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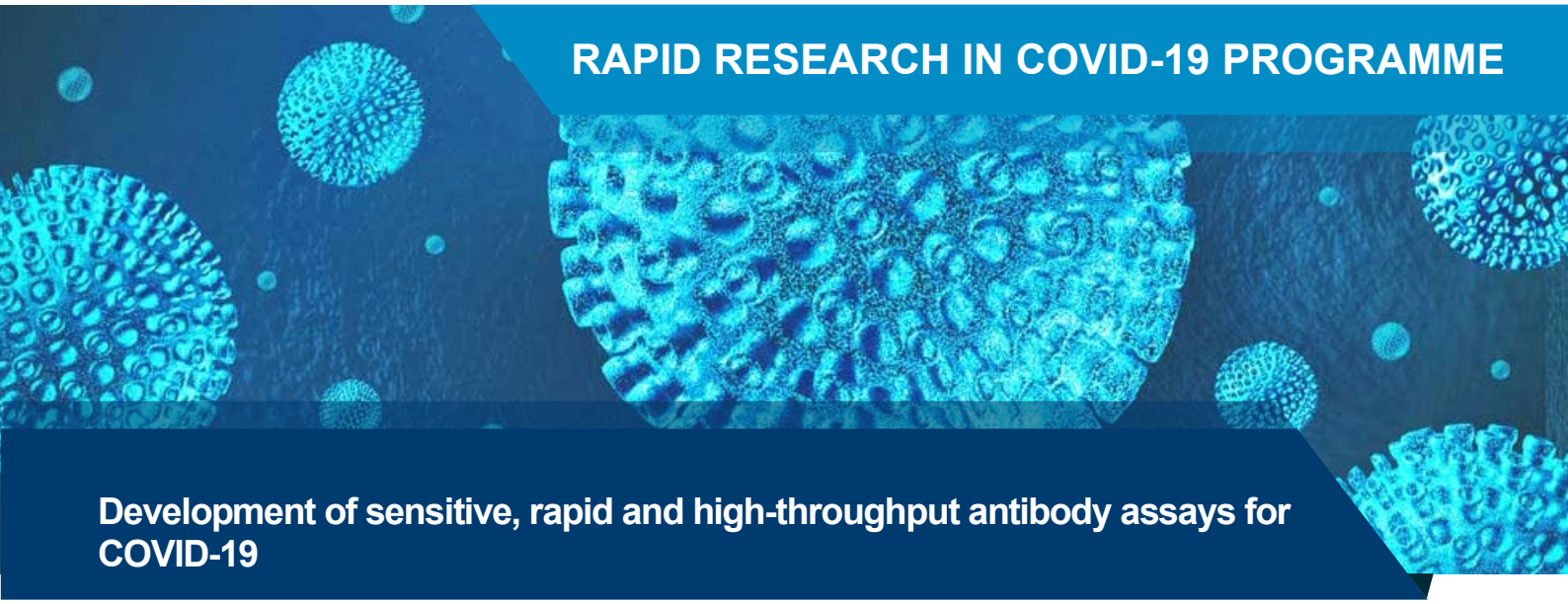

RAPID RESEARCH IN COVID-19 PROGRAMME

Development of sensitive, rapid and high-throughput antibody assays for COVID-19

AIMS

The aim of this project was to produce a sensitive, rapid, high-throughput antibody-based test for COVID-19, which is superior to those currently on the market.

KEY FINDINGS

- In the laboratory we successfully manufactured two of the proteins that make up the SARS-CoV-2 virus (virus that causes COVID-19); these are termed the N protein and S protein.
- In addition to the SARS-CoV-2 N protein we also produced N protein of other similar human coronaviruses, which are the constituents of the current tests, such as those causing common colds. This were necessary to allow us to test the specificity of our test.
- We detected antibodies against the N and S proteins of SARS-CoV-2 virus in 85.4% and 72.5% COVID-19 positive samples, respectively.
- When testing the common cold N protein, we detected antibodies in all the pre-COVID samples and in 170 out of the 171 COVID-19 samples, suggesting a common prevalence of common cold coronaviruses.
- **These results highlighted that the current antibody tests that are based on detecting the N and S proteins of general coronaviruses are not specific and that there is an urgent need for the development of specific antibody tests against COVID-19 (SARS-CoV-2) in order to control the infection and assess prevalence in general population.**
- We used an artificial intelligence (AI) approach which identifies very specific protein components on the SARS-CoV2 virus (known as “epitopes”) and which are unique to SARS-CoV-2 only, in order to solve the shortcoming of existing tests.

- In the laboratory we manufactured over 50 of these and each epitope was tested using COVID-19 samples from multiple centres (Grampian, Tayside and USA) to represent heterogeneity of the population. Epitopes with best performance were taken forward to the next step of testing (after using the artificial intelligence software to inform on the best combinations).
- We have now produced a mix of the best-performing epitopes to create an antibody-based test with superior sensitivity and specificity.

WHAT DID THE STUDY INVOLVE?

We manufactured, on a large-scale, regions of the SARS-CoV-2 virus that represent its main parts; namely, the nucleocapsid (N) protein and spike(S) protein. We also manufactured regions from other similar coronaviruses, such as common cold, to test for specificity and sensitivity of the antibody test. We obtained 100 pre-COVID samples from the SHARE biorepository in Tayside (samples collected in 2018), as well as 171 COVID-19 positive samples and 20 samples negative for COVID-19 but positive for other common coronaviruses, such as common cold.
Testing in the laboratory revealed that **there was a low specificity of the currently available antibody tests, because 38% of the pre-COVID samples were positive for the N protein (commonly used in current tests), and around 20% to the S protein (also a constituent of the current tests)**.

If both were combined (S+N, as is the case in some current available antibody tests), over 40% of pre-COVID samples showed positivity to the test. In addition,100% of the pre-COVID samples were positive for common cold N proteins we produced.

This revealed that we required a novel approach to design a better and more specific test to SARS-CoV-2. We used our artificial intelligence (AI) software which identifies parts of the virus (epitopes) only present in SARS-CoV-2 and different enough from common cold viruses (*i.e. not cross-reactive*). The software predicted which parts of the virus were best used to allow an antibody response needed to solve the shortcoming of existing tests. To achieve this, we had to engineer new parts and combinations of the virus in the laboratory, which we then tested. This approach allowed us to find a very specific and sensitive test against COVID-19. Our engineered regions of the virus (which became our “antigens”), showed no cross-reaction with common cold viruses.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

This study revealed that the current antibody tests are not specific to SARS-CoV-2 virus, which causes COVID-19, and therefore not reliable, as individuals can be shown to be "positive" for antibodies against COVID-19 whilst the test can actually also pick up common cold coronaviruses. This means that if an individual had a common cold earlier on in the year (eg. Dec 2019 -March 2020) they would also be shown as "positive" for COVID-19, which may not be the case (Table 1).

Table 1. Antibody positive rate (%) detected by ELISA test using SARS-CoV-2 N and S proteins, or N proteins of other human coronavirus causing common cold.

Region of the virus	COVID-19 samples	Pre-COVID samples
SARS-CoV-2 N protein	85.4%	37.9%
SARS-CoV-2 S protein	72.5%	16.8%
N and S proteins combined	87.7%	43.2%
N proteins of common cold	99.4%	100.0%

This project used an innovative approach whereby an artificial intelligence software identified short viral parts on SARS-CoV-2 surface (epitopes), which the software predicted may be identified by antibodies present in COVID-19 patients, resulting in a positive test result. These short regions were unique to SARS-CoV-2 virus only, in order to eliminate the detection of antibodies that may have developed after a common cold infection. These short viral parts were manufactured in the laboratory to appear like viral-proteins and therefore used to test the sensitivity of blood-based tests, comparing them to existing tests. Sensitivity is the ability of a test to correctly identify those with the disease and to test each short viral region (epitope), we used COVID-19 positive samples from Grampian, Tayside and USA. Epitopes with high sensitivity were taken forward to evaluate their specificity. Specificity is the ability of the test to correctly identify those without the disease. In order to do that, we screened several epitopes both individually and in combination, on pre-pandemic samples (samples collected in 2018). Only those epitopes with 100% specificity, meaning they didn't detect common cold samples, were selected.

Three epitopes emerged as promising candidates with sensitivity and specificity range around 95%. The final part of the project is to mix these three epitopes together to generate a routine and a point-of-care test to detect COVID-19. Fusing these three viral elements together is anticipated to boost specificity to near 100%. Results obtained during this project will allow us to progress the production of the specific and sensitive point-of-care antibody tests; this will require around two more months of work/optimisation.

WHAT IMPACT COULD THE FINDINGS HAVE?

This project will lead to the development of a specific and reliable test against SARS-CoV-2 virus which causes COVID-19, and will allow us to understand the prevalence of, and the transmission profiles, of the virus. The reagents generated will also allow us to generate more sensitive tests against other infections (i.e. seasonal coronaviruses) as well as identifying prognostic signatures between patients with other underlying conditions.

A sensitive, rapid and high-throughput COVID-19 antibody test will drastically change the government's strategy for dealing with/and monitoring the virus and could have important utility in screening the general population in order to determine prevalence. This data is critical in order to get an accurate picture of the outbreak and its evolution, providing information that will influence public health measures. The rapid test could be utilised in airports to control the number of cases. The test will be employed for population screening to identify asymptomatic carriers, individuals who were exposed to the virus and those who haven't and provide data on future targeted vaccination programme. Accurate and high-throughput capacity will allow for mass testing of NHS staff, keyworkers, sport venues, universities and schools screening during the COVID-19 pandemic. Additional advantages are a reduced manufacturing cost and lead production time which means the cost for end users is much less.

HOW WILL THE OUTCOMES BE DISSEMINATED?

We plan to publish our results imminently in open source journals and they will be available to all of the community, scientific and general public. We will target traditional media (newspapers, TV, radio) through a press release and share our research on our website, Twitter, LinkedIn. We will also produce short podcasts and/or videos. For example, we recently recorded outcomes from this project for the public engagement event, Explorathon which will be available online and through Twitter.

CONCLUSION

Employing a novel and innovative approach, the team was able to provide a breakthrough solution for improving existing blood-based tests. The team has identified and validated key viral elements (epitopes) that provided highest sensitivity and specificity levels. When combined, these epitopes will generate an accurate and rapid test which we hope to complete by the end of 2020. Furthermore, the team has developed a diagnostic platform built on our AI technology that can be employed to combat future outbreaks and even used to aid the diagnosis of autoimmune disorders.

ADDITIONAL INFORMATION

This project was completed on 31st Oct. 2020 and work to develop a high throughput assay is ongoing. The project was supported by an award of £101,903 from the Chief Scientist Office and an in-kind contribution of £38K from Vertebrate Antibodies Ltd.

RESEARCH TEAM & CONTACT

Professor Mirela Delibegovic (PI), Dr T Wang, Professor G Murray, Dr N Mody (University of Aberdeen). Dr A Alnabulsi & Dr A Alnabulsi (Vertebrate Antibodies Ltd (VAL), Dr R Brittain Long NHS Grampian.	Email address: m.delibegovic@abdn.ac.uk
Institute of Medical Sciences School of Medicine, Medical Sciences & Nutrition University of Aberdeen	Phone number 01224 437587