EDCTP Contract N^0 RIA2020EF-2918

Rapid diagnostics for COVID-19: manufacturable in Africa to increase affordability, improve epidemic preparedness and strengthen local resilience

Deliverable report

Deliverable number	D5.2 and 5.3
WP no/title	WP5: Proof of Concept Serological Test Development
Deliverable title	Purified covid coat protein fragments
Responsible partner	UCAM
Dissemination level	PU
Due date	30 March 2021
Actual submission date	03 April 2021

History of changes:

Date	Version no	Comments
03/4/2021	1.0	
30/04/2022	2.0	Additional data added obtained later.

Delivery Type

R	Report	
DEM	Demonstrator, pilot, prototype, plan designs, new or revised health policies etc	✓
DEC	Websites, patents filing, press & media actions, etc	
OTHER	Other	

Dissemination Level

PU	Public	✓
RE	Restricted to a group specified by the consortium.	

List of content

Partner	Contribution to this deliverable
SW Hamaia	SWH carried out described work in collaboration with AP Jackson and L Hall.

1 Status of the Deliverable

The key goal of the current project is to lay the foundations for the future development of a rapid lateral flow and/or ELISA-type technology to detect exposure to SarsCov2 virus. The assay should be suitable for use in Africa and- critically – should be manufacturable, at scale in an African setting.

The assay originally envisaged using recombinant viral spike protein as single capture agent for the serum immunoglobulins, which would subsequently be detected by scFvs raised against human immunoglobulins. Generation and modification of scFvs against human antibodies – both IgM, to detect early exposure to the viral antigens, and later Ig classes – was successful – see Deliverables, 5.1.

However, during the experimental phase it became clear that using purified recombinant spike protein, at scale and in good yield as capture agent was not practical. This is due to the technical difficulty of producing recombinant spike proteins in high yield. Furthermore, it has now become clear that a typical immune response to SarsCov2 does not just relate to the spike protein and indeed the spike protein is not even the most immunogenic. In practice, typical polyclonal immune responses to SarsCov2 include antibodies against other viral protein including, the viral nucleocapsid (estimated to be ~ 100-fold higher than against spike protein) and ORF8 protein (~ 10-fold higher than against spike protein) (Hachim, A et al., Nature Immunology, Vol 121, October 2020, 1293-1301).

In view of these facts and the requirements of the project, we created a recombinant 'universal antigen' which contains all the key established and characterised viral epitopes recognized by immune serum. These include multiple epitopes from spike protein, nucleocapsid, ORF8 and viral membrane proteins (Hachim, A et al., Nature Immunology, Vol 121, October 2020, 1293-1301).. The selection was also based on selection of non-glycosylated epitopes that can easily be expressed in bacteria at high yield and at low cost.

2 Summary of the results

The recombinant 'universal antigen' was successfully cloned into Gateway cloning vector and large amount of recombinant protein produced and purified. When tested using available commercial antibodies against

multiple viral epitopes from spike and nucleocapsids, positive signals were elicited using this capture antigen.

On this basis, we will proceed to assemble a rapid detection system for exposure and successful immunological reaction against SarsCov2 infection in patients.

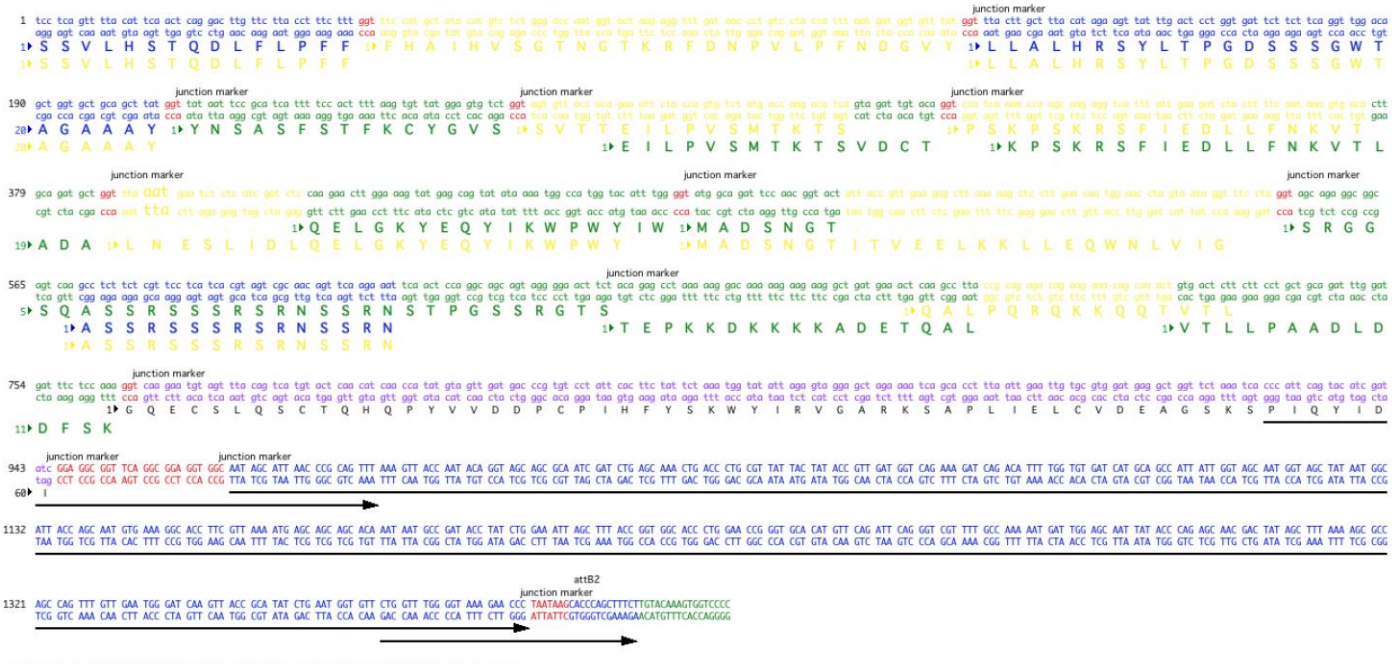
3 Description of work performed and obtained results

(Describe the work performed and obtained results towards the deliverable).

A recombinant universal antigen has been designed, following identification of key epitopes (see appendix) which were included into the construct to generate the universal antigen.

Generation of the universal antigen: The core UA with distinct epitopes are illustrated :

UA with added cellulose binding domain to facilitate binding to lateral flow paper.



These cDNA constructs were cloned into the E Coli expression vector pET-Dest-42-N110 plasmid and transfected into E Coli The universal antigen is isolated from ~ 1 l media, by a simple affinity purification step Ni columns. Typical yields are: 2-3mg/L (10 His at N-ter and Cellulose binding domain at C-ter).

To this end, we have now recloned the universal antigen to include a C-terminal cellulose-binding domain that facilitates attachment of the antigen to paper in the correct orientation to allow serum antibodies to bind.

Conclusions: This work described in deliverables 5.2 is important as it establishes the key concept behind the universal antigen as a pragmatic solution to generating antigen for use in large scale ELISA and lateral flow assays. Subsequent and continuing work is being carried out, using these key reagents to produce such assays.

As a result of the project we have established an on-going collaboration with NMIMR and Dr Kofi Bon and a postdoctoral researcher (funded by Royal Society UK) and in this subsequent work, we have shown that the universal antigen can indeed bind to anti-(spike protein) and anti-(nucleocapsid) human immunoglobulins.