UNIVERSITI SAINS MALAYSIA
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LAPORAN AKHIR

PRODUCT AND PROCESS VALIDATION FOR COLD CHAIN FREE, LIVE ATTENUATED CHOLERA VACCINE

PENYELIDIK

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Monitoring › **Product and Process Validation for Cold Chain Free, Live Attenuated Cholera Vacc...**

Progress Summary

Project Progress : 100.00%

Budget Used : 9

Human Capital :

Current Outcome

	Number
Activities	10
Publication	0
Exhibition	0
Intellectual Property	1
Product	0

Milestone

No.	Description	Project Completion Contribution	Expected Completion Date	Completed Percentage	Actual Completion Date	Contributed Progress
1	Scale up and optimization of the fed batch fermentation process conditions for high cell density cultivation of VCUSM14P strain at 10 lt fermentor (Bench scale)	10	31/05/2016	100	16/04/16	10.00%
2	Validation of large scale preparation of liquid vaccine formulation and evaluation of its purity, potency and viability by phenotypic and genotypic methods	10	30/06/2016	100	29/05/16	10.00%
3	Scale up and optimization of the fed batch fermentation process conditions for high cell density cultivation at 100 lt fermentor (Pilot scale)	5	31/08/2016	100	15/08/16	5.00%
4	Optimization of down stream processing unit operations	15	31/08/2016	100	26/08/16	15.00%
5	Colonization and protective efficacy of V. cholerae vaccine strain in animal models	15	30/04/2017	100	07/08/2017	15.00%
6	Accelerated storage stability testing	15	31/05/2017	100	14/07/2017	15.00%
7	Limited pre-clinical and toxicological studies of vaccine formulation at GLP Compliant Test Facilities, Malaysia	25	30/09/2017	100	10/08/2017	25.00%
8	Product and process validation of cold chain free, live attenuated cholera vaccine. And Research write up	5	30/11/2017	100	15/01/2018	5.00%
	Overall Progress					100.00%

Research Abstract

A Fed - batch fermentation process was standardized for high cell density cultivation of the attenuated cholera vaccine candidate (VCUSM14P). The fed-batch operation resulted in the highest cell density of 1.86 g cells / lt. The concentrated biomass was used to formulate 480 vials of liquid attenuated vaccine containing (6 x 10^8 CFU / ml). The storage stability of the vaccine formulation was evaluated for its purity, potency and viability by phenotypic and genotypic methods over an extended storage period of 140 days at 25°C ± 2°C and 60% ± 5% relative humidity in the Binder ICH compliant Stability Chamber.

The viability of the vaccine strain (VCUSM14P) in the formulation after 140 days of storage was recorded with 6 x 10^6 CFU /ml which is 2 logs lower as compared to its storage at room temperature (25 ° C ± 2°C) ie; 6 x 10^8 CFU /ml and the reduction of CFUs might be attributed to the high humidity. To ascertain the genetic purity of VCUSM14P culture in the formulation, the PCR was conducted by using two different primer sets. The results indicate that the VCUSM14P strain is intact and comparable to the positive control VCUSM14P (unformulated). The vaccine culture purity was validated by series of biochemical tests that are specific for Vibrio cholerae. Since colonization is critical for elicitation of the immune response, the VCUSM14P formulation was examined for their colonization in the infant mouse model. The colonization ability of formulated VCUSM14 strain was good and recorded with two logs higher than that of unformulated VCUSM14P strain. It effectively colonized and induced a higher titer of antibodies.

In order to assess whether the vaccine formulation is causing reactogenicity, studies were performed in ligated ileal loops of unvaccinated rabbits and analyzed for the fluid accumulation in comparison with wild type and unformulated VCUSM14P. The vaccine formulation was found to be non-reactogenic at doses of 10^5-10^6 in rabbit ileal loop model. The protective efficacy of vaccine formulation was determined by challenging immunized rabbits by the Reversible Intestinal Tie Adult Rabbit Diarrhoea (RITARD) model. Rabbits vaccinated with vaccine formulation or unformulated VCUSM14P survived the challenge and showed no signs of diarrhoea and other symptoms of disease or death up to 5 days of the observation period. Whereas, 100% mortality was observed in unvaccinated (control) rabbits within 18 hours post challenge with wild type. In conclusion, our results validate the cold chain free formulation of VCUSM14P is non-reactogenic and immunogenic in vivo, and protects animals from lethal V. cholerae O139 challenge.

A Patent for "A monovalent vaccine formulation and a method for preparation thereof" was filed on 09/01/2018. Reference: Patent Application No: PI 2018700106 at Intellectual Property Corporation of Malaysia (MyIPO).

Summary of Research Findings

The storage stability of the Live Attenuated Cholera Vaccine (LACV) formulation was evaluated for its purity, potency and viability by phenotypic and genotypic methods over an extended storage period of 140 days at 25°C ± 2°C and 60% ± 5% humidity. The glass test tubes/vials containing the LACV formulation were incubated in the Binder ICH compliant Stability Chamber and monitored regularly on weekly basis.

1) Accelerated stability testing of the Live Attenuated Cholera Vaccine (LACV) formulation at 25 °C ± 2°C and 60% ± 5% humidity:

The viability of the vaccine strain (VCUSM14P) in the formulation after 140 days of storage at 25 °C and 60% humidity was recorded with 6 x 10^6 CFU /ml. The viability of the strain in the formulation was 2 logs lower after 140 days of storage as compared to its storage at room temperature (25 ° C ± 2°C) ie; 6 x 10^8 CFU /ml and the reduction of colonies might be attributed to the high humidity.

2) Genetic purity verification of vaccine candidate in the formulation by Polymerase Chain Reaction (PCR):

To identify the intact VCUSM14P culture in the formulation after 140 days storage at 25 °C and 60% humidity, PCR was performed by using two different primer sets. PCR and gel electrophoresis was conducted as the genetic purity of VCUSM14P culture in the cold chain free formulation. The first PCR reaction conducted was the Mctx reaction using MctxA112MS-F and ctxBCDS-R primer for the detection of the presence of the mutated ctxA gene in the samples to verify that the mutated ctxA gene does not revert back to the toxigenic form. All the samples containing culture in the formulations as well as the positive control which is the VCUSM14P strain from the glycerol stock have shown the band at 700bps region, while no band is observed in the negative control column. This indicates that mutated ctxA gene is present in the culture and it is free from contamination. In the second PCR reaction, KanFse reaction using KanFse-2F and KanFse-R primers were used to detect the