BactiVac Catalyst Projects CONFIDENTIAL

Final Project Report: July 2022

All recipients of catalyst project funding are required to submit a final end of project report as stated in the terms and conditions of award. These reports must be submitted to BactiVac for review and approval before the final payment, as detailed in your award letter, can be released. Failure to complete and submit a final report may cause the final milestone payment to be delayed, and/or BactiVac to refuse to consider further grant requests.

You should ensure that the contents of this Report are reviewed and accepted by all co-applicants prior to submission to BactiVac.

This Final Project Report has been pre-populated with the details that we currently hold for your project. If any of these details have changed, please update them as required. The main focus of the final report is on delivery against objectives and milestones as provided in your original proposal and expenditure against the funding awarded. We also need to collect/confirm information on the following for **inclusion in our onward reporting to our funders and to complete our mandatory annual return for all activity supported by BactiVac via ResearchFish**:

- Matched funding/in kind contributions
- Collaborations
- Dissemination of research findings
- Challenges faced
- Future plans and sustainability

Deadline for receipt of Final Project Report: 31 JULY 2022

Main project details:

Please advise us of any changes that relate to the main project details provided in your original proposal by updating the 'Changes' column below, e.g. any changes in co-applicants, host organisations, etc.

1. Project details:		* Changes?
Reference no.:	BVNCP5-07	
Project Title:	Kick-starting production process development of a porin-based NTS vaccine: Strain selection	
Lead Applicant:	Tania Rivera-Hernandez	
Co-applicant 1	Constantino Lopez-Macias	
Co-applicant 2	Edmundo Calva	
Co-applicant 3	Adam Cunningham	
Confirmed start date	31 October 2021	
Project end date	30 June 2022	
Project duration	8 months	
Total funding awarded	£49,000.00	
Total project cost (100% fEC)	**£49,000.00**	

2. Project aims and technical summary (max 500 words)

Our laboratory is unique world-wide for its ability to produce highly purified porins from *Salmonella*. We have ample experience working with several serovars, including *Salmonella* Typhimurium (STm) which causes non typhoidal Salmonellosis (NTS). However our current production process uses the commercial strain *Salmonella enterica* serovar Typhimurium ATCC 14028, this detail which represents a hurdle for the eventual scale up of the production process aiming for commercialisation due to IP. On the other hand we have a large collection of clinical isolates that could potentially be used for porin production, removing any IP constrains.

The overall aim of this project is to select a *Salmonella* Typhimurium strain for the production of a porin-based candidate vaccine against non-typhoidal salmonellosis.

The five specific aims of this project are:

(1) Selection of producing strain

(1a) Characterise the growth kinetics of five candidate *Salmonella* Typhimurium strains. Within the preselected Mexican STm clinical strains include representative isolates from four different and widely separate geographical locations in Mexico over a several year period (1). As an example, the ST213 genotype has now been found in other distant locations although they are not necessarily confined to and widely distributed in LMICs. The preselected strains caused either invasive, diarrheal or asymptomatic course of symptoms (1) and have different laboratory phenotypes such as degree of invasiveness in tissue culture cells (unpublished). Despite this diversity, their genes coding for the outer membrane proteins that provide immunity (OmpC, OmpD and OmpF) are highly conserved, as shown in a multiple sequence alignment of three preselected Mexican isolates (33676, YU15 and SO3) and other relevant isolates from Malawi (D23580) and Vietnam (VNB151)(Supplementary Figure 1). This analysis suggests that porins produced by the preselected strains will provide coverage against circulating strains within Mexico and other LMICs.

(1b) Test the candidate strains for their ability to produce porins and select the producing strain.

(1c) Full genome sequencing of the selected strain.

(1d) Generate *msbB and pagP* mutants from the selected strain.

(2) Produce *Salmonella* Typhimurium purified porins from the selected strain and characterise the purified product.

(2a) Produce *Salmonella* Typhimurium porins from the selected strain and from its corresponding *msbB* and *pagP* mutants in our laboratory scale pilot plant.

(2b) Characterise purified product integrity using SDS-PAGE and MALDI-TOFF and determine the LPS content using the LAL assay.

(3) Characterise the immune response development following immunisation with purified *Salmonella* Typhimurium porins in mice

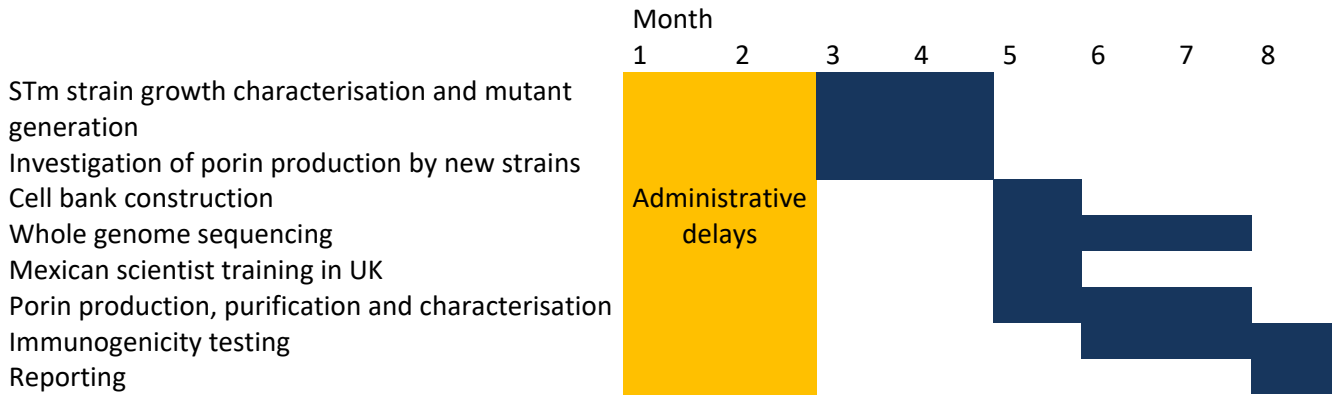
(3a) Measure porin-specific antibody responses (IgG and IgM) following immunisation and compare to porins purified from our reference strain.

(3b) Characterisation of T cell recall responses following immunisation and compare to porins purified from our reference strain.

(3c) Measure bactericidal antibodies against MDR strains. We have selected the multi-drug resistant 33676 human-invasive strain, isolated from a previously healthy 15-year-old young woman, with a 4-day duration clinical picture characterized by fever, vomiting, severe abdominal pain and watery bloody diarrhea.

3. Project Objectives and Milestones – please list objectives & milestones and include Gantt chart (or equivalent) detailing the related timescales for achieving these

3.1



3.2 Delivery against objectives and milestones:
Please indicate status for delivery made against project objectives & milestones:

***AMBER:** Some issues were experienced which impacted delivery against planned objectives & milestones, but the mitigation plans that were implemented addressed the issues

3.3 If status for delivery against planned objectives is not GREEN, please detail what actions were being taken to address any issues that impacted on project delivery (approx. 200 words).

Support from additional UIMIQ staff was dedicated to this project, mainly to complete the production process using the new strains. This significantly helped to keep the project progressing appropriately. Even though aims (3b) and (3c) are still work in progress, we were able to deliver most of the work planned in the initial proposal. In addition, the team decided to test the WT strains, before generating and testing the mutants proposed in aim (1d). Once all functional assays are completed, we will generate and test the proposed mutants.

3.4 Please provide a lay summary of key project findings (non-confidential; max 500 words – this information will be used for publication on the BactiVac website)

STm porins represent promising vaccine candidates against NTS. Our team is currently developing a scaled-up production process in order to be able to manufacture this vaccine candidate at an industrial scale. As part of this scale-up process, selection of a suitable producing strain is critical. In this work we selected two strains from a panel of STm clinical isolates, representative of the ST213 genotype firstly reported in Mexico. Two novel STm strains were successfully used for STm porin production and the purified products were mainly composed of OmpC and OmpD, as shown by LC-MS analysis. One strain in particular had a significantly high yield of purified protein and low levels of LPS in the final product. The purified porins were highly immunogenic in mice, even in the absence of adjuvants, and antibody titres were higher in those mice immunised with porins purified from the novel strains compared to our reference strain. Studies regarding the protective efficacy of these porin preparations are still underway and will be critical to push forward the use of either one of this STm strains.

3.5 Please provide a short sentence that we could use to summarise a key finding from your project via Twitter (approx 200 ***characters*** long. If you have a suitable image that could be used in the tweet, please paste this image in below)

The results from this project set up the foundations of the production of a porin-based NTS vaccine at an industrial scale.

3.6 Please provide a detailed description of delivery against planned objectives & milestones, including results achieved and key project findings.

The team first decided to focus on two STm strains as porin production strains, SLHS-03-15 (SL7) and YUHS-04-50 (YU33). Both strains were isolated in Mexico and belong to the ST213 genotype, which has now been found in other distant locations, although they are not necessarily confined to and widely distributed in LMICs. The selected strains caused either invasive, diarrheal or asymptomatic course of symptoms (1) and have different laboratory phenotypes such as degree of invasiveness in tissue culture cells (unpublished).

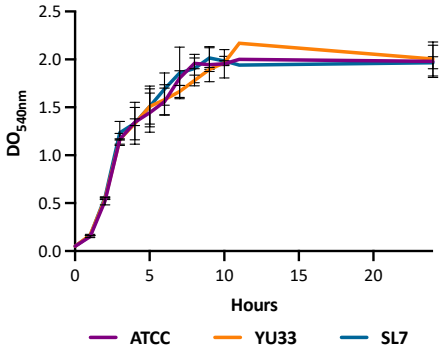


Figure 1. Bacterial growth in MMA

Aims 1a and 1b. The UIMIQ team currently produces STm porins using the ATCC 14028 strain; thus it was used as a standard to compare the new strains being investigated in this project. All strains had similar growth patterns and kinetics when grown in MMA media (Figure 1). Outer membrane proteins from bacterial pellets were extracted through sonication in the presence of Triton X-100 (2) and analysed by SDS-PAGE to check for the presence of OmpC and OmpD (results not shown). Once the expression of both porins was confirmed, master and working cell banks were generated for both STm strains.

Aims 2a and 2b. Porin production was carried out at UIMIQ using the method described by Nikaido (3). Porin purity was assessed by SDS-PAGE (Figure 2) and LPS content was measured using the LAL assay. In total, 84 mg of protein were purified from the STm YU33 strain and the LPS content was 0.12 EU/10μg of porins. On the other hand, for the STm SL7 strain we were able to obtain 57 mg of purified total protein with an LPS content of >100 EU/10μg of porins. Purified products were sent for LC-MS analysis, in order to characterise the protein products contained in the final formulation. This analysis showed that the main products present in porins purified from our reference strain (ATCC 14028) as well as the novel strains (YU33 and SL7) are OmpC and OmpD (Table 1). In addition, the final product obtained from STm YU33 strain contained 2 uncharacterised protein products, with estimated molecular weights of 21.4 and 6.7 kDa. The 21.4 kDa product corresponds to a protein from *Salmonella* phage SPFM7, while no relevant information was found for the 6.7 kDa product. Porins from the STm SL7 strain also contained an uncharacterised protein with an estimated molecular weight of 36.1 kDa. No further information was found regarding this product in public databases such as UniProt. It is important to highlight that even though additional proteins were found in the final products, the most abundant proteins were OmpC and OmpD.

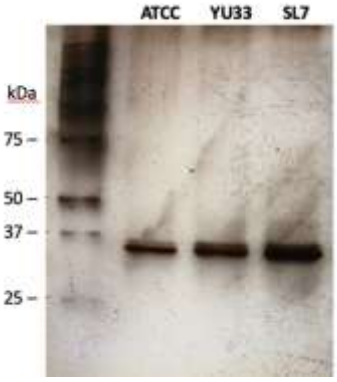


Figure 2. SDS- PAGE analysis of STm purified porins

Table 1. Protein identification LC-MS

Strain	Protein	MW (Da)
ATCC 14028	OmpC	41241
	OmpD	39713
YU33	OmpC	41213
	OmpD	39713
	Uncharacterised protein	21442
	Uncharacterised protein	6786
SL7	OmpC	41255
	OmpD	39625
	Uncharacterised protein	36107

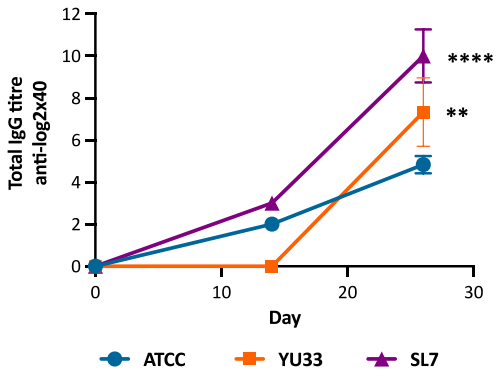


Figure 3. IgG antibody titres in mice

Aim 3a. Female BALB/c mice (n=6) were immunised on days 0 and 14 via IP injection with 10 µg of purified porins from our reference strain (ATCC 14028) and the novel STm strains (YU33 and SL7). Serum samples were collected at days 0, 14 and 26 and anti-STm porins IgG titres were determined by ELISA. Porins from the ATCC strain were used as coating antigen for comparison purposes. Porins purified from the novel strains were immunogenic. IgG titres of sera from mice immunized with our test porins was higher than those found in control (ATCC) sera. However, functional assays are still pending to determine the protective efficacy of the immune responses elicited by these formulations. We also need to perform *Aim 3b*.

A visit by a Mexican scientist to the Cunningham lab was planned as part of this proposal. This visit took place in the second half of June, with a young scientist (María García-Valeriano) spending 2 weeks in the UK receiving training to study B and T cell responses by ELISPOT. With this training, María will be able to set up the technique at UIMIQ and train other scientists. In addition, María had the opportunity to discuss other techniques critical for the continuation of this project, such as the bactericidal antibody assays.

Aim 1c. In parallel with the work previously described the team at the Instituto de Biotecnología UNAM (IBt carried out genome sequencing of both strains and continues to do the curation and analyses of the obtained sequences. Nevertheless, significant progress has been made in identifying the presence/absence of genes associated with antibiotic resistance (Figure 4), as well as plasmid replicons and phage sequences present in the STm strains, as well as various other laboratory phenotypes (results not shown). With this information, the team has also decided to use the 33676 strain to carry out proof of concept functional assays, such as bactericidal assays and challenge experiments against a relevant MDR strain (*Aim 3c*).

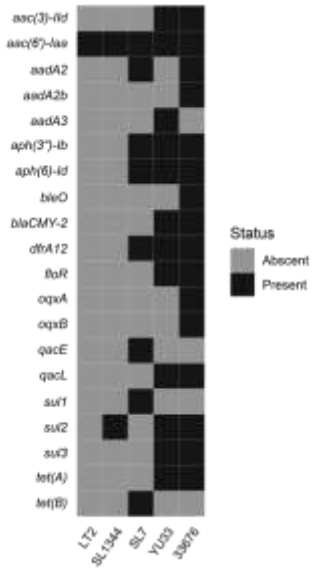


Figure 4. Antibiotic resistance genes

References

1. Wiesner, M., et al. Association of virulence plasmid and antibiotic resistance determinants with chromosomal multilocus genotypes in Mexican Salmonella enterica serovar Typhimurium strains. BMC Microbiol 9, 131 (2009).
2. Puente JL, et al. The Salmonella ompC gene: structure and use as a carrier for heterologous sequences. Gene 156(1):1-9 (1995).
3. Nikaido H (1983) Proteins forming large channels from bacterial and mitochondrial outer membranes: Porins and phage lambda receptor protein. in Methods in Enzymology, pp 85-100.

4. Expenditure against budget:

4.1 Please provide details for expenditure against funding awarded for each applicant by completing the 'Final Expenditure Statement' Excel spreadsheet. You should also complete the summary table below using the appropriate figures from the Excel spreadsheet. BactiVac does not require receipts to be submitted with invoices raised to evidence expenditure, but these must be kept by the host institution as they may be required for future audits. In the event that the Lead Applicant completes the funded project without

spending the full funding awarded, the Lead Applicant Institution must repay all unspent sums and/or submit a final invoice to reflect the actual total expenditure.

Applicant	Award (100% fEC)	Actual (100% fEC)	Variance (100% fEC)
Lead	**£43,000.00**	£40393.17	£2606.83
Co-applicant 1	£0	£0	£0
Co-applicant 2	**£6,000.00**	£4,668.45	£1,331.55
Co-applicant 3	£0	£0	£0
Total (100% fEC)	**£49,000.00**	**£45,061.62**	**£3,938.**

4.2 Expenditure against budget:
Please indicate the status for expenditure against budget:

***AMBER:** Expenditure up to 20% above/below plan for the project

4.3 Please provide a summary of expenditure status against budget (approx. 250 words).

We had planned to pay for WGS service; however, this was done by the team at IBt with funds from another project. Thus, the funds budgeted for this particular aim were not spent.

In addition, items such as the visit to the UK and the LC-MS analyses were significantly cheaper than initially expected, therefore money was not entirely spent.

We had some variation in various items, mainly due to changes in the exchange rate between the GBP and the MXN, between the beginning and the end of the project.

4.4 If status for expenditure against budget is not GREEN, please provide details of how any overspends will be met or confirmation that any unspent funds are reflected in the final invoice and/or will be returned to BactiVac (approx. 250 words).

We can confirm that unspent funds will be reflected in the final invoice.

5. Matched funding/in kind contributions – please include details of funding leveraged (if applicable) or in kind contributions from other sources that are supporting this project.

This information will be submitted in reports to the funders of the BactiVac Network to evidence the level of matched funding/in-kind contributions provided by applicants and their host organisations and is a key metric of catalyst funding performance. Please complete the table below as fully as possible so that we can accurately report on the additional funds that have been leveraged by this catalyst award.

Type	Description	No. hrs per wk	Actual/estimated value	Comments
Matched funding	Tania Rivera-Hernandez	10	£2,000	Lead applicant supervision project – costs covered by CONACYT
	Constantino Lopez-Macias	1	£400	Supervision project – costs covered by IMSS
	Edmundo Calva	1	£400	Supervision project – costs covered by Instituto de Biotecnología UNAM
	Adam Cunningham	0.2	£100	Supervision project – costs covered by University of Birmingham
In kind contribution	IMSS		£5000	Core facility (flow cytometry, confocal microscopy, GLP pilot plant and porin production

				equipment maintenance) project e.g., access to reagents, technical or other expertise, etc.
In kind contribution	UNAM		£500	STm Strains
Matched funding	Circulating water bath		£1,120	We were able to purchase this equipment with funds from another project (funds provided by CONACYT)
Matched funding	Genome sequencing (UNAM)		£1,000	The team at IBt were able to cover sequencing costs with funds provided by UNAM
Other				
Total		**12.2 hrs**	**£10520**	

6. Collaborations: please detail all collaborations for this catalyst project award

This information will be submitted in reports to the funders of the BactiVac Network to evidence how the Network has supported the establishment of new collaborations between the lead applicant and co-applicants from different sectors/research disciplines as well as highlighting the extent of the collaborations between them.

Type	**Description**	**[1] Sector**	**[2] Discipline**	**[3] New/existing**	**[4] Extent of collaboration**
Co-applicant	Constantino Lopez-Macias	Academic	Immunology	Existing	High
Co-applicant	Edmundo Calva	Academic	Bacteriology	New	Medium
Co-applicant	Adam Cunningham	Academic	Immunology	New	Medium

[1] **Sector -** please state if this is academic, industry, policy and/or LMIC
[2] **Discipline -** please state research discipline/area of expertise (e.g. immunology, bacteriology, epidemiology, vaccine manufacture, etc.)
[3] **New/existing -** please state if this collaboration is new or existing prior to the catalyst project funding award
[4] **Extent of collaboration -** please indicate the extent of the collaboration with this partner as 'E1' (low), 'E2' (medium) or 'E3' (high)

7. Dissemination of research findings: please provide details of any ways in which you have communicated and/or disseminated your catalyst project research findings to date

7.1 Peer-reviewed publications (include articles in press and journal impact factors):

None

7.2 Non peer-reviewed publications (including expert opinions, book chapters etc):

None

7.3 Workshops or conference presentations:

None

7.4 Public engagement activities and media coverage:

None

8. Challenges faced: Please detail any issues experienced during the set up and delivery of this catalyst project and how these were overcome (approx. 250 words).

Challenges were mainly administrative, which were already mentioned in the first project report. The work with FUNSALUD as our administrative partner was efficient and helped significantly to deliver the proposed project.

9. Future plans and sustainability (approx. 500 words):

9.1 There is an expectation that recipients of catalyst project funding will secure further substantive follow-on funding to continue to progress the research that the initial project funding catalysed. This is another key metric to evidence the success that our catalyst project funding scheme has supported to deliver.

Please indicate the current status for future plans and sustainability for your project:

***RED:** No plan in place to secure follow-on funding

Despite not having a clear plan, we are currently looking for funding opportunities, national and international (e.g. IMSS, CONACyT, ESCMID)

9.2 If an application has been submitted and/or awarded, please provide details (include funder, scheme, applicant details, value, duration and planned start date - approx. 250 words).

N/A

9.3 If an application has not been submitted, please highlight the funding bodies that will be targeted and likely timescales for application submission:

Funder:	**Proposed timeline to apply:**
CONACYT	**Depending on calls for applications**

9.4 If status for future plans and sustainability is not GREEN, please detail what actions are being taken to address this and to progress the research (approx. 250 words).

We are currently searching for local and international sources of funding. This is something that we as researchers are constantly doing, and we are prepared to submit a proposal once opportunities are identified.

10. In what ways might this project benefit society and/or the economy? :

Please provide a comment: (approx. 100 words).

The project is already having an impact through the training of young scientists. As we continue to work in this project, we could have a promising vaccine candidate against NTS that could have an effect in disease prevention, but also could boost vaccine development and manufacture in Mexico.

11. Commercialisation **Confidential: Please note that BactiVac will liaise directly with you regarding the status of Intellectual Property if further details are required, and prior to updating any information on ResearchFish or providing details to our funders.** **We are keen to learn of any patents/patent applications, whether published, granted, allowed to lapse or rejected. We also want to capture details of any spin out companies and/or other commercial outputs from your project.**	
11.1 Have you submitted a patent application?	☐ Yes ☑ No If **yes**, *please complete section **11.2*** If **no**, *please complete section **11.3***
11.2 If you have submitted an application:	Date submitted: …… Patent application number: …… ☐ Yes **Successful**: Date awarded …… ☐ Yes **Unsuccessful:** Outcome date: …… ☐ Yes **Outcome pending**, date expected…….. *Now please complete section **11.4***
11.3 Do you plan to submit a patent application?	☐ Yes ☑ No If yes please provide expected submission date: ……
11.4 Has a spin out company been created?	☐ Yes ☐ Plans for future ☑ Not applicable
11.5 If you have any further information regarding commercialisation please provide details here:	This subject will be further discussed by the team in the near future.

12. Technology readiness level (TRL) **We are keen to identify the TRL of your project and to capture if the catalyst project funding awarded has supported increasing your project's TRL.** **The definitions for each TRL are provided in Appendix 1 below.**	
12.1 TRL provided for Start of Project	**TRL-3**

12.2 TRL provided in Interim Project Report	**TRL-3**
12.3 Please indicate the current TRL of your project (this should be stated as TRL-1 to 9, with reference to the definition provided in Appendix 1). ***Please only provide one whole number.***	**TRL-3**

Appendix 1 – Definitions of technology readiness levels (TRLs)

Stage Technology	Readiness Level	Definition
Ideation	TRL-1	Need identified, Basic principles observed and reported (Scientific research begins to be translated into applied research and development)
Proof of Principle	TRL-2	Epidemiologic study, Research ideas developed, hypothesis formulated and protocols developed (Initial level in vitro studies, Development of working Cell Bank)
Proof of Concept demonstrated	TRL-3	Hypothesis testing and initial proof of concept (PoC) is demonstrated in a limited number of in vitro models and limited in vivo efficacy studies (Formulation development, complete in-house testing of the formulated vaccine by in vitro model studies and In vivo efficacy in limited number of animals)
Proof of concept established	TRL-4	Efficacy & safety of vaccine candidate is demonstrated in a defined animal model (Results of serological studies in different animals at preliminary level and efficacy in defined in vivo model, Manufacturing and QC release of vaccine for Studies, Scale up Development)
Early stage validation	TRL-5	Pre-clinical studies, including GLP efficacy, acute and chronic toxicity, all the studies mandatory for safe exposure to humans such as repeat dose toxicity (RDT) and safety in animal model producing sufficient data for DCGI application for clinical trials
	TRL-6	Material produced in GMP facility of clinical trials. Phase I Clinical trials done and results & safety of the vaccine candidate reviewed by DCGI for approving Phase II Clinical trials
Late stage Validation	TRL-7	Phase II Clinical trials completed and data reviewed by DCGI and Phase III Clinical trial plan approved
Pre-commercialization	TRL-8	Phase III Clinical trials completed successfully. DCGI approves the vaccine and provides commercial manufacturing license for market introduction
Commercialization and post market studies	TRL-9	Commercial launch of the new vaccine, Post marketing studies and surveillance (Phase IV clinical trial)

13. Overall status of project:
13.1 Please indicate the current **overall** status for your project: ***AMBER:** Some issues were experienced which impacted on the ability to deliver the full scope of the project within
13.2 Please provide comments regarding the overall status of your project below (approx. 250 words).

We have some pending data to be generated, however the main objectives of the project have been completed. We will continue on the development of a NTS vaccine candidate and the scaling-up of the production process.

14. Signed – Lead applicant to please sign and date this form before submission. Electronic signatures are acceptable if the report is submitted from the Lead Applicant's e-mail address	
Signed:	
Dated:	30/08/22

Deadline for receipt of Final Project Report: **31 JULY 2022**

Please submit your completed Final Project Report to our BactiVac Admin Team at bactivac@contacts.bham.ac.uk. Please do send any queries about the report to this address. All reports must be submitted in 'Word' format. Reports submitted in any other format (including pdf documents) will not be accepted.

Conditions

The award payment schedule is linked to progress made against planned objectives and milestones. We need to ensure that satisfactory progress was being made on the project and that expenditure against the budget awarded was appropriate and as planned before releasing of the final payment. This also provides the necessary information for onward reporting and for audit purposes.

The Final Project Report must be submitted by the date specified. Failure to complete and submit a report may cause milestone payments to be delayed, and/or BactiVac to refuse to consider further funding requests.

All original peer-reviewed published research papers directly associated with your award must comply with Research Council UK's open access policy – details can be found here: https://www.ukri.org/funding/information-for-award-holders/open-access/.

Any publications, outputs or downstream funding must acknowledge the catalyst funds awarded through the BactiVac Network as follows:

"This work was supported by the Bacterial Vaccines (BactiVac) Network funded by the GCRF Networks in Vaccines Research and Development which was co-funded by the MRC and BBSRC. Additional support was provided by The Department of Health and Social Care as part of the Global AMR Innovation Fund (GAMRIF), a UK aid programme that supports early-stage innovative research in underfunded areas of antimicrobial resistance (AMR) research and development for the benefit of those in low- and middle-income countries (LMICs), who bear the greatest burden of AMR. The views expressed in this publication are those of the author(s) and not necessarily those of the UK Department of Health and Social Care."

What happens next?

The submitted report will undergo an initial check by the BactiVac admin team to ensure that it is appropriately completed, after which they will e-mail you to confirm receipt. The Final Project Report will then be passed on for review and approval by the Directors of the Network. The approval process should be completed within 2 weeks of confirmation of receipt of a report. Once the report is approved, the final scheduled payment for the award will be authorised, subject to receipt of an invoice for the agreed/correct amount as stated in the original award letter. Once approved, copies of this report may be shared with our funders.

If there are any concerns raised by the Directors during the review of the Final Project Report, these will be discussed with you directly.