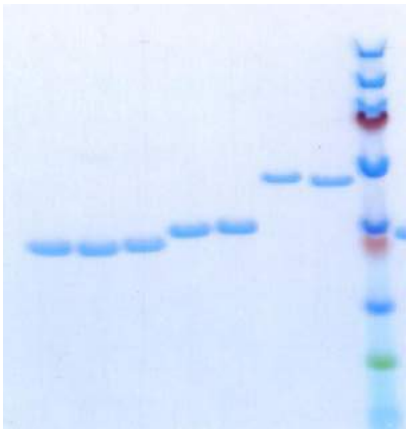
Nucleocapsid protein
(YP_009724397)



Epitope locations

Composite Nucleocapsid EpitoGen **N6**

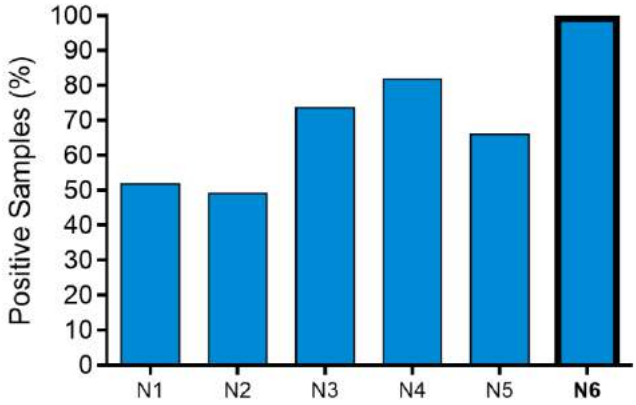
Composite Nucleocapsid EpitoGen **N6m**
19 mutations from N6



N1 N2 N3 N4 N5 **N6** **N6m**

Expression of single epitogens (N1 to N5), the composite epitogen (N6) and its corresponding prevalent mutants (N6m) using EpitoGen Technology visualised using SDS-PAGE.

EpitoGen demonstrated high expression levels and stability.

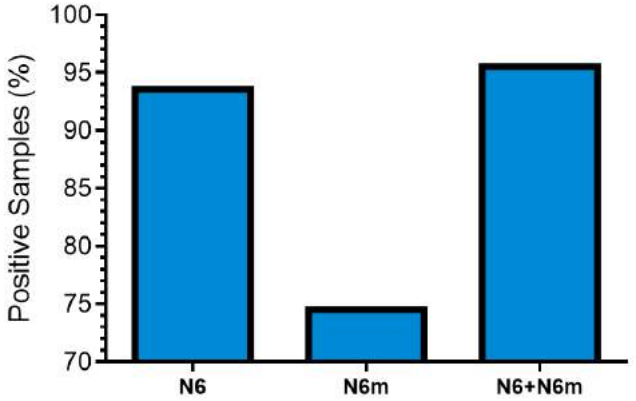


Percentage of COVID-19 samples which tested positive for SARS-CoV-2 specific antibodies by each individual epitogen. The composite epitogen P6 showed **99.1%** sensitivity (n=110).

EpitoGen works well with either a single epitope or composite epitopes.
Complexing of epitopes enhanced sensitivity of the test.

Percentage of COVID-19 samples which tested positive for SARS-CoV-2 specific antibodies by composite epitogen P6, P6m, and N6 + N6m combined. **The inclusion of mutants-complex N6m N6m increased the assay sensitivity to 95.5% (n=110).**

It is imperative to include circulating SARS-CoV-2 mutants into future diagnostic tests to maintain high levels of detection.



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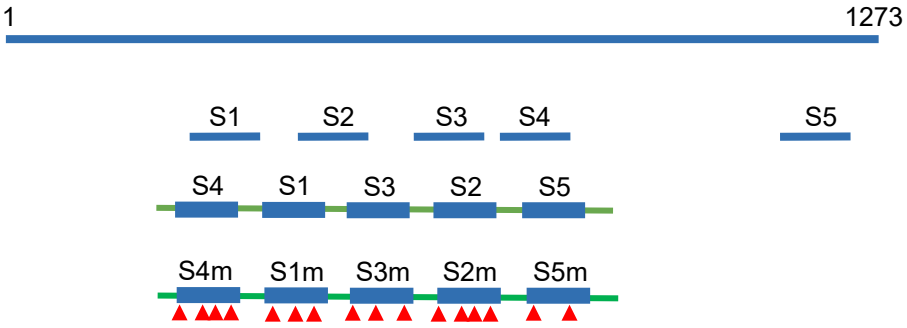
Composite EpitoGen boosts sensitivity of SARS-CoV-2 ELISA assay

EpitoGen Technology

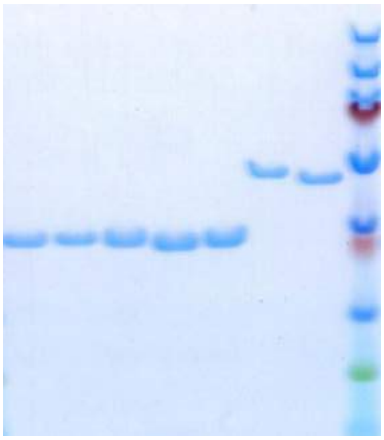
Spike protein
(YP_009724390)



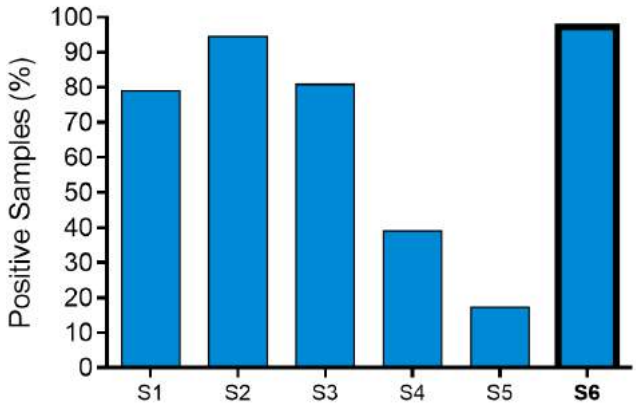
Epitope locations

Composite Spike EpitoGen **S6**

Composite Spike EpitoGen **S6m**
16 mutations from S6



Expression of single epitogens, composite epitogen (S6) and its corresponding prevalent mutants (S6m) using EpitoGen Technology visualised using SDS-PAGE.



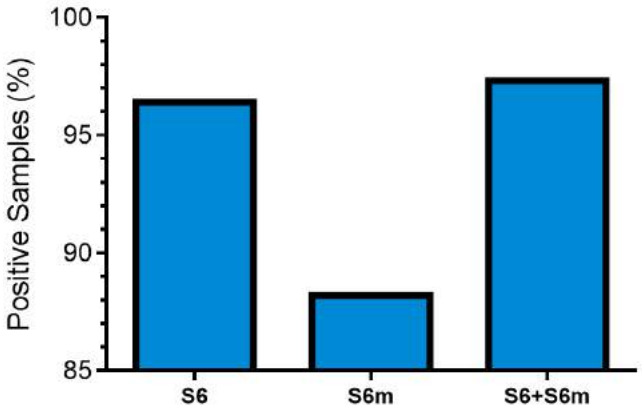
Percentage of COVID-19 samples which tested positive for SARS-CoV-2 specific antibodies by each individual epitogen. The composite epitogen S6 showed **97.3%** sensitivity (n=110).

Complexing of epitopes enhanced sensitivity of the test.

Percentage of COVID-19 samples which tested positive for SARS-CoV-2 specific antibodies by epitope-complex S6, epitope-complex S6m, and S6 + S6m combined. **The inclusion of mutants-complex S6m increased the assay sensitivity to 97.3% (n=110).**

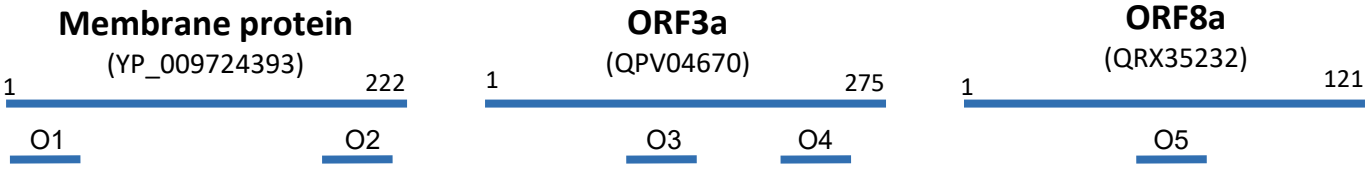
Accumulation of mutations in the SARS-CoV-2 spike protein has impacted negative on the antibody response.

It is imperative to that future vaccines and spike-based diagnostic tests include spike mutants.



Composite EpitoGen boosts sensitivity of SARS-CoV-2 ELISA assay

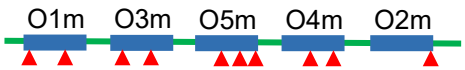
EpitoGen Technology

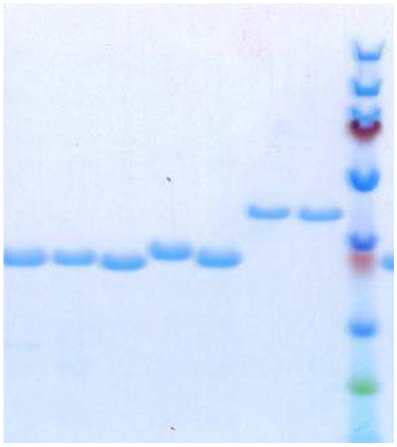


Composite EpitoGen **O6**

Composite EpitoGen **O6m**

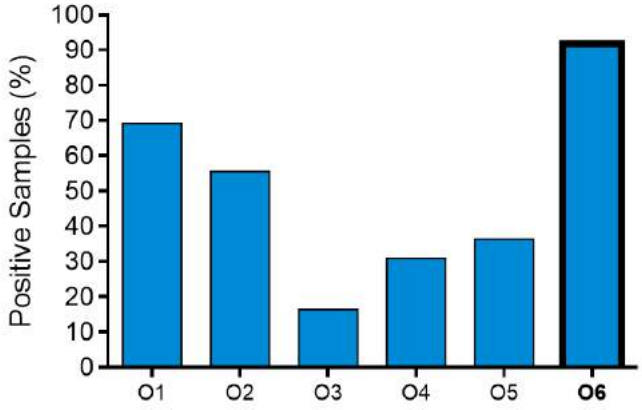
10 mutations from O6





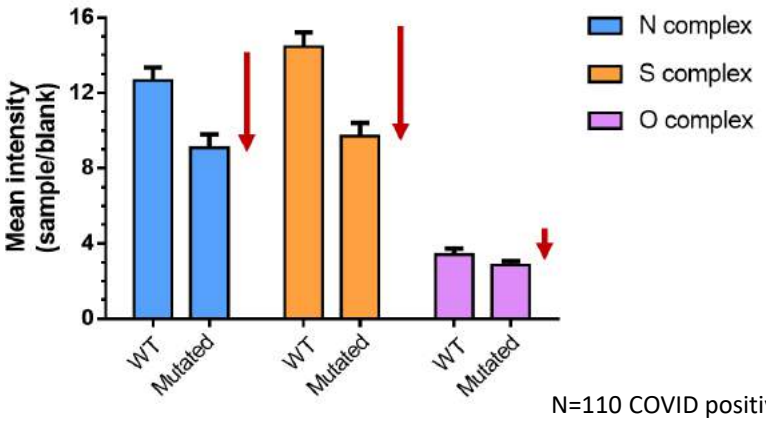
O1 O2 O3 O4 O5 **O6** **O6m**

Expression of single epitogens, composite epitogen (O6) and its corresponding prevalent mutants (O6m) using EpitoGen Technology visualised using SDS-PAGE.



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

Complexing of epitopes enhanced sensitivity of the test.



Emerging variants of SARS-CoV-2 (mutated) **reduces antibody response** among COVID patients compared to wild type (WT) strain.

N=110 COVID positive patients; SEM

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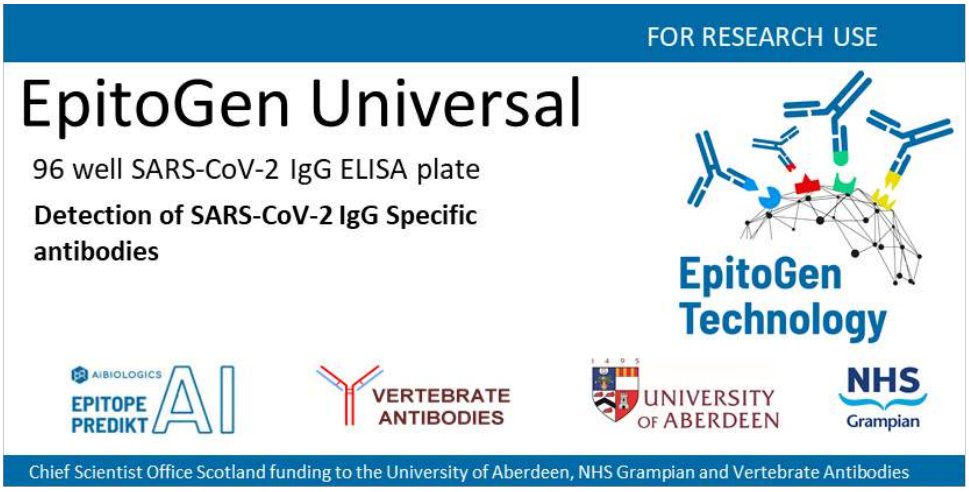
Universal EpitoGen COVID-19 ELISA plate

At a population level, the humoral response to SARS-CoV-2 is heterogeneous. In the course of our validation we observed that significant numbers of samples were only reactive for either the spike or nucleocapsid antigen alone. Therefore, traditional antibody assays using either the Spike or Nucleocapsid antigen are less than effective. Also, accumulation of SARS-CoV-2 mutations will further reduce sensitivity.

To overcome this, the Universal EpitoGen® COVID-19 ELISA test was designed for maximum sensitivity. **The antigen consists of a set of 15 immunodominant epitopes from N, S, M, ORF3a and ORF7a viral proteins, and their corresponding major circulating mutants (45 prevalent mutations)**. It is designed for the detection of IgG antibody response to SARS-CoV-2 infection and immunisation.

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.

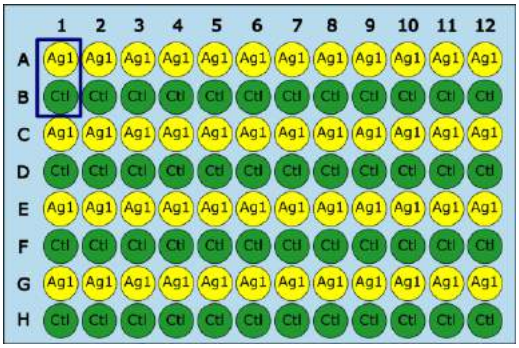
The combinations of these epitogens in the assay offers the ability to detect broadest range of human anti-SARS-CoV-2 antibodies.



Ag1 **Antigen 1 (Ag1)** = A set of composite epitogens of 15 epitopes and their major mutants from five proteins (S, N, M, ORF3a and ORF7a) of SARS-CoV-2.

Ctl **Control (C)** = The Epitogen scaffold protein lacking the specific SARS-CoV-2 epitopes but containing the linker peptide which links epitopes to the scaffold and separate epitopes from each other.

Test Sample = 44 single tests or 22 duplicate tests per plate

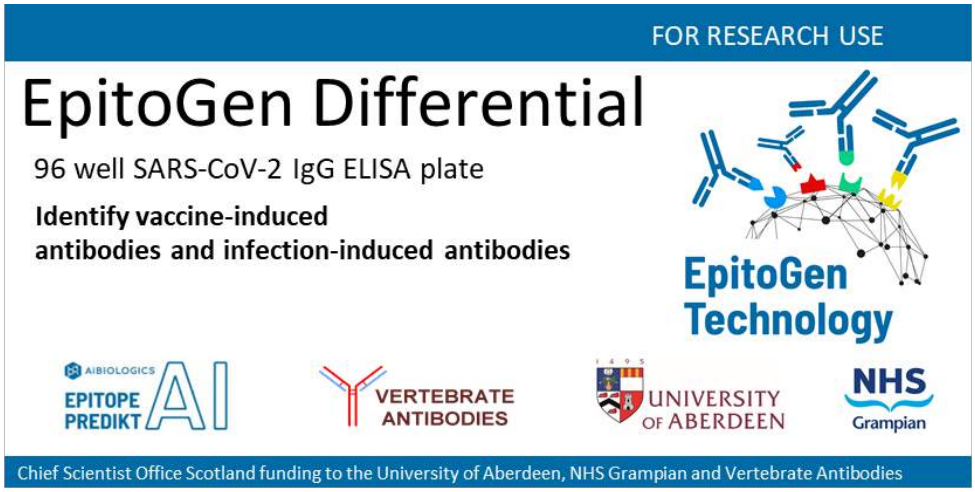


ELISA test plate

Differential EpitoGen COVID-19 ELISA plate

The Differential EpitoGen COVID-19 ELISA test offers the ability to differentiate between humoral responses to vaccine and infection with SARS-CoV-2. SARS-Cov-2 vaccines, thus far, are based solely on the spike protein so therefore detection of antibodies reactive with the other viral proteins is indicative of a natural infection with SARS-CoV-2. The plate contains two set of antigens. Antigen 1 consists of a composite epitogen of **5 epitopes** and their corresponding mutants (**16 prevalent mutations**) from the spike protein. Antigen 2 contains **10 non-spike epitopes** and their corresponding mutants' forms from N, M, ORF3a and ORF7a (**29 prevalent mutations**). The assay is designed for the differentiation of vaccine-induced and infection-induced IgG antibody response to SARS-CoV-2. The test can be used to detect IgM and IgA to evaluate active SARS-CoV-2 infection or mucosal immunity.

The combinations of these epitopes expressed as antigens in the assay offers the ability to detect the broadest range of human spike or non-spike SARS-CoV-2 antibodies.

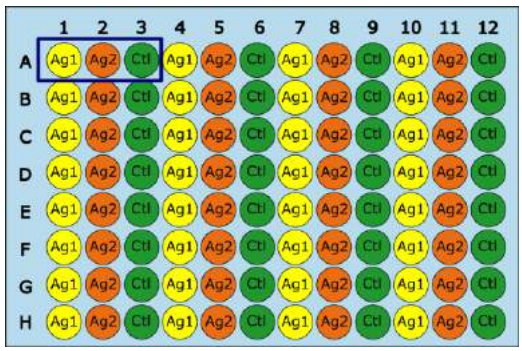


Ag1 **Antigen 1 (Ag1)** = A set of composite epitogens of 10 epitopes and their mutants from four non-spike proteins (N, M, ORF3a and ORF7a) of SARS-CoV-2

Ag2 **Antigen 2 (Ag2)** = A composite epitogen of 5 immunodominant epitopes and their major mutants from the vaccine target spike protein.

Ctl **Control (C)** = The Epitogen scaffold protein lacking the specific SARS-CoV-2 epitopes but containing the linker peptide which links epitopes to the scaffold and separate epitopes from each other.

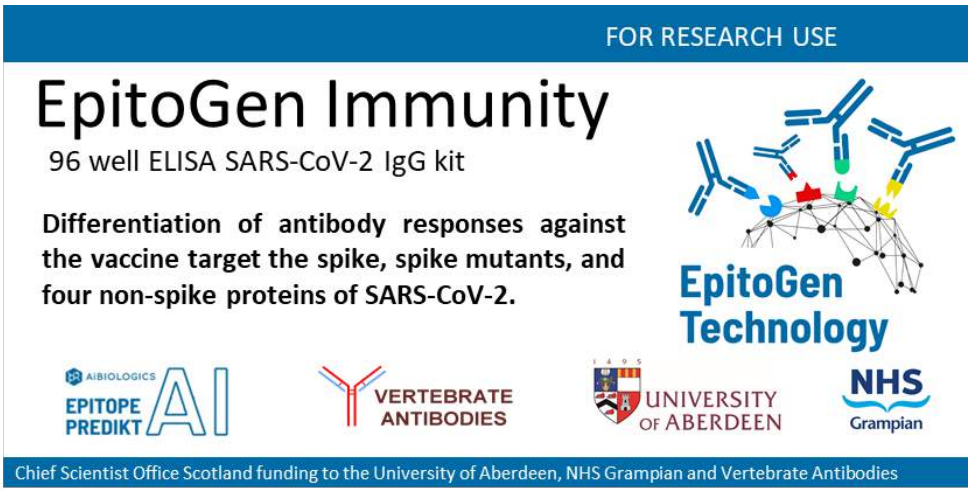
Test Sample = 28 single tests or 14 duplicate tests per plate



ELISA test plate

Immunity EpitoGen COVID-19 ELISA plate

One of the more challenging aspects of containing SARS-CoV-2 is the ability of the virus to mutate and evade acquired immune responses. The Immunity EpitoGen® COVID-19 ELISA test is designed to detect IgG antibody responses to non-spike proteins and their corresponding mutants (N, M, ORF3a and ORF7a**, 29 prevalent mutations**), the reference spike protein used in vaccine development, and major mutants of the spike protein (**16 prevalent mutations**). It can be used before & after SARS-CoV-2 infection or vaccination to provide a readout of immunity against further infection by the reference strain or mutated variants. The plate can also be used to detect IgM and IgA to evaluate active SARS-CoV-2 infection or mucosal immunity.



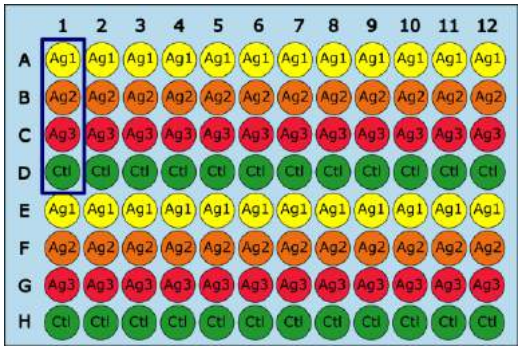
Ag1 **Antigen 1 (Ag1)** = A set of composite epitogens of 10 epitopes and their mutants from four non-spike proteins (N, M, ORF3a and ORF7a) of SARS-CoV-2.

Ag2 **Antigen 2 (Ag2)** = A composite epitogen of 5 immunodominant epitopes from the vaccine target spike protein.

Ag3 **Antigen 3 (Ag3)** = The mutant versions of the epitogen Ag2.

Ctl **Control (C)** = The Epitogen scaffold protein lacking the specific SARS-CoV-2 epitopes but containing the linker peptide which links epitopes to the scaffold and separate epitopes from each other.

Test Sample = 20 single tests or 10 duplicate tests per plate



ELISA test plate

Spike Mutant EpitoGen COVID-19 ELISA plate

The spike protein is the most vital structural protein contained within the proteome of SARS-CoV-2. Its binding to the ACE2 receptor is responsible for the entry of the virus into the host cell. As crucial as the protein is to the virus, it is similarly crucial to controlling the pandemic. All the major approved vaccines use the spike protein as a basis for design. Increasingly sequencing studies have shown that several mutations have emerged within the spike protein leading to the designation of new "variants" of SARS-CoV-2. Monitoring the prevalence of these variants is vital. The Spike Mutant EpitoGen® COVID-19 ELISA test contains two antigens to detect antibodies specific to original SARS-CoV-2 strains and several prevalent variants. **The test is useful to evaluate the impact of emerging mutants on vaccine performance and acquired immunity.**

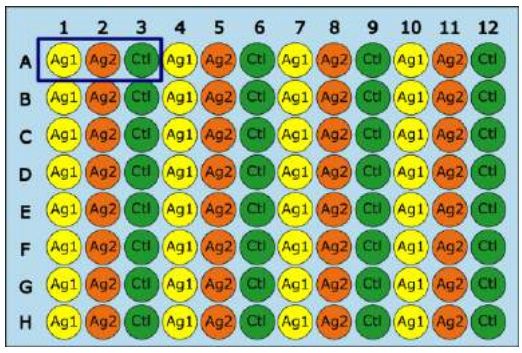
Antigen 1 is a composite set of 5 immunodominant epitopes from the original spike protein. Antigen 2 is a set composite mutant epitopes from the most common SARS-CoV-2 variants (**16 prevalent mutations**). The test is designed for evaluating IgG antibody response to potential SARS-CoV-2 spike variants.

Ag1 **Antigen 1 (Ag1)** = A composite set of 5 immunodominant epitopes from the vaccine target spike protein expressed using the EpitoGen scaffold.

Ag2 **Antigen 2 (Ag2)** = The mutant versions of the epitopes of Ag1 expressed using the EpitoGen scaffold.

Ctl **Control (C)** = The EpitoGen scaffold protein lacking the specific SARS-CoV-2 epitopes but containing the linker peptide which links epitopes to the scaffold and separate epitopes from each other.

Test Sample = 28 single tests or 14 duplicate tests per plate

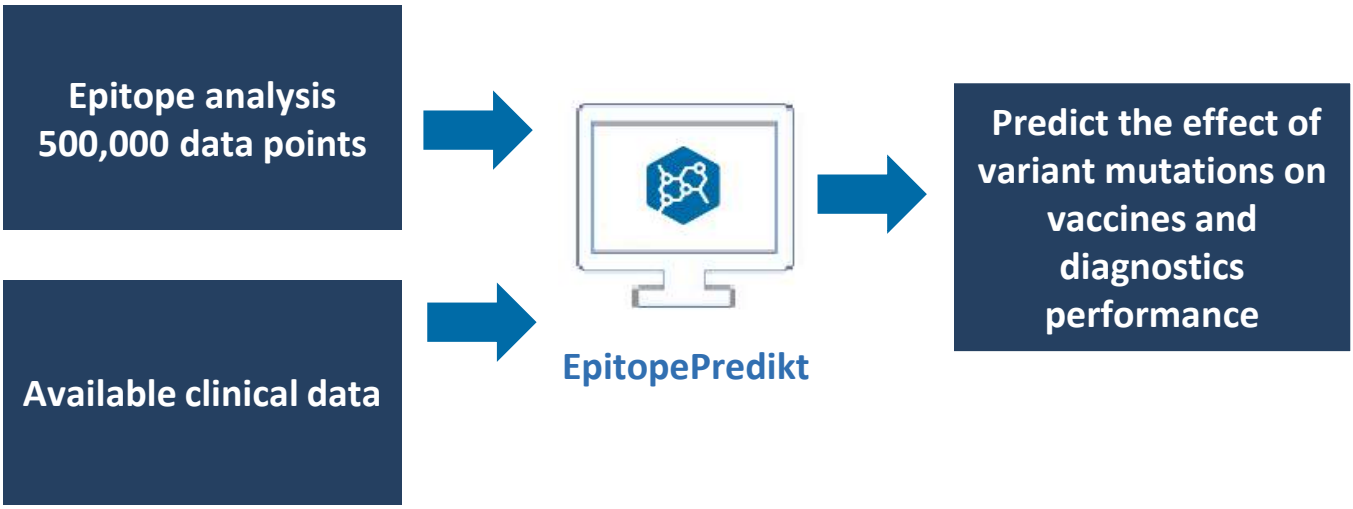


ELISA test plate

EpitoGen Technology and EpitopePredikt

Vertebrate Antibodies Ltd has developed EpitoGen technology, a novel epitope display platform to produce Epitogens, in order to create more accurate and consistent COVID serological tests. As part of the development of a SARS-CoV-2 assay an overlapping epitope library of the major structural proteins of SARS-CoV-2 was constructed. The binding of antibodies from SARS-CoV-2 positive sera were measured at each of the constructed epitogens. At strongly reactive epitopes the epitope length was reduced and a sliding window was created which progressed one amino acid at a time through the sequence.

This would eventually provide approximately 500,000 data points. In order to make use of all this data it was determined to create an i*n silico* model of the humoral response to SARS-CoV-2 response to infection. The data was uploaded into AiBiologics machine learning capable platform EpitopePredikt. Using a number of algorithms including random forest and neural networks combined with genetic algorithms a predictive model emerged. This model is now capable of determining the humoral immune response to SARS-CoV-2. The model is also useful to evaluate IVDs and vaccines post market performance across different geographical areas and ethnic groups, especially in relation to emerging variants.



We plan to, wherever possible, include the data from all the EpitoGen assays used by our customers for further expand and train our model. With the addition of more clinical data we believe the disease-specific model will identify clinical indicators for diagnosis and prognosis.