Joint Canada-Israel Health Research Program

Technical reporting guidelines

The JCIHR technical reports and accompanying documents should be submitted in electronic form to IDRC and ISF. Principal investigators are responsible for submitting technical reports on or before the due dates, as stated in the grant agreement.

The table below identifies the coverage, due dates and audience for the two reports.

	Period of work covered in the report	Report due	Audience
Mid-term Report	From the start to the midway point of the grant	One month after the midway point of the grant (June 1st, 2018)	An internal report circulated amongst the funding agencies
Final Report	The entire period of the grant	One month after the conclusion of the grant (January 1st, 2020)	A public report

- Technical reports should not exceed 20 spaced pages, excluding tables and appendices.
- Please ensure that report reflects the experience and activities across the different research sites.
- Plan time for discussion among team members from different project sites.

These guidelines apply to both the mid-term and the final technical report. The guidelines specify which questions should be answered in the two reports.

IDRC File #: 108404-001 ISF File #: 2641/16	Project Title: Epitope focused immunogens and recombinant antibody therapeutics for the control of tropical diseases	
Report completed by[1]: (add rows if needed)		
Family Name Sidhu	Given Name Sachdev	Initials S.
Organization name and address: University of Toronto, The Donnelly Centre, 160 College Street, Toronto, ON M5S 3E1, Canada		
Primary E-mail: sachev.sidhu@utoronto.ca		
Role in the project: Oversees the whole project, supervises staff and trainees in Canada, communicates with collaborators, analyzes & communicates the data.		
Family Name Gershoni	Given Name Jonathan	Initials
Organization name and address: The School of Molecular Cell Biology & Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel		
Primary E-mail: gershoni@post.tau.ac.il		
Role in the project: Co-leader for project, supervises staff and trainees in Israel, communicates with collaborators, analyzes & communicates the data.		
Family Name Nene	Given Name Vishvanath	Initials
Organization name and address: International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi 00100, Kenya		
Primary E-mail: V.Nene@cgiar.org		

[1] List only those people who were involved in writing the report. Those listed confirm that they have read the entire report before submitting it.

Role in the project: Contributes to the East Coast fever part of the project, supervises staff and trainees in Kenya, communicates with collaborators, analyzes & communicates the data.		
Family Name Chaudhuri	Given Name Amitabha	Initials
Organization name and address: SciGenom Labs, 43A SDF 3rd floor, CSEZ, Kakanad, Kochin, Kerala, 682-037, India		
Primary E-mail: amitc@scigenom.com		
Role in the project: Contributes to the development of Dengue reagents, diagnostics and therapeutics, supervises staff and trainees in India, communicates with collaborators, analyzes & communicates the data.		
Family Name Fellouse	Given Name Frederic	Initials A.
Organization name and address: University of Toronto, The Donnelly Centre, 160 College Street, Toronto, ON M5S 3E1, Canada		
Primary E-mail: fred.fellouse@utoronto.ca		
Role in the project: Scientific manager for the project, directly supervises all staff, trainees and experiments performed in Canada, responsible for technology transfer to India and Kenya.		
Family Name Gopalsamy	Given Name Anupriya	Initials
Organization name and address: University of Toronto, The Donnelly Centre, 160 College Street, Toronto, ON M5S 3E1, Canada		
Primary E-mail: anupriya.gopalsamy@utoronto.ca		
Role in the project: Performs experiments towards anti-dengue virus synthetic antibody development, analyzes and presents results.		

Family Name	Given Name	Initials
Piller	Chen	
Organization name and address: The School of Molecular Cell Biology & Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel		
Primary E-mail: pillerchen1@gmail.com		
Role in the project: PhD candidate working on the development of peptide immunogens for dengue virus.		
Period covered in this report: 11/16 to 04/20		
Date Submitted: 31/07/2020		

Section A: Project Description

1. Project abstract
Note: Your abstract should be no longer than ½ page. Infectious diseases have enormous impacts on global health and economics. This study focused on developing therapies and vaccines against two devastating diseases, dengue fever (DF) and East Coast fever (ECF). Currently 3.6 billion people are at risk of dengue virus infection. Endemic in 100 countries with an estimated 390 million annual infections and ~500,000 hospitalizations yearly makes DF a worldwide challenge for public health officials and policy makers. ECF is a tick-borne disease affecting cattle, caused by the protozoan parasite *Theileria parva*. With a 3-4 week incubation time and 100% mortality rate in exotic cattle, it has a devastating impact on pastoralists and smallholder farmers in Africa. This collaborative research program, with experts from around the world, aimed to develop affordable antibody-based therapies for dengue patients and improved vaccines for the control of DF and ECF in both humans and animals. The team combined cutting-edge immunology and protein and antibody engineering methods to generate antibodies and proteins with therapeutic and vaccination potential. Importantly, our core technologies reduce the use of research animals and are easy and affordable to implement. This made these approaches particularly appealing to research groups in low and middle income countries. With this collaboration, we shared technology developed in Canada and Israel with research partners at the International Livestock Research Institute, Kenya and SciGenom, India, who excel in cattle research and antibody production,

respectively. The exchange of trainees and information was the foundation of our program and helped achieve our goals of disease prevention and control.

2. Summarize your main lines of work and achievements during the reporting period as they relate to the goals of the funding opportunity (e.g., advance research and discovery; deepen international scientific collaboration and build capacity).

Our work had two broad goals: to develop novel diagnostics and therapeutics for infectious diseases, that can be produced in a cost-efficient manner; and to transfer technology developed in Canada and Israel to low and middle income partner countries – India and Kenya.

Infectious tropical diseases can cause death and are a huge economic and social burden in low and middle income countries. Our project aimed to alleviate this burden by:

1. Developing therapeutic and preventive solutions for dengue fever (DF), a life-threatening human disease caused by the dengue virus (DV). We aimed to develop affordable recombinant antibody therapies; *this project was undertaken mainly by the Indian and Canadian groups*. Synthetic antibodies were selected against DV-like particles and soluble non-structural protein 1 (sNS1) of all 4 DV serotypes. We produced, epitope-grouped and characterized these antibodies and identified extremely serotype-specific antibodies as well as 4 serotype-cross-reactive antibodies against DV and sNS1. Some of the anti-DV antibodies showed neutralization activity in a cell-based assay, suggesting that they will potentially be useful for therapeutic development. Overall, we greatly progressed towards our goal of producing a new DF treatment. Additional funding will be required to affinity mature the most promising antibodies, and to undertake further validation of the potency of these antibodies in cell-based assays and animal models. Towards diagnostics, we also finalized a set of antibodies and a sandwich-ELISA protocol to detect either DV or sNS1 in a serotype-specific manner.

2. Improving vaccine efficacy through engineering of protective immunogens. We used directed evolution technology to tackle both DF and East Coast fever (ECF), a lethal disease of cattle in sub-Saharan Africa; *this project was undertaken mainly by the Kenyan, Canadian, and Israeli groups*. Mouse-derived antibodies against p67C, a promising immunogen for ECF vaccination, were produced recombinantly. We also successfully expressed the full-length soluble p67 (FL-p67) protein in HEK-293T cells. FL-p67 as well as fragments of p67 were used to select synthetic antibodies to either discover novel neutralizing antibody epitopes or obtain novel conformation-specific antibodies against FL-p67 to assist epitope focused immunogen (EFI) development. Two epitopes were targeted and conformer libraries were generated and selected for proper reconstitution. One of the two reconstituted epitope showed binding against several antibodies. We selected new synthetic antibodies against the reconstituted epitopes themselves. The binding of these antibodies to DV virus-like particles (VLPs) is still being assessed. The fact that antibodies raised *in vitro* against the reconstituted epitopes bind to the bona fide antigen suggests that the reconstituted epitopes should be able to generate a productive immune response in animals or humans.

EFI development is greatly facilitated by knowing the 3-dimensional structure of the targeted protein. Hence, we focused on the structural determination of p67. We obtained the structure of a fragment of p67C bound to one of our selected synthetic antibodies, B11. We measured the binding kinetic of the B11 antibody to full length p67, as well as p67C, and a series of alanine mutants by bio-layer interferometry (BLI). This allowed the functional mapping the B11 epitope on p67C. Finally, a panel of

73 fragments of the p67 protein were generated, which are valuable reagents for epitope mapping of conformational-epitope antibodies.

3. Building capacity to enable scientists in the affected countries to take a lead role and independently explore these cutting-edge technologies for disease control. *All four groups were involved in this project*. We addressed this third objective with an exchange of trainees between the laboratories involved and with 2 dedicated training workshops and one international symposium in Africa. A total of 5 international exchanges of personnel enabled the transfer of the recombinant antibody production, antibody phage display library selection and screening technologies to the Kenyan and Indian groups. Both groups successfully used elements of the technologies.

Section B: Objectives & Research Problem

3. State the general and specific project objectives.

We aimed to (1) develop affordable recombinant antibody therapies for dengue fever (DF) and (2) improve dengue virus (DV) vaccine efficacy through engineering of protective immunogens. We also aimed to use the latter technology to tackle East Coast fever (ECF), a lethal disease of cattle in sub-Saharan Africa. Our final objective was to build capacity to enable scientists in the affected countries to take a lead role and independently explore these cutting-edge technologies for disease control. We addressed this last objective with an exchange of trainees between the laboratories involved, technology transfer between laboratories, and dedicated training workshops in Africa.

Our plan combined the expertise in combinatorial phage display of the Canadian (antibodies, Sidhu) and Israeli (peptides, Gershoni) laboratories. Moreover, the experimental plan involved exchange of materials between laboratories, namely peptide antigens produced by the Gershoni group were used to generate antibodies in the Sidhu group, and reciprocally antibodies developed in the Sidhu group were used to identify antibody targets from peptide libraries in the Gershoni group.

The DF objectives were to:

- Produce DV envelope proteins (E-proteins) of all 4 serotypes as recombinant proteins and virus-like particles (VLPs) and use these as antigens for selection of synthetic antibodies; then characterize and screen the selected antibodies for therapeutic and diagnostic potential.
- Produce and use DV non-structural protein 1 (NS1) as a target for antibody selection; then characterize promising antibody candidates and screen them for therapeutic and diagnostic potential.
- Engineer epitope focused immunogens (EFIs) to be used as a DF vaccine. The goal was to reconstitute epitopes of DV proteins into immunogens to elicit DV infection-neutralizing antibodies without eliciting infection-enhancing antibodies (*Am J Trop Med Hyg.* 4: 444, 1989). Initially, conformer peptide libraries of two neutralizing epitopes were planned and constructed (E1, E2), but in view of results (see section 10), we decided to focus our attention on E2. Thus far over 25 E2 conformers have been isolated, at least 4 of which are cross reactive with multiple neutralizing antibodies. These conformers were used as bait to isolate E2-specific phage-displayed antibodies, which are currently being characterized. Moreover, the cross-reactive E2 conformers are being further developed as vaccine immunogens. Finally, additional efforts will be made to isolate and develop potential immunogens from epitope E1.

- Use the antibodies that bind the targeted epitopes in the native E protein to fish out their corresponding epitope conformers to reveal the specific linker that best drives reconstitution of a physiologically-relevant conformation, and thus presents a valid candidate EFI for vaccine development.

ECF objectives: Our goal was to improve the immune response to a p67-based immunogen:
- Domain scan libraries of *T. parva* antigens: Express extensively overlapping tiled peptides of p67 and PIM as domain scans using the Gershoni group phage display vector system and as GST fusions (the latter was not originally planned but was done).
- Screen for synthetic antibodies to several versions of recombinantly produced p67 protein.
- Determine the 3-dimensional structure of p67 with antibodies acting as chaperones in the crystallization process. This would allow identifying positive domains of p67 that can be used as affinity ligands to fish out serum and synthetic antibodies to be tested for their neutralizing capacity. Peptides recognized by neutralizing antibodies constitute potential EFIs for further vaccine development.

Overall, our goal was to produce bona fide vaccine candidate immunogens. The advantage of our approach, in which we develop epitope-based vaccines, is that it allows one to precisely focus the immune response on the most relevant and potent neutralizing surfaces of the pathogens. Moreover, it concomitantly reduces the dangers of producing non-neutralizing antibodies. Our experiments involved constant communication and exchange of materials between the groups, allowing sharing of expertise, and involved conformer libraries or domain scans, antibody selections, antibody characterization, identification of those antibodies that define native configurations, and isolation of the cognate immunogens to be used for further vaccine development.

Technology transfer objectives:
All members of the research teams developed vibrant and meaningful collaborations and exchanged technologies and reagents, which was promoted by several visits to the various laboratories in Canada, Israel, India and Kenya, as well as exchange of personnel for technology transfer and training, and training workshops in Kenya. This enabled successful application to targets of investigation, and will in the future, also be applied to other infectious agents.

4. Describe the rationale of the project and the research problem being addressed.

Tropical diseases can cause death and are a huge economic and social burden in low and middle income countries. In this project we focused on two infectious diseases, dengue fever (DF) affecting humans, and East Coast fever (ECF), which affects cattle. Our goal was to develop therapeutic and preventive solutions for these diseases.
Dengue virus (DV) is endemic in 100 countries with an estimated 390 million annual infections and 3.6 billion people at risk. Infection by the virus is either asymptomatic or causes febrile illness (DF) with different levels of severity, with lethal illness in 1% of cases. Due to DF's debilitating effects, outbreaks have a severe economic impact in affected countries. Because several viral epitopes can lead to the disease, it is critical for effective treatment that therapeutic agents target all viral epitopes. Current vaccines are only partially effective, and thus there is an unmet need for effective vaccines for use as next-generation primary vaccines or as boosting agents for existing vaccines.

Considering the complexity of DV infection and the risk of induction of enhancing antibodies with sub-optimal vaccines, an attractive alternative approach is to use subunits of DV as boosting agents or next-generation vaccines. Our goal here was to develop novel vaccine candidates – engineered epitope focused immunogens (EFIs) that specifically focus the immune response towards neutralizing epitopes of the virus. To avoid developing immunogens that do not recapitulate native antigens, as is often the case with isolated proteins fragments, our EFIs were designed such that they would reconstitute minimal requirements of targeted antigens and adopt a conformation similar to the natural conformation.

ECF is caused by the protozoan pathogen *Theileria parva* and ranks first among tick-borne disease constraints to smallholder cattle agriculture in sub-Saharan Africa. A live parasite-based vaccine that utilizes an infection and treatment method (ITM) of immunization is available but it is sub-optimal in nature and needs. The fact that long-lasting immunity to ECF can be generated by ITM (*Curr. Opin. Microbiol.* 11: 369, 2008; *Parasitology* 136: 1415, 2009) provides a strong rationale for undertaking research to develop subunit vaccines as sustainable and scalable alternatives to the live vaccine. The Sidhu (Canada) and Nene (Kenya) groups have been collaborating to generate antibodies against *T. parva* immunogens, which were used here in combination with the EFI platform to identify pathogen EFIs (Gershoni, Israel) that could trigger a protective response. Specifically, the project aimed to map regions of the *T. Parva* protein p67, which has been shown to induce weak immunity in cattle and can be used as immunogenic fragment. In addition to phage display technologies, structural studies aided in the design of a good immunogenic candidate, with which vaccines could be prepared. An added value of this collaboration was that it resulted in the transfer of the antibody phage technology to the African site, where it can be applied to other livestock diseases and could have transformative benefits to global health.
Overall, the project will benefit human health and subsistence, as well as the economy, by providing novel treatments and diagnostics against viral diseases that affect human and agricultural animals in low and middle income countries, while at the same time, transferring know-how from Canada and Israel to Indian and Kenyan scientists.

5. Has your understanding of the project objectives and rationale changed since the grant was awarded? If so, describe this evolution and the reasons behind it.

Note: To be completed for mid-term technical report only

N/A

Section C: Project implementation and management

6. Briefly outline project planning tools you used to support collaboration and implementation, including the frequency of their occurrence. Such tools may include teleconference meetings, face-to-

face planning meetings; lab visits/exchanges; student exchanges between labs; or institutional agreements.

Note: To be completed for mid-term technical report only

N/A

7. Discuss project implementation to date indicating what lines of work are progressing as planned and those lines that are ahead or behind schedule. Outline project management issues (e.g., team coordination, human resources, grant administration by IDRC and ISF) that affected implementation and discuss how you plan to mitigate such challenges if they are likely to persist.

Note: To be completed for mid-term technical report only.

N/A

8. Discuss how your management of the project influenced project implementation. What worked well and what would you do differently.

Note: To be completed for final technical report only.

The whole team was in frequent communication throughout the project, using email, phone (including bimonthly teleconference meetings), and site visits.

Notably, we had a chance to meet face-to-face at meetings in Kenya (January 2017 and July 2019), India (October 2017), Israel (December 2017), and Canada (May 2019).

The exchange of trainees also enhanced the transfer of technologies between the participating groups, between Canada, Kenya, India and Israel.

Therefore, most aspects of the proposed management plan progressed as planned. The only delays occurred with the organization of the workshop in Kenya and with the training of some of the Kenyan trainees and staff. The original plan was modified and extended to allow the training of 2 ILRI personnel in Dr. Gershoni's laboratory in Israel. This was a very productive visit allowing further capacity building for the Kenyan group: next generation sequencing, data pipeline optimization, immune finger printing.

The workshop and symposium were a very valuable experience for all the participants, and some of them have been offered material transfer to enable the use of the technology in their host institution. However, we found it surprisingly difficult to recruit trainees to attend the events. In the future, we would place more emphasis on methods for trainee recruitment.

One major hurdle was the administration of the project. Indeed, each institution has their own financial procedures and requirements. Having to satisfy these from different institutions increased

the administrative burden dramatically and forced the Kenyan group to incur unexpected costs. This is very unfortunate and a solution to the problem would be to have a more integrated administrative approach.

Section D: Project outputs and outcomes

Objective 1: To advance research and discovery

9. Update your complete bibliography of research outputs in your IDRC Connect account, and please upload the papers through ISF Online.
The results of this project have not yet been published.

Table 1: Number of research outputs by type

Journal articles (published and/or accepted)	Journal articles (submitted)	Conference papers	Presentations (non-academic)	Books	Book chapters	News-papers /Other	Theses
0	0	6	0	0	0	0	0

10. Discuss the significance of your research findings achieved to date to advancing knowledge and discovery (e.g., new methods, findings, technologies). Be sure to reference (hyperlink, if possible) published material where the full findings are reported.
NB: all figures and tables are at the end of the report. **1. Therapeutic and preventive solutions for dengue fever** *1A. Targeting of the envelope protein of dengue virus* We have produced dengue virus (DV) E-glycoprotein and virus-like particles (VLPs) for all 4 DV serotypes. Hundreds of synthetic antibodies against these antigens were selected. Antibodies with broad reactivity against all 4 serotypes as well as serotype-specific antibodies were identified. After several campaigns of screening, the most promising candidates were tested for their recognition of purified DV (**Table 1**). Pairs of these antibodies were then used to detect actual DV samples provided by the Public Health Agency of Canada (PHAC), as shown in **Figure 1.** Preliminary experiments showed that one of the pan-specific antibodies neutralized DV infectivity at 62.5nM to 125nM, depending on the serotype.

In summary, we developed a set of antibodies specifically recognizing DV either in a serotype-specific or a pan-serotype-specific manner. Both sets have therapeutic potential. These antibody sets will also be investigated for the development of a serotype-specific dengue diagnostic kit.

1B. Targeting of non-structural protein 1 (NS1)

DV non-structural protein 1 (NS1) is present at high levels in the blood of DV-infected individuals. The blood level of NS1 has also been linked to pathology (*J. Infect. Dis.*, 186:1165, 2002). Thus, we also focused on the production of antibodies against NS1 in a serotype-specific and pan-serotype-specific manner. **Table 2** and **Figure 2** show the affinity and the serotype specificity of the antibodies we generated against NS1.

Our antibody sets recognize recombinant NS1 either with high serotype specificity or with pan-serotype cross-reactivity (**Table 2**). They also detect NS1 secreted by DV-infected cells as shown in **Figure 3**. These antibodies will be great tools to assess the validity of an NS1-targeting therapeutic approach. A serotype-specific DF diagnostic based on these antibodies is ready to be licensed for commercialization.

Both the NS1 antibodies and DV antibodies will be further developed as potential therapeutics.

2. Epitope focused immunogens

In this collaborative part of the program, we focused on developing novel immunogens for vaccination against tropical pathogens. The Israeli team has thus far focused on the development of epitope-based immunogens specific for the DV spike protein. Thus far, two comprehensive conformer libraries have been constructed as fusions of the P3 protein of the filamentous bacteriophage fd. Both libraries have been screened against select neutralizing antibodies. No functional conformers were successfully isolated for the E1 epitope target. The E2 target has proven to be extremely successful and multiple potential immunogens are being developed and characterized.

2A. Epitope focused immunogens for dengue fever vaccination

The DV spike protein consists of three structural domains (D1, D2 and D3) and is presented on the viral coat as a dimer of envelope proteins oriented head to tail, with the D2 domain of one subunit is juxtaposed across from the D3 domain of the opposing subunit and the D1 domain bridging D2 and D3. Identifying specific neutralizing epitopes on the viral envelope and reconstituting them as potential vaccine immunogens is extremely important as DV tends to elicit enhancing antibodies (*Am. J. Trop. Med. Hyg.* 40.4: 444, 1989). Thus, focusing on discrete neutralizing epitopes should obviate the production of these enhancing antibodies. Here, two specific neutralizing epitopes were targeted. There is ample evidence that these can elicit the production of neutralizing antibodies, some of which have been shown to be broadly cross-reactive among the 4 DV serotypes.

2B. Construction of combinatorial conformer libraries

Our technical approach was to produce comprehensive conformer libraries of selected epitopes using filamentous phage display. Because the two epitopes are greater than 60 amino acids in length, the libraries were constructed as N terminal fusions with the bacteriophage protein 3. Each of the epitopes was constructed with diverse linkers, and NNK codons were used to enable the incorporation of all 20 amino acids at each residue position.

2C. *Epitope E1 – residues K64 to K120 of the D2 domain.*
The construction of the conformer library was conducted using a modified fth1 expression vector in which a cloning cassette was introduced into the 5' terminal end of the Protein 3 gene. The cassette consisted of two asymmetric *Bst*XI sites, which ensure that the cut vector cannot close on itself and that the inserts are introduced only in the correct orientation. Inserts were introduced into the cut vector by Gibson assembly.
The design of the library consisted of 0-3 NNK codons at the 5' end of the construct as well as 0-3 NNK codons at the 3' end (**Figure 4A**). The four cysteines included in this region were maintained to enable the formation of the two disulfide bridges (74-105 and 92-116, **Figure 4B**) that are thought to stabilize the epitope structure.
In order to produce the combinatorial complexity of all possible linkers, a total of 16 libraries were produced by 16 PCR reactions. The libraries were then mixed to generate a master library that contained all possible linker lengths. The library was confirmed by cloning and sequencing 10 randomly selected clones. As can be seen in **Table 3**, good representation of the various linkers was obtained.
The library was then screened with a total of three neutralizing antibodies (A11, B7 and C8). Each of the antibodies was used in 1-2 independent panning experiments and underwent as many as 5 capture/amplification rounds. Clonal phage picking was performed using aliquots of amplifications #3, #4 and #5. Clones were tested by dot-blot for binding to the antibody used during the selection. Screening over 3200 clones did not produce any positive constructs.
In view of this negative result, we turned our efforts to the construction and analysis of Epitope E2.

2D. Epitope E2 – residues M301 to E370 of the D3 domain.
This construct differed from E1 in that it contained an internal linker that bridges the gap between residues I335 and P356. Each construct could contain as many as three combinatorial linkers: 0-3 NNK at the 5' end, 0-5 NNK internally and 0-3 NNK at the 3' end. One dedicated construct contained 5' and 3' linkers and a native 20 amino acid internal loop instead of the bridging linker.
Construction of this library was conducted using 5'-sense and 3'-antisense combinatorial PCR primers that contained 0, 1, 2 or 3 NNKs. These primers were mixed and used for PCR of 7 different templates. One template contained the native complete sequence from 301 to 370 - including the loop (335-356). The second template started at 301 through 370, however the loop was omitted and residue 335 was linked directly to 356 (i.e., no internal linker). Templates 3-7 contained 1-5 NNK codons, respectively, bridging residues 335 to 356 (**Figure 5**). The PCR products were introduced into the *Bst*XI cloning sites of the modified fth1 vector by Gibson assembly as was done for Epitope E1. The libraries were confirmed for expected complexity as shown in **Table 4**.

2E. Isolation of functional reconstituted versions of Epitope E2
The libraries were screened against two neutralizing antibodies (4E5A and 1A1D) multiple times and as many as 4 capture and amplification cycles. For each sample 400 clones were picked and tested by dot-blot for antibody recognition. Positive clones were validated and sent for sequencing. **Table 5** shows the linker compositions for 7 positive clones isolated with the two antibodies tested. The question arose as to whether the positive constructs are cross-reactive for the two antibodies, i.e. do 4E5A specific clones bind 1A1D antibody and vice versa? As shown in **Figure 6**, no cross-binding was detected.
Thus we conducted specific experiments with the intent to isolate constructs that might be cross-reactive. The library was panned against one antibody and the eluted phage were panned against the

other antibody. This was repeated for a number of cycles with a number of library aliquots. Moreover, this experiment was conducted once starting with 4E5A and once with 1A1D.
A total of 52 positive clones were isolated and validated. Sequence analyses showed that: (i) one clone was previously seen for antibody 4E5A; (ii) a total of 13 unique clones were found; (iii) 3 clones appeared to be cross-reactive (**Table 6**). The three potential cross binders are currently being further characterized.

3. Epitope focused immunogens for East coast fever (ECF) vaccination

Theileria Parva (*T. parva*) is an intracellular protozoan parasite that causes ECF in cattle. Among the various *T. parva* antigens, the p67 surface protein was identified as the most protective antigen. p67 induces weak antibody responses during the "infection and treatment method" but immunization of cattle with recombinant p67 induces neutralizing antibodies and immunity in ~50% of cattle vaccinated under laboratory conditions (*Proc. Natl. Acad. Sci. U.S.A.* 89: 514, 1992). Some of the neutralizing antibodies reported are AR 21.4, AR 22.7, and AR 23 F, targeting different regions of p67. In order to generate an improved p67-based immunogen, we aimed to produce the recombinant version of these antibodies, producing recombinant full-length (FL) p67, identifying synthetic antibodies against p67, and determining the 3-dimensional structure of p67.

3A. Recombinant antibody production and characterization

Recombinant single-chain-Fab-IgG (21.4 & 22.7) were cloned, produced, purified and analyzed for their binding to the C and N-terminal regions of p67 (p67C and p67N, respectively as defined in Nene V., et al. (*Infect. Immun*. 67: 1261, 1999) in comparison to the binding of the same antibodies issued from mouse ascites. As shown in **Figure 7**, the recombinant 21.4 was expressed well and showed better binding affinity to the antigen compared to 22.7. The 23F antigenic fragment is unknown and hence the binding studies were not performed for 23F.
Recombinant scFab-IgG 21.4 showed similar binding to p67N as the ascite-derived antibody does; however scFab-IgG did not show as much binding to p67C as the ascite-derived antibody does. We are in the process of making the antibodies in IgG format rather than producing in scFab-IgG format, in case the chain linking the light and heavy chains is hindering the binding of 22.7 to its antigen.

3B. Recombinant full length p67 (FL-p67) production

The DNA sequence of FL-p67 without the predicted transmembrane domain was codon optimized for human cell expression and cloned into our in-house IgG expression vector (pSCTA) with a His-tag at its C-terminus. The protein produced was concentrated up to ~2 mg/ml (**Figure 8A**) and was found to be stable at 4ºC for several weeks (**Figure 8B**). The theoretical molecular weight of the protein is ~68 kDa but in SDS-PAGE it appears at a higher molecular weight, which is consistent with the data reported by Tebaldi et al. (*PLoS. Negl. Trop. Dis.* 11: e0005803, 2017). We identified synthetic antibody binders for the FL-p67 protein we produced (described in 3D) (**Figure 8C**), in order to uncover novel neutralizing antibodies leading to the discovery of novel neutralizing epitopes.

3C. p67 protein fragments

In order to identify novel epitopes so that we could engineer p67-based epitope focused immunogens, fragments of p67 protein-coding DNA were synthesized to produce GST fusions of 100 amino acids at 10 amino acid intervals as shown in **Figure 9A**. These fragments were tested for their

expression in *E. coli*. **Figure 9B** shows the validation of a subset of these fragments. Unfortunately, these fragments did not allow the identification of the binding region of the previously identified 23F neutralizing antibody, for which peptide-based epitope mapping was unsuccessful (*Infect. Immun.* 67: 1261, 1999). High-throughput selection of antibodies against all p67 protein fragments was conducted, leading to the identification of more than 10 antibodies. These antibodies are being assessed for their neutralization potential by the Kenyan group. The corresponding immunogen(s) could be either more potent than p67C or have an additive or synergistic effect when combined with p67C.

3D. Synthetic antibodies against p67C and p67C/FL-p67 structure determination
Unlike is the case of DV, there is no structure available for ECF antigens. Hence we focused on determining the structure of p67C and FL-p67 to further optimize p67C as an immunogen and help the design of epitope focused immunogens. As discussed above, p67C provides the same level of protection as FL-p67. We used p67C to identify synthetic antibodies to help the crystallization of p67C and to characterize the mechanism of T. parva neutralization by antibody response. From four rounds of selection, three unique binders were identified. One of the binders, Fab B11 showed the best binding (**Figure 10A**) and hence was used for crystallization studies (**Figure 11**). The interaction of Fab B11 with p67C was characterized kinetically by bio-layer interference (BLI) and the functional epitope was determined by alanine scanning experiment. This information enables greater understanding of the neutralizing antibody – antigen interaction allowing the design of further epitope focused immunogens.

3E. Bovine B-cell receptor repertoire sequencing using high throughput sequencing
We aimed to use high-throughput sequencing technologies to characterize the bovine functional antibody repertoire and discover specific responses to the p67C antigen – a candidate sub-unit vaccine against ECF. Due to the presence of ultra-long CDR3 antibodies that are unique to bovine species, we had to develop new methods for capturing these ultra-long sequences using short-read Illumina technology.
We purified PBMCs from African indigenous (Ankole, Boran and Ndama) and exotic (Friesian) cattle breeds. We established a pipeline to analyse B cell receptor (BCR) profiles using high throughput sequencing technologies. We analysed BCR from immunized and non-immunized cattle to characterize p67C-specific antibody sequences. We detected and identified genetic features distinguishing BCR profiles among the indigenous and exotic breeds that may contribute to differences in disease response and resilience. We also identified antigen-specific antibody sequences from the immunized animals that may contribute to the improvement of the candidate vaccine.

Among the antigen-specific antibody sequences that we identified, a total of 34,258 transcripts that we assembled were bearing ultralong CDRH3 loops – a unique antibody structure only found in bovine species. Of those bearing ultralong CDRH3 loops, 27 transcripts were found to represent the most abundant clones (clones are defined as groups of sequences that are >95% identical to each other) or present in multiple sequencing libraries derived from the same sample. These 27 transcripts were taken forward for recombinant antibody expression. Through this work, we were able to develop a robust technology for immune repertoire profiling and establish a bioinformatic pipelines for analysis at ILRI, thereby creating a crucial biotechnology platform within the region. This project

also contributed to capacity development in this new field of immunogenetics. The data generated will contribute information needed to improve the candidate vaccine.

3F. Deep panning of phage-displayed random peptide libraries and machine learning to identify signature features of protective immunity

The Gershoni group has coined the term IgOme to describe the repertoire of antibodies present in a serum sample. A method based on affinity selection of random recombinant peptides that bind serum antibodies was used to infer their epitope specificities and, with machine learning, to characterize an IgOme. Comparative typing of several IgOmes representing well defined biological conditions (e.g., infected vs naïve or protective vs failing immunity) led to the identification of similar and discriminatory signature-features, which were linked with meta-data associated with the antibody samples. Proof-of-principle was developed using a mixture of four different antibodies (**Figure 12A**) and then extended to demonstrate that the method can be used to discriminate serum samples from HIV and HCV human patients (**Figure 12B**). It also discriminated samples from HIV patients who control disease from those who do not. Hence, we showed that the IgOme platform offers a generic discovery driven approach in vaccine development research.

The p67C antigen, which has been tested as soluble protein and in nanoparticle formats as candidate vaccine antigens. On challenge, about half of the vaccinated cattle were immune to ECF, but conventional assays (ELISA antibody titers, T-cell responses and sporozoite neutralization assays) did not reveal strong correlates of immunity. Since the Kenya group holds a biobank of sera from 87 vaccinated cattle that were classified as immune or susceptible to ECF and are associated with clinical data and a score as indicator of disease severity, we analyzed these sera and samples from naïve cattle using the IgOme platform.

Before typing the entire p67C serum bank, we generated proof-of-principle data based on assessing panels of sera derived from 21 and 18 cattle that were elite controllers (score <2) and ultra-sensitive (score >7) to disease, respectively. The phage peptide libraries developed in the Gershoni lab were subjected to serial rounds of deep panning with serum samples, followed by next generation sequencing to generate hundreds of millions of data-points per sample, which were processed using IgOme computational tools.

We anticipate that with machine learning we will be able to define peptide signatures:

1. that discriminate an immune from a susceptible phenotype in vaccinated cattle
2. to identify the evolution of profiles associated with immune/susceptible status
3. as probes to assess the functional significance of discriminatory peptide sequences
4. that will accelerate the design and optimization of immunization methods

3G. Immune profiling of bovine sera from animals vaccinated with p67C.

Sera from 48 animals immunized with one of the formulations of the p67C vaccine trialled at ILRI over the years were shipped to the Gershoni group for deep panning using the protocol described in 3F. The 48 serum samples comprised pre-immunization (day 0) and post-challenge (day 77) samples from animals that were elite controllers i.e. having ECF score < 2 (N=14) and ultra-sensitive i.e. having ECF score > 7 (N=35) to ECF upon challenge both in the immunized and control animal groups. In addition, aliquots of purified polyclonal p67C specific antibodies and D3, B11 and AR21.4 monoclonal antibodies

were sent. The Gershoni laboratory has the capacity to process 8 samples in triplicates in one deep panning round, which takes around two weeks to complete.
So far we have bio-panned 20 samples in triplicates and prepared libraries for deep sequencing. These samples will be sequenced on Illumina HiSeq in Dr. Gershoni's lab. 9 of these samples have been sequenced on Illumina MiSeq at ILRI. MotifAI, an algorithm to analyse affinity selected peptides developed by the Gershoni group, was modified to be compatible with multiple computing infrastructures by the Israeli IT team and adjusted for ILRI's High Performance Cluster (HPC) by ILRI's team.
Dr. Sam Oyola from ILRI spent two weeks in Dr. Gershoni's laboratory, to learn experimental procedures of deep-panning technology. These involved bio-panning (binding of antibody/phage mixtures, washing, elution and neutralization procedures), phage amplification, phage extraction, PEG-precipitation and PCR and library preparations for high throughput sequencing. Dr. Sonal Henson from ILRI also trained in the Gershoni laboratory to set up MotifAI on ILRI's HPC; she gained an in-depth understanding of the processes underlying MotifAI and tested the pipeline on the HPC using test data from Gershoni group. MotifAI is computationally very intensive and contains several inefficiencies. For these reasons, the sequences generated at ILRI are still undergoing analysis, with the Gershoni group continuously improving it, and major improvements being implemented at ILRI.
Therefore, deep panning technology has been successfully transferred to ILRI and plans are underway to apply the technology to support various vaccine development initiatives. The technology will also be applied in other epidemiological studies to discriminate between infected and vaccinated subjects in cases where a DIVA vaccine is absent.

11. Are there intended or unintended applications or potential applications in terms of technology or clinical practice emerging from your research?
Note: Final technical report only The ability to create neutralizing antibodies, and to make new vaccines with neutralizing epitopes, should greatly improve the development of preventive measures for dengue fever and East Coast fever, and provide new avenues of therapeutic development.

Objective 2: To encourage international scientific collaboration

12. List the researchers involved in the project, their organizational affiliations, and location. Has this list changed since you submitted your research proposal? If so, discuss who left and/or joined, why, and their role in the project.
Canada: **Research Institution:** The Donnelly Centre, University of Toronto, 160 College Street, Toronto, ON M5S 3E1 **Members of Research Team:** • Dr. Sachdev Sidhu, Professor: Oversaw the whole project, supervised staff and trainees in Canada, communicated with collaborators, analyzed & communicated the data.

• Dr. Frederic Fellouse, Senior Research Associate: Scientific supervisor of whole project, supervised staff and trainees in Canada, communicated with collaborators, was responsible for technology transfer to India and Kenya.
• Dr. Alexander Singer, Senior Research Associate: trained Dr. Gopalsamy and participated in the crystal structure characterization of the antibody-antigen interaction.
• Dr. Shane Miersch, Senior Research Associate: responsible for the molecular characterization of the antibody-antigen interaction.
• Dr. Anupriya Gopalsamy, Post-doctoral Fellow: Worked on development of synthetic antibodies.
• Dr. Guillermo De La Rosa, Post-doctoral Fellow: Worked on development of synthetic antibodies and analysis of binding peptides.

Israel:
Research Institution: The School of Molecular Cell Biology & Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel
Members of Research Team:
• Dr. Jonathan Gershoni, Professor: Co-leader for project, supervised staff and trainees in Israel, communicated with collaborators, analyzed & communicated the data.
• Ms. Chen Piller, PhD candidate: Worked on peptide phage display technology.
• Ms. Smadar Neeman, PhD candidate: Worked on peptide phage display technology.
• Dr. Yael Ottolenghi, Research Associate: Worked on peptide phage display technology.

India:
Research Institution: SciGenom Labs, 43A SDF 3rd floor, CSEZ, Kakanad, Kochin, Kerala, 682-037, India
Members of Research Team:
• Dr. Amitabha Chaudhuri, VP Research & Development: Contributed to the development of Dengue reagents, diagnostics and therapeutics, supervised staff and trainees in India, communicated with collaborators, analyzed & communicated the data.
• Dr. Sangeetha Mohan, Research Scientist: Participated in technology transfer between Canada and India.

Kenya:
Research Institution: International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi 00100, Kenya
Members of Research Team:
• Dr. Vishvanath Nene, Director & Program Leader: Contributed to the East Coast Fever part of the project, supervised staff and trainees in Kenya, communicated with collaborators, analyzed & communicated the data.
• Ms. Elizabeth Kibwana, Research technician: Worked on the ECF project and participated in technology transfer from Canada to Kenya.
• Dr. Sonal Henson, Scientist, worked on BCR and deep panning.
• Dr. Samuel Oyola, Senior Scientist, worked on BCR and deep panning.

USA:
Research Institution: Department of Biochemistry, Albert Einstein College of Medicine, Forchheimer 320, 1300 Morris Park Avenue, Bronx, NY 10461
Members of Research Team:
• Dr. Jonathan Lai, Associate Professor: Contributed to the development of viral disease reagents, supervised staff and trainees in USA, communicated with collaborators, analyzed & communicated the data.

13. Describe how you promoted research collaboration among the researchers involved in your project. For example, did you pursue complementary research (distinct experiments carried out in different sites that complement each other); undertake the same experiments at one or several research sites; or build a shared research database?

The entire project depended on the transfer of materials back and forth between teams. For example, a novel approach of "reciprocal cloning" was used, where the Gershoni group constructed libraries of DV and *T. parva* fragmented peptide antigens. These were then used to scan antibody libraries developed by the Sidhu group. Selected antibodies were screened against native proteins to identify those that recognized the native antigens and then for neutralizing activities. Back in the Gershoni group, neutralizing antibodies were used to identify their cognate peptide targets from phage-displayed peptide libraries. This "back and forth" exchange produced potent and very relevant epitope focused immunogen candidates for further vaccine development. Thus, the overall scheme combined two well-established phage display technologies and expertise perfected in the two collaborating labs. The success of this project stems from the ensuing genuine synergy. Reciprocal cloning is totally new and banks on the ability of the collaborators to produce extensive antigen arrays that drive the selection of candidate antibodies, which are then used not only to back-screen for native cross-reactive physiologically relevant conformers, but are also considered for therapeutic potential.
Another example is the IgOme technology developed in the Gershoni group; they used serum provided by the Nene group to design and test a method for analyzing the peptides bound by antibodies in the serum. This technology was then transferred back to Kenya and will be used to analyze other infected sera.
Yet another example is the serotype-specific dengue fever diagnostic based on the NS1 antibodies generated in Canada, which is now being developed in India by SciGenom with support from the SciGenom Foundation.
Overall, the research teams shared and transferred reagents and technologies with the other groups, which enabled the leveraging of expertise for the development of directly applicable solutions to infectious disease prevention and treatment.

14. From the perspectives of the Canadian, Israeli and international researchers, what is the value added of this collaboration?

This joint funding has enabled the Canadian and Israeli PIs to concretise a project and work on developing products that could greatly improve the health of millions of people. It enabled these PIs to strengthen their relationships with key collaborators and create novel interactions with scientists in

India and Kenya. For the Canadian PI, the funding enabled an expansion of the program of research in the area of infectious diseases, an area in which synthetic antibodies could have a great impact.

The senior research associate from the Canadian group also acquired training experience in two international locations and strengthened the exchange of ideas between collaborators in India and Kenya.

This project enables the Israeli and Canadian state-of-the-art technology platforms to be applied to global health issues with a potentially high impact on patients.

Objective 3: To strengthen capacity in biomedical science

Table 2: List of trainees funded through the project

Name (SURNAME, First)	Male / Female	Home University	Degree Program (MSc, PhD, other)	Citizenship[2]	Use of funds[3]
GOPALSAMY, Anupriya	Female	University of Toronto	PDF	India	Stipend (including benefits)
RAMKRASHAN	Male	University of Toronto	PDF	India	Stipend (including benefits)
DE LA ROSA, Guillermo	Male	University of Toronto	PDF	Mexico	Stipend (including benefits)
KIBWANA, Elizabeth	Female	ILRI	N/A (technician)	Kenya	Airfare and living expenses while in Canada for training

[2] Citizenship: The funding agencies are interested in understanding how this program contributes to building scientific capacity, and where that capacity resides. As such, the funding agencies are requesting information on the citizenship of trainees involved in the project, an indicator of where the trainee may reside after the project. For example, if a Kenyan student is pursuing a post-doctoral fellowship at a Canadian university, they should be listed as a Kenyan. If this post-doc obtains Canadian residency during their period of work, the person's citizenship should be changed to 'Canadian', as they seek to remain in Canada.

[3] Use of funds: List the main trainee costs supported by the grant. Options may include tuition; conference participation; research assistantship; short-term training / workshops; internships or fellowships; international exchange.

PILLER, Chen	Female	Tel-Aviv University	PhD	Israel	Stipend (including benefits)
NEEMAN, Smadar	Female	Tel-Aviv University	PhD	Israel	Stipend (including benefits)
OTTOLENGHI, Yael	Female	Tel-Aviv University	N/A (Research Associate)	Israel	Salary (including benefits)

(add rows if needed)

Table 3: Trainee skills acquired

Using the individuals listed above, identify the kind of skills the trainees acquired.[4]

Name (SURNAME, First)	Research design	Data Collection & Analyses	Mobilizing / communicating research	Managing research activities / organizations
GOPALSAMY, Anupriya	X	X	X	X
RAMKRASHAN	X	X		
DE LA ROSA, Guillermo	X	X	X	X
KIBWANA, Elizabeth	X	X		X
PILLER, Chen	X	X	X	X
NEEMAN, Smadar	X	X	X	X
OTTOLENGHI, Yael	X	X	X	X

15. From the perspectives of Canadian, Israeli and international researchers, explain how involvement in this project has strengthened their capacity. Consult Appendix 1 for examples of how capacity can be strengthened.
Capacity building in India and Kenya Our goal was to transfer of the antibody generation technology from the Canadian/Israeli groups to the Kenyan/Indian groups. The technologies are well established in the Gershoni and Sidhu laboratories,

[4] Research Design skills acquired may include framing research questions, developing methodology/experiments. Data Collection and Analyses skills acquired may include data collection protocols, data analysis, database management. Mobilization and communication skills acquired include conference presentations, consulting with non-technical stakeholders, writing technical or media reports. Research management skills acquired may include organizing conferences / workshops; financial/technical monitoring and reporting, supervising/mentoring trainees.

but technology transfer is complex in nature and there are many "tricks of the trade". So, to transfer the technology to ILRI, Kenya, and SciGenom, India, there have been several staff exchange visits.

SciGenom, India, technology transfer

Dr. Frederic Fellouse, senior research associate from the University of Toronto (UofT), trained a total of 7 people (listed below) from SciGenom (India) in year 1.

List of people involved in the training: Sangeetha Mohan – Scientist (F), Aju Antony – Scientist (M), Rajesh Devasia – Associate Scientist (M), Lizebona August – Research Associate (F), Ambika Rajendran – Research Associate (F), Sweety Abraham – Research Associate (F), Mony Kuriakose – Research Associate (F).

Dr. Fellouse went step by step through the procedures of antibody selection and screening. Hands on training was given to screen a positive control antigen (DENV4-NS1) using the phage display library transferred from UofT. This training was useful in setting up the screening process at SciGenom and the process is now being used to identify high affinity binders to priority antigen proteins. Dr. Fellouse was also available, in person and/or through on-line meetings, for discussing the data, which helped the team to refine the process and set up a pipeline in the Indian lab facility. The SciGenom laboratory now routinely selects and produces recombinant antibodies for various applications. Dr. Sangeetha Mohan was one of the trainers during the workshop we organized in ILRI, Kenya.

ILRI, Kenya, technology transfer

In year 1, Elizabeth Kibwana, an ILRI research associate, was trained at the University of Toronto for 3 months. The training covered (a) phage display selection on phage-displayed synthetic antibody libraries against recombinant p67C protein (b) clonal phage ELISA and PCR sequencing (c) recombinant antibody production and purification in IgG and Fab format, and (d) flow cytometry training. Elizabeth was actively involved in the organization and execution of the 2019 workshop, giving Ms. Kibwana a valuable teaching opportunity.

Two members of the ILRI went to Israel to train in the IgOme technology from the Gershoni group, so that they were able to set it up in Kenya. Drs. Sonal Henson and Samuel Oyola spent a number of weeks in the Gershoni Lab training in two technologies: (i) running the Deep Panning protocol in which serum samples are screened against random peptide phage display libraries; and (ii) Computational analyses of the affinity selected peptides using the Tel Aviv algorithm MotifAI. For this, serum samples from Kenya were sent to Israel via and according to Israel Ministry of Agriculture import license regulations. The samples were screened against the Tel Aviv phage display library and affinity selected phages were used for NGS analyses. In parallel, the MotifAI algorithm were transferred to ILRI and Dr. Henson along with the Israeli IT team set up the cluster in Kenya to run the mega data for the identification of discriminating features corresponding to defined biological conditions. The transfer of these technologies has already proven successful and an ongoing exchange and collaboration has been established.

Section E: Future collaboration

16. Outline your priorities as they relate to your project objectives for the remainder of the grant and what you hope to achieve during that period. Include the dates and location on any significant events you may be planning. Please indicate Significant deviations from the Original research plan.
Note: Mid-term report only N/A

17. Have you or your collaborators developed **new** collaborations that either widen or deepen your research program or network? If so, briefly discuss the project, who is involved, and source of funding.
The Canadian team has established a novel and productive collaboration with the Public Health Agency of Canada (PHAC), which enabled the binding assays of the anti-dengue virus antibodies on virus particles and the evaluation for therapeutic applications. This collaboration will continue and may extend to other projects in the future.

Section F: Observations / Recommendations

18. The funding agencies invite any observations you or your collaborators would like to share on the design or our administration of the funding opportunity (scope, duration, or budget).
Note: Your comments are confidential in the mid-term report and public in the final report. As per correspondence with our contact at IDRC (Dr. Fabiano Santos), we have requested and been granted a 12-month embargo on the publication of the final technical report, which will enable our groups to file appropriate patents based on the data shown in the report within the next 12 months. We thank the agency for this delay in publication that will protect key intellectual property created in the project.

Figures and Tables

Table 1: Binding affinities of identified binders against VLPs from 4 dengue virus serotypes. ELISAs were performed using 4E5A as capture IgG, saturating amount of VLPs and then dilution series of analyzed Fab/IgG as detection antibodies. The EC_{50} values represent concentrations of Fab or IgG that yielded 50% of saturation signal. The EC_{50} values were obtained by fitting the data using 4-parameter non-linear regression model in GraphPad Prism 7. The EC_{50} values are shown as best-fit values with their standard deviations (SD). NF – represents either the data sets that we were unable to fit with the model or the fits that showed higher than 100% SD of EC_{50} value.

	clone ID	EC_{50} for Fab [nM]				EC_{50} for IgG [nM]			
		VLP-DV1	VLP-DV2	VLP-DV3	VLP-DV4	VLP-DV1	VLP-DV2	VLP-DV3	VLP-DV4
Serotype-specific	**F21**	3.1±0.1	NF	NF	NF	0.24±0.04	NF	1.2±0.5	NF
	3F1	NF	0.82±0.04	140±30	90±40	NF	0.06±0.01	0.60±0.09	0.54±0.06
	7F1	NF	NF	6.2±0.2	NF	NF	NF	0.33±0.05	NF
	6H3	NF	NF	NF	1.21±0.04	NF	NF	NF	0.38±0.07
Pan-specific	**7H1**	0.8±0.1	120±20	1.5±0.1	3.7±0.5	0.048±0.003	0.078±0.006	0.052±0.003	0.050±0.003
	3F3	1.7±0.4	120±40	1.7±0.1	15±6	0.151±0.008	0.18±0.01	0.058±0.002	0.50±0.02
	7F3	37±6	22±2	19±3	350±90	0.36±0.03	2.7±0.3	0.139±0.006	0.41±0.03
	6H4	25±3	70±9	24±1	10.6±0.6	0.21±0.01	3.1±0.2	0.178±0.009	0.171±0.008

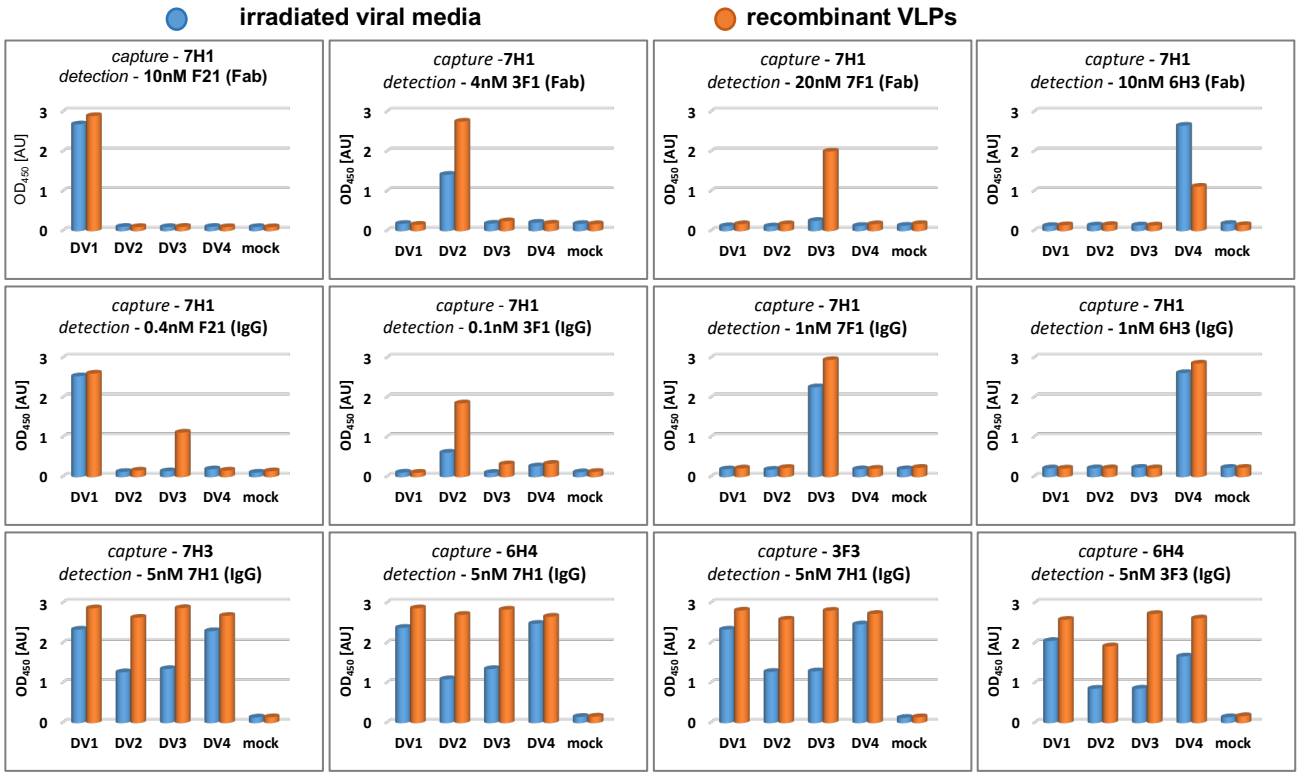


Figure 1: Single point specificity binding assay analysis of selected ELISA sandwiches against recombinant VLPs and irradiated media from dengue-infected Vero cells. The ELISAs were done by immobilizing capture IgG on the plate followed by incubation with either 3x diluted media from dengue-infected Vero E6 cells (blue bar) or with recombinant VLPs (orange bar; 100x diluted stock solution in case of DV1/2 and 25x diluted in case of DV3/4). Then the biotinylated detection antibody (either in Fab or IgG format) was added to the well. Afterwards, the NA-HRP was used for signal detection.

Table 2: Binding affinities of identified IgGs against recombinant NS1 proteins of 4 DV serotypes. The analyzed antibodies in IgG format were immobilized on ELISA plates and then 4 different concentrations (100/10/1/0.1nM) of recombinant His-tagged NS1 protein of a given DV serotype was added. Afterwards, the signal was developed with an anti-His HRP conjugated antibody. The NS1 affinity value represents a concentration of NS1 protein that yielded 50% of a saturation signal and corresponds to the EC_{50} parameter of the fitting model. The values were obtained by fitting the data using 4-parameter non-linear regression model in GraphPad Prism 7 with a "top" parameter of the model set by default to a value of 3.0. The values are shown as best-fit values with their standard deviation. NF – represents either the data sets that we were unable to fit with the model or the fits that showed higher than 150% SD of EC_{50} value.

clone ID	NS1 affinity [nM]			
	DV1	DV2	DV3	DV4
13H1	5±2	28±5	380±70	50±10
24F1	4.6±0.7	210±30	122±7	50±20
16H1	NF	2.3±0.4	130±20	NF
16F2	NF	0.04±0.02	600±100	NF
24H2	1.8±0.4	2.1±0.4	1.3±0.2	0.9±0.2
24F16	1.1±0.3	1.1±0.3	0.9±0.1	1.2±0.3
22H1	0.15±0.03	NF	0.13±0.01	NF
22H3	NF	NF	1.4±0.2	NF
13H8	NF	NF	NF	1.0±0.1
19F1	300±100	NF	900±400	0.23±0.01
19F2	NF	NF	1100±400	2.6±0.6
19H1	3.8±0.4	200±50	33±8	2.4±0.6

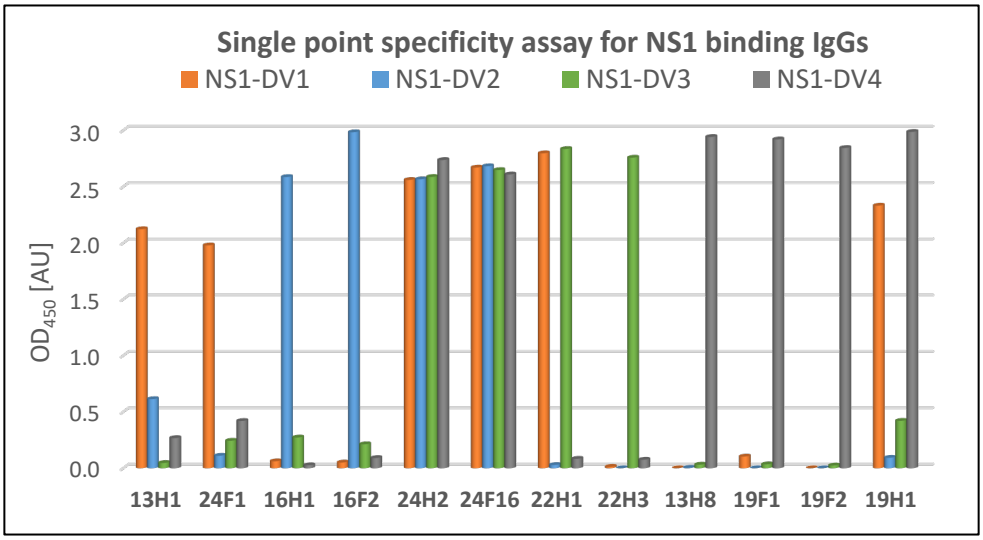


Figure 2: Single point specificity binding assay analysis of selected IgGs against recombinant NS1 protein from 4 DV serotypes. The ELISAs were done by immobilizing analyzed IgGs (final concentration 4 µg/mL in PBS) on the plate followed by incubation with 10nM recombinant His-tagged NS1 protein of respective DV serotype and developed with an anti-His HRP-labelled antibody. The readout signal corresponds to the well absorbance at 450nm.

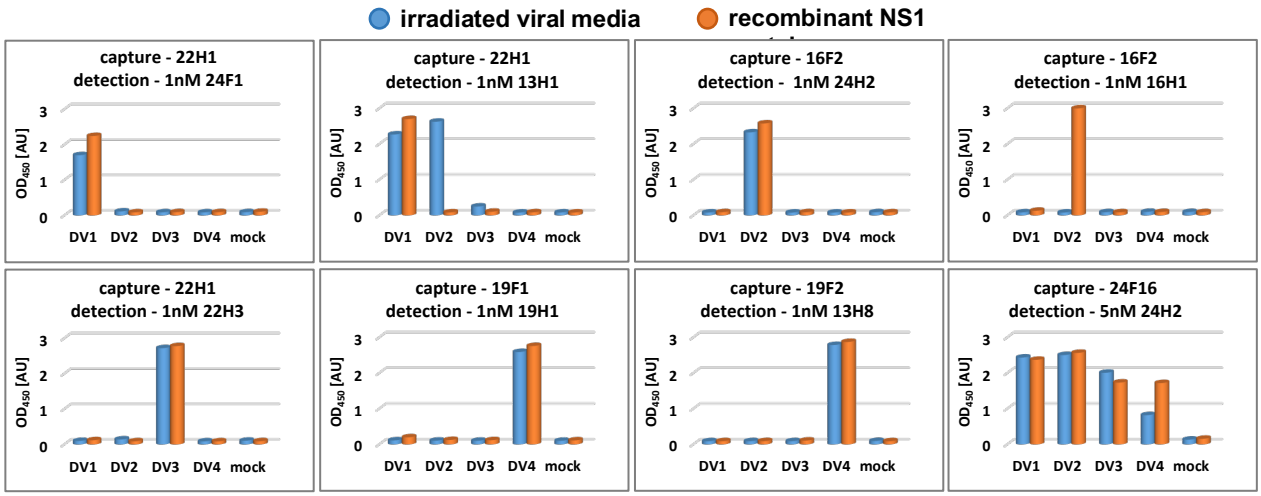


Figure 3: Single point specificity binding assay analysis of the selected ELISA sandwiches against recombinant NS1 protein and irradiated media from DV-infected Vero cells of 4 DV serotypes. The ELISA was done by immobilizing IgG on the plate followed by incubation with either 3x diluted media from DV-infected Vero E6 cells (blue bars) or with 30nM recombinant NS1 protein (orange bars). Then the biotinylated detection IgG was added to the well. Afterwards, the NA-HRP was used for signal detection. Y axis values refer to optical density at 450nm and are shown in AU units.

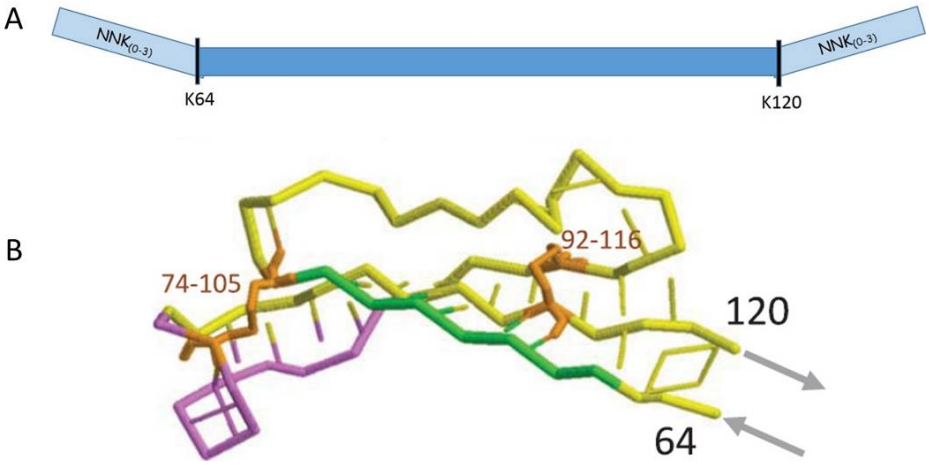


Figure 4: Construct of *Epitope* 1. A. Epitope with combinatorial linkers introduced just preceding residue 64 and following residue 120. **B.** Residues 64-120 produce a compact and highly structured feature, supported by extensive hydrogen bonding and further locked into position by two disulfide bonds (74-105 and 92-116).

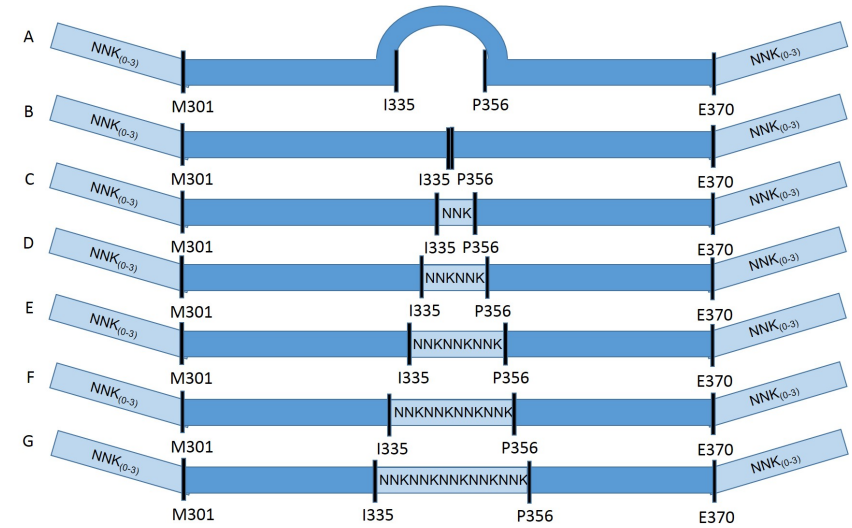
Table 3. Sequencing results of randomly selected clones from Epitope 1 master library.

Clone ID	5' NNK sequence	3' NNK sequence
EP1_1	F R	TSR
EP1_2	ALH	T
EP1_3	S	H
EP1_4	IKR	P
EP1_5	R	ST
EP1_6	HLL	TT
EP1_7	NAP	-
EP1_8	G	TRT
EP1_9	SQI	-
EP1_10	CAL	HT

Table 4. Sequencing results of randomly selected clones from Epitope 2 library.

Clone ID	5' NNK sequence	Internal linker	3' NNK sequence
EP2_1	QSD	R	-
EP2_2	IWE	GSGGS	K
EP2_3	RS	A	-
EP2_4	GG	-	PNV
EP2_5	A	-	-
EP2_6	D	-	P
EP2_7	VR	-	QKD
EP2_8	RRV	Original loop	-
EP2_9	L	TEY	G*
EP2_10	-	ELV	PT

A



H I

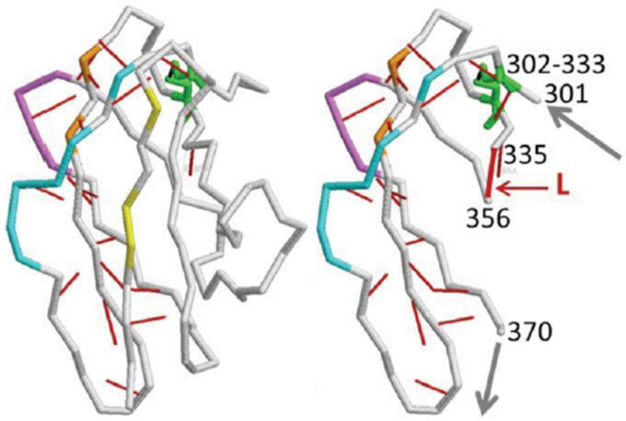


Figure 5: A. Templates of the Epitope *2* library. A. Native complete sequence of DV envelope protein from 301 to 370 - including the loop (335-356). **B.** Sequence of DV envelope protein from 301 to 370 with the loop omitted and residue 335 linked directly to 356 (i.e. no internal linker). **C-G.** Templates containing 1-5 NNK codons bridging residue 335 to 356. **H** depicts the extensive hydrogen bonding (shown with red lines) and disulfide bond between residues 302 and 333. Residues inserted in place of residues 336-355 are highlighted in red and labeled "L" as "linkers". Removal of the F and G strands at residue 370 further reduces the bulk of the proposed reconstituted epitope (shown in C). **I** shows a model of the scaffold sequence used for the library design described in **A-G** with a start at Met 301 and an end with Glu 370 (grey arrows). [Rasmol depiction of PDBID:2R69]

Table 5. Linker composition for 7 positive clones isolated with 1A1D or 4E5A.

Detected by antibody	Clone ID	5' NNK sequence	Internal linker sequence	3' NNK sequence
1A1D	G10	N	SKRG	T
	C9	SR	WRLG	Y
	B5	GR	QTGW	L
	E3	K	GGWG	-
	F6	P	RRL	RS
4E5A	B12	S	GGG	RL
	H9	RGA	NNG	YL

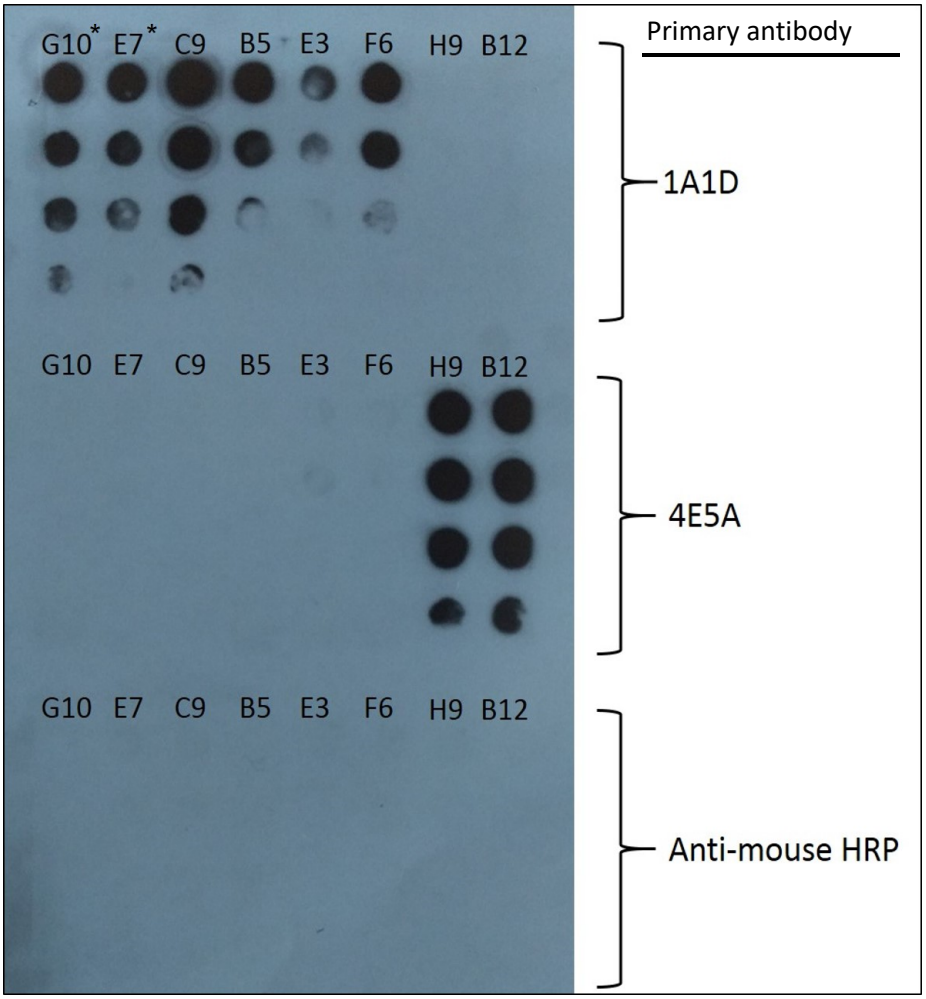


Figure 6: Testing positive clones for cross-binding with 1A1D and 4E5A. *Clones G10 and E7 are duplicates.

Table 6. Linker composition of positive clones isolated from the cross-reactivity experiments.

Clone #	Frequency	Clone ID	5' NNK sequence	Internal linker	3' NNK sequence	4E5A binding	1A1D binding
1	13	F8	TSR	GLRG	TL	+	
2	12	A11	G	PFGSS	-	+	+
3	9	C7	R	KGG	NLA	+	
4	6	D12	GFP	PLGDH	RPV	+	
5	2	H1	PA	GRGG	LL	+	+
6	2	E4	P	AGIDH	-	+	
7	2	H9 (*)	RGA	NNG	YL	+	
8	1	C1	P	SPKG	-	+ (**)	+ (**)
9	1	C2	H	QGG	TW	+ (**)	+ (**)
10	1	C6	TGL	YSGQW	TTQ	+	+
11	1	D3	P	RFG	YMR	+	
12	1	E2	VP	EWN	T	+	
13	1	H10	R	GDWG	NI	+	

(*) H9 Clone was previously seen for antibody 4E5A.

(**) Still to be confirmed.

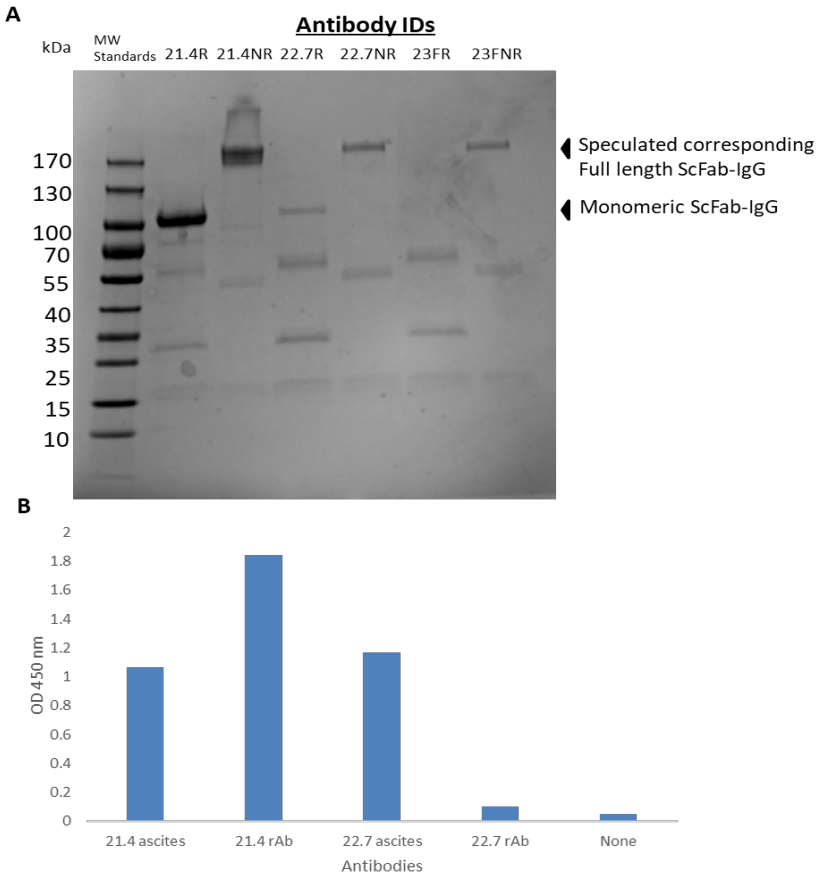


Figure 7. Production and characterization of recombinant versions of antibodies against p67. A. Purified 21.4, 22.7, and 23F antibodies in reducing (R) and non-reducing (NR) conditions were analyzed by SDS-PAGE. **B.** The binding of these antibodies for their cognate antigens is compared to the binding of the antibodies issued from mouse ascites.

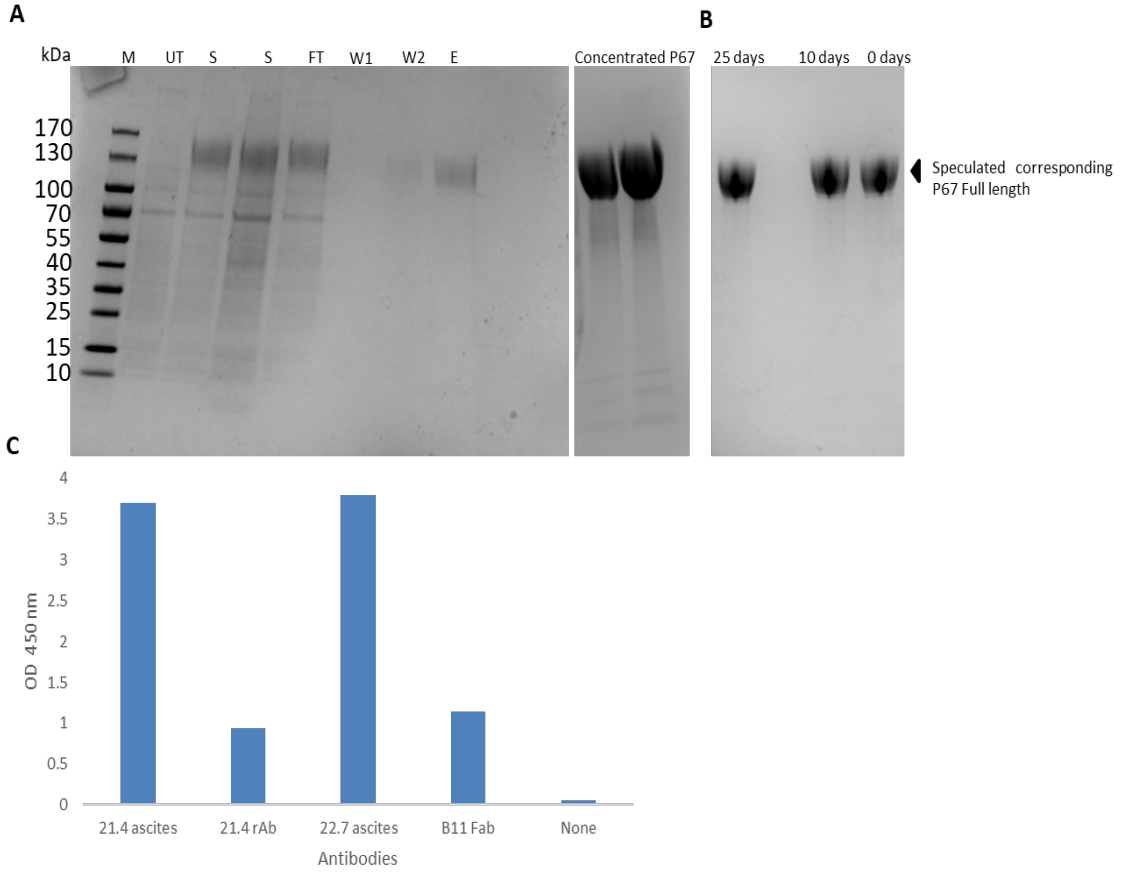


Figure 8. Production of full length p67. A. Expression and purification profile of FL-p67 in HEK cells. Marker (M), Untransfected (UT), Supernatant (S), Flow through (FT), wash (W), Eluate (E). **B.** SDS-PAGE analysis to monitor the degradation of FL-p67 at 4ºC at different time points. **C.** Binding of recombinant (r) FL-p67 with antibodies by ELISA (B11-Fab is the synthetic antibody described in Fig. 10).

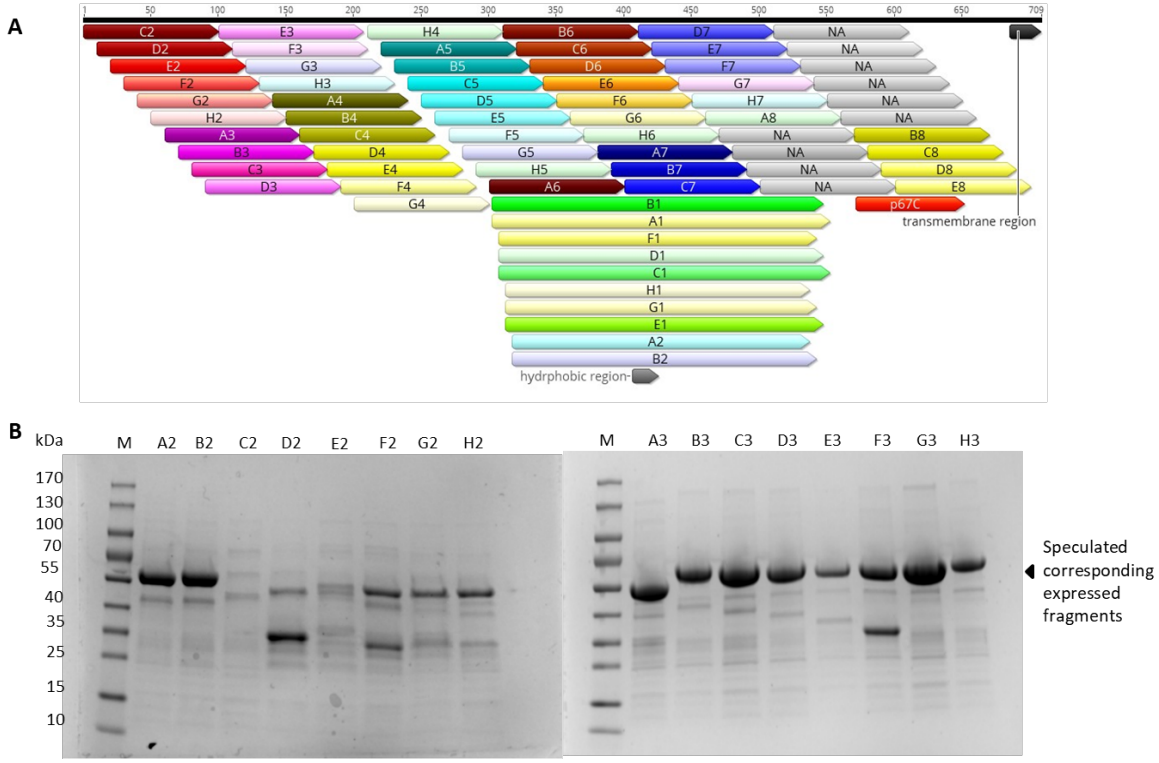


Figure 9. p67 fragments. A. Diagramatic representation of the p67 protein fragments produced (amino acid numbers showed on the top X-axis). **B.** Purified fragments expressed in *E. coli* analyzed by SDS-PAGE.

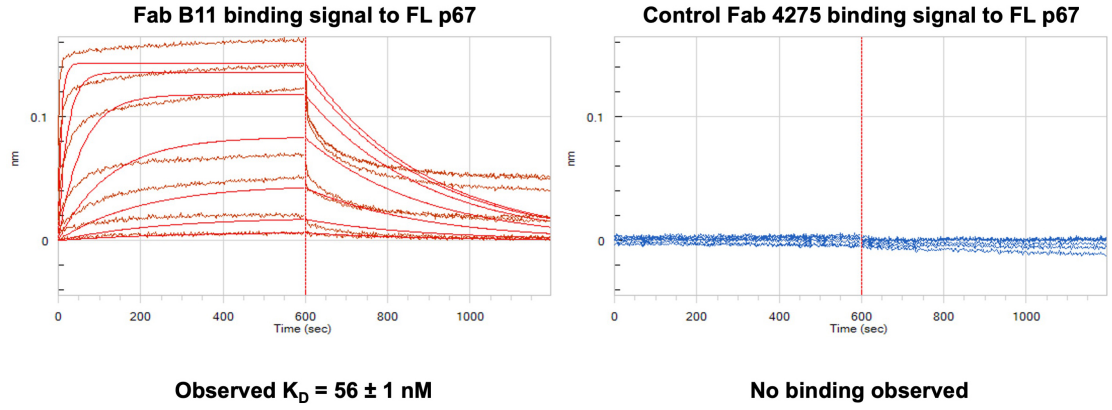
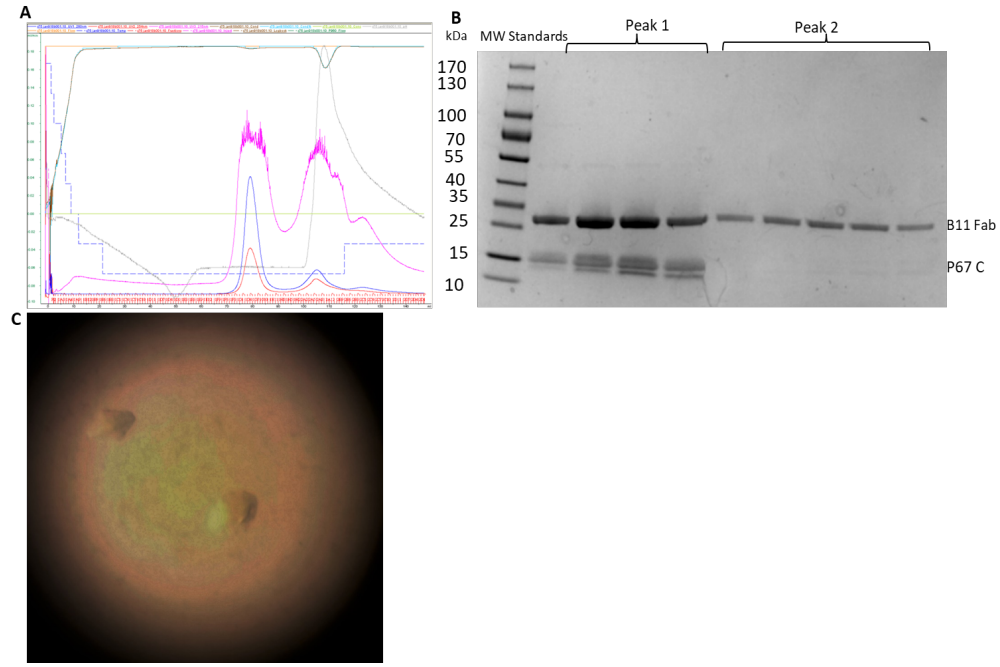


Figure 10. Synthetic antibodies against full length p67. Binding kinetics of antibody fragment (Fab) B11 for full length (FL) p67 expressed (conditions of the experiments are being optimized to obtain better fit between the model and the experimental data).


A
B
kDa
MW Standards
Peak 1
Peak 2
170
130
100
70
55
40
35
25
15
10
B11 Fab
P67 C
C

D

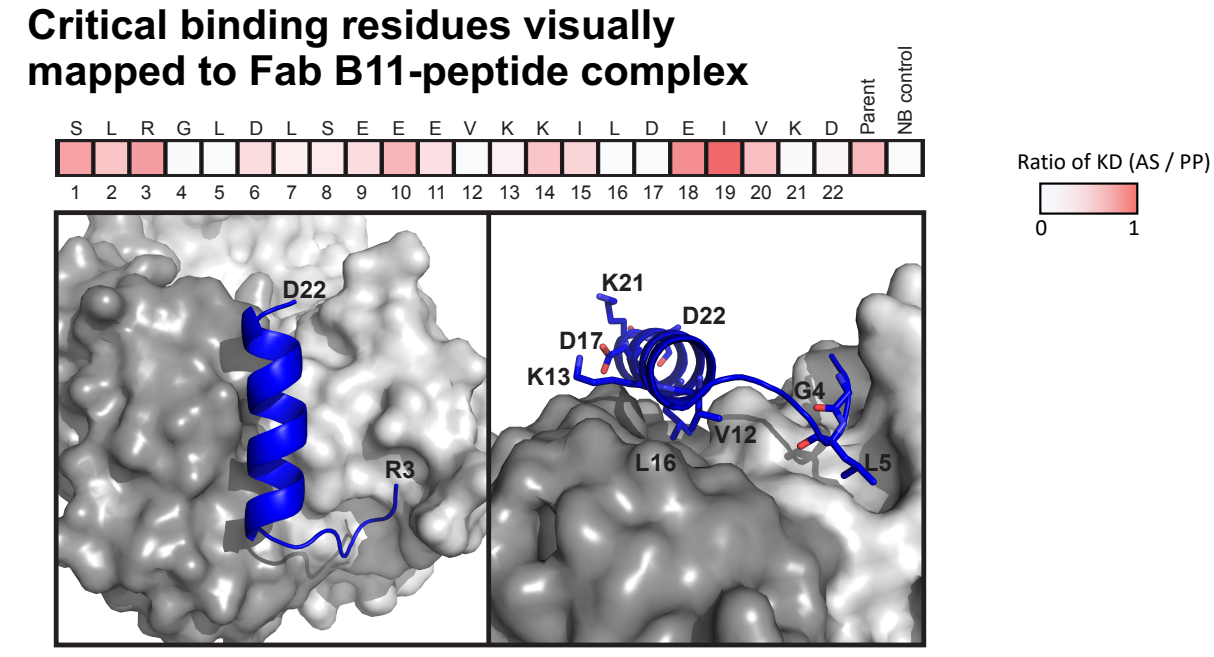
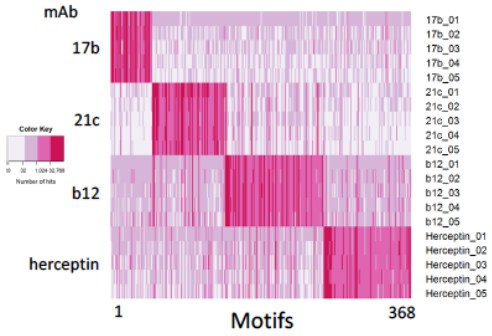

Critical binding residues visually mapped to Fab B11-peptide complex
S L R G L D L S E E E V K K I L D E I V K D
Parent
NB control
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
D22
R3
K21
D22
D17
K13
G4
V12
L16
L5
Ratio of KD (AS / PP)
0
1

Figure 11. p67 crystallization. A. Gel filtration profile of B11 Fab in complex with the p67C antigen. **B.** Fractions from gel filtration chromatography analyzed by SDS-PAGE. **C.** Relatively small crystal formation in 28% PEG 1k, MES, and ZnCL. **D.** p67 fragment bound to synthetic antibody fragment B11. The portion of p67 for which we found corresponding electron density in the crystal structure is shown as a cartoon coloured in blue in the left and right bottom images. The antibody light and heavy chains are shown as surface representation and coloured in light and dark gray, respectively. The important residues for binding to B11 are defined by a loss of binding affinity greater than 5 fold upon mutation to an alanine and these are labeled and shown as stick in the right side image. The top graph shows the amino acid sequence of the p67 peptide fragment used to determine the crystal structure. The scale underneath this sequence depicts the energetic contribution of each residues of p67 fragments for the binding to B11 antibody based on the alanine mutant scan and BLI affinity measurement (KD ratio between the alanine scanned peptide (AS(and the parental peptide (PP)).

A



B

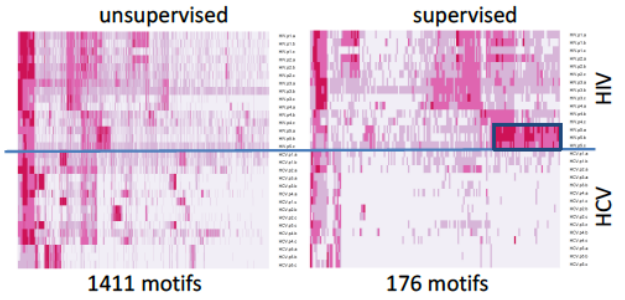


Figure 12. A method for characterization of the IgOme. A. Peptide sequences bound by antibodies. **B.** Serum samples from HIV and HCV patients.

Section G: Only for final Report

19. Please write a short descriptive laymen report up to one page including :
    - Description of the general field
    - Research Question
    - Research Achievements
    - Key words and associated (or complementary) fields

Infectious tropical diseases can cause death and are a huge economic and social burden, especially in low-and-middle income countries. In this project, we focused on two devastating diseases, dengue fever and East Coast fever. Currently 3.6 billion people are at risk of dengue virus infection. Existing in 100 countries with an estimated 390 million annual infections and ~500,000 hospitalizations, dengue fever is a worldwide challenge for public health officials and policy makers. East Coast fever is a tick-borne disease that affects cattle and is caused by a parasite. It has a devastating impact on pastoralists and smallholder farmers in Africa.

This collaborative research program, with experts from around the world, aimed to develop affordable antibody-based therapies for dengue patients and improved vaccines for the control of dengue fever and East Coast fever. The team combined cutting-edge scientific expertise to advance the development of therapies and vaccines that are easy and affordable to implement, which is important for low-and-middle income countries. Through this collaboration, we shared technology developed in Canada and Israel with research groups at the International Livestock Research Institute, Kenya and SciGenom, India. The exchange of trainees and information was the foundation of our program and helped us achieve our goals.

Our first goal was to develop novel diagnostics and therapeutics for dengue fever. We used our established protein engineering technologies to make antibodies that can neutralize the dengue virus. These antibodies represent candidate therapies that will be further developed, and they can also be used to develop new, reliable diagnostic tests.

Our second goal was to improve the efficiency of vaccines by finding which parts of the disease-causing organisms (called epitopes) are most likely to elicit a protective immune response. These epitopes can then be used to generate new, more effective vaccines. We have successfully identified epitopes for both dengue virus and the East Coast fever parasite that can be used for new vaccine development.

Our final goal was to transfer technology developed in Canada and Israel to low-and-middle income partner countries – India and Kenya, and to build capacity to enable scientists in these affected countries to take a lead role and independently explore these cutting-edge technologies for disease control. We addressed this objective with an exchange of trainees between the laboratories involved and with dedicated training workshops in Africa.

Overall, our project significantly advanced the development of new therapies and vaccines for two infectious diseases that affect a large number of people and animals throughout the world. Moreover, it enabled laboratories in India and Kenya to gain new expertise in technologies that will allow them to improve therapies and vaccines for several infectious diseases endemic to their regions.

Key words: dengue fever; East Coast fever; infectious diseases; human health; cattle farming; antibody-based therapies; vaccines; protein engineering.

Fields: Immunology, virology, protein engineering, molecular biology, cell biology

Appendix 1: Types of Capacity Strengthening[5]

Capacity strengthening is the process by which individuals, groups, organisations, institutions and societies increase their ability to identify and analyse challenges, and to conceive, conduct, manage and communicate research that addresses these challenges. These kinds of capacity are developed further below.

1. *The capacity to conduct research*: This refers to the technical, disciplinary and/or sectoral knowledge, mastery of research methods and analytical skills appropriate to conducting either a current or an evolving research investigation. Specifically, it includes capacities to:
    - work effectively within a research paradigm;
    - access to and operation of research equipment;
    - conduct technical/scientific lab work at a level of expertise and independence appropriate to the research activity;
    - conduct fieldwork (social, biological) data collection and analysis;
    - communicate ideas to, and collaborate with, peers and supervisors; and
    - interpret and present results appropriate to external audiences.

2. *The capacity to manage research*: This refers to the professional knowledge and practical experience of management principles, processes and procedures within the research context appropriate to conceiving, initiating, facilitating implementation and ensuring monitoring of a research activity, programme or institution. Specifically, it includes capacities to:
    - negotiate research activities appropriate to available/potential human and infrastructure resources in the programme, organization or wider environment;
    - identify technical and fieldwork requirements of the research;
    - develop and oversee execution of work plans, including monitoring and assessment systems;
    - select, direct and supervise researchers, support staff, resource people;
    - facilitate internal co-ordination and external liaison;
    - plan and report financial expenditures and technical findings required by funding agencies.

3. *The capacity to conceive, generate and sustain research*: This refers to such capacities as:
    - analyze and synthesize complex ideas and data;

[5] Adapted from "IDRC-Supported Capacity Building: Developing a Framework for Capturing Capacity Changes", February 2007, and from Mapping Capacity Development at IDRC (2005) http://idl-bnc.idrc.ca/dspace/handle/10625/47541

- perceive problems or issues in researchable terms;
- challenge existing research paradigms, and create new ones;
- formulate theory and concepts, think laterally;
- initiate first-order questions and set them within a research design;
- generate/implement data gathering, analysis and synthesis procedures;
- articulate implications of results in policy and/or use-oriented terms;
- serve as independent/senior resources regionally and globally on matters of theory, policy and practice; and
- manage teams of researchers, co-ordinate networks, generate/catalyze research and exchange activities.

4. *The capacity to position or use research results -- in clinical, technical, policy-making or other professional settings,* this refers to the skills needed to communicate research, articulate the potential risks and benefits, constraints and opportunities for users, often required to move from the generation of ideas and analysis to enabling their dissemination and application. Specifically, it includes capacities to:
    - tailor research designs, methods and the articulation of results in terms of specific application for specific users;
    - analyze the types of attitudes, knowledge and skills needed by users to put the research innovation into practice and the capacities they need to maintain it e.g. to overcome bureaucratic, technological or socio-economic barriers to sustainability;
    - conceive and execute dissemination strategies;
    - present/disseminate research and results in clear actionable terms;
    - plan and execute “risk-mitigating” strategies in introducing innovations;
    - facilitate user access to/practice with research products and ideas;
    - design and implement participatory research/adult learning methodologies;
    - design and manage on-site and post-research extension activities;
    - act co-operatively in putting time and imagination into joint project development and application with practitioner/users; and
    - tolerate ambiguity of the real world, using incremental, iterative approaches.