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Stability Characteristics of Freeze-Dried IVRI-CSF-BS Classical Swine Fever Vaccine

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A B S T R A C T

Freeze-drying (lyophilization) of biologically active materials including attenuated live virus vaccine is an well accepted method for ensuring stability and preserving the vaccine potency. As freeze- drying does not ensure complete stability, studies for stability of freeze-dried vaccines after exposure at different temperatures are required. Heat stability of classical swine fever (CSF) virus vaccine is also urgently needed in tropical countries including India where the disease is still endemic. In this study, the thermostability of freeze- dried vials of live attenuated CSF vaccines were investigated at 4℃ and 25℃ for 30 days. This lyophilized vaccine containing combination of sucrose, lactose and lactalbumen hydrolysate gives good heat stability and viral infectivity was reduced by a factor of 0.2 to 1 $Log_{10}TCID_{50}$/mL at the end of freeze drying. In this experiment, we have studied storage stability of lyophilized classical swine fever vaccine produced at Division of Biological Standardization, IVRI using IVRI-CSF-BS vaccine strain which can be stored at -20℃ for more than a year without any loss. Stability at 4℃ and 25℃ may ensure easy transportation and minimize expense related to that.

Introduction

Classical swine fever (CSF) is an OIE notifiable infectious viral disease of domestic and wild pigs. The etiological agent, classical swine fever virus belongs to the genus *Pestivirus* in the family *Flaviviridae* (Becher *et al.,* 2003). Since 1964, a lapinized vaccine virus was in use in India for controlling the disease (Bett *et al.,* 2012). To avoid ethical issues of killing of rabbits, earlier lapinized Weybridge strain was adapted in cell culture and used for vaccine production (Dhar *et al.,* 2008). Alive attenuated cell culture vaccine for CSF (IVRI-CSF-BS) has been developed very recently in India by adapting an indigenous virulent isolate. Although IVRI-CSF-BS has a great advantage of having a very high yield up to the titre of $10^{9.5}$ $TCID_{50}$/mL like any other CSF virus vaccine it is also very much

susceptible to storage conditions at different temperatures. Even exposure to ambient temperature may result in decrease of infectious titer due to loss of virus integrity. To solve the problem of cold chain maintenance and to prevent damage in viral integrity in tropical countries, stable forms of vaccines are essential for storage and transportation (Chen and Kristensen, 2009).

Maintenance of cold chain accounts for 80% of vaccination cost in tropical countries (Zhang *et al.*, 2016). Freeze drying or lyophilization could be an easy solution where addition of stabilizer increases the thermal stability of product (Cardoso *et al.*, 2017). For lyophilization, most common stabilizer formulations include carbohydrates or sugar alcohols (Bovarnick *et al.*, 1950) and disaccharides are reported to be more effective than monosaccharides (Adams, 1996) and thus freeze drying of CSFV is not an exception.

Freeze drying protocol influence crystallization of additives which can reduce stabilizing effects of amorphous stabilizer producing vastly different freeze-dried products, both in appearance and viability of the micro-organism. An well optimized lyophilization protocol will provide efficient drying while maintaining the integrity of the virus (Morgan *et al.*, 2006).

Our aim was to determine viral infectivity up to 1month at +4°C or +25°C in freeze dried vials, conditions mimicking transportation without cold chain preservation. The performance of the protocols is evaluated by measurement of the infectivity of the resulting freeze-dried material using a representative panel of viral specimens.

Materials and Methods

Cells and viruses

Porcine kidney-15 (PK-15) cells (ATCC, USA), free from mycoplasma and pestivirus contamination, maintained at Division of Biological Standardization, ICAR-IVRI, Izatnagar were used for the study. The cells were cultured in Eagle's Minimal Essential Medium (HiMedia, India) supplemented with 10% pestivirus free foetal bovine serum (Invitrogen, USA) and 100X antibiotic and antimycotic solution (HiMedia, India) and kept at 37°C under 5 % CO_2 tension and 100% relative humidity. Porcine kidney (PK)-15 cell culture adapted classical swine fever virus (CSFV) developed from indigenous isolate (IVRI-CSF-BS) vaccine strain was used in this study.

Preparation of virus for freeze drying

PK-15 cells were infected at a multiplicity of infection (moi) of 0.01 by adsorption of CSF virus and incubated at 37℃, 5%CO_2. Virus was harvested after 66 hours of incubation after two cycles of freezing at -20℃ and thawing. Stabilizer was formulated by adding combination of sugars and dissolving in HBSS. pH was adjusted and filtered through 0.1 micron syringe filter.

Lyophilization of virus

Two (2x) stabilizer was prepared using 7% Sucrose, 5% Lactalbumen hydrolysate (LAH) and 3% Lactose and pH of the stabilizer was set to 7.4. Then equal volume of titrated CSFV wet virus was added and 1ml was dispensed into each 2 ml vials after mixing well.

Vials were lyophilized in Labconco freeze drier and following freeze drying condition was optimized where the temperature ramping rate was 1°C/minute during thermal cycling.

Storage and thermo-stability of freeze-dried virus

After freeze drying vials were stored at Separate freeze-dried virus vials were stored at - 20°C. Vials were taken out and exposed to different storage temperatures (+4°C and +25°C) in a light-protected environment and then vials (2 for each condition) were taken out after 10 days, 20 days and 30 days for both the conditions. Each vial was reconstituted in 1 ml of serum free media and used for titration.

Virus titration and comparison

Virus infectivity was quantified by estimating the 50% tissue culture infectivity dose ($TCID_{50}$). Being a non-CPE virus, titration of CSF virus was done in cover slip culture in 24-well cell culture plate. Prior sterilized cover-slips were transferred to 24-well tissue culture plate using sterile forceps.

Cells were sub-cultured and transferred to the plate at a seeding density of $2x10^5$ cells per well (400 µL of cell stock of 5 x 10^5/mL). The plates were incubated at 37°Cwith 5% CO_2 until the virus dilutions were prepared. Ten fold serial dilutions of vaccine virus were prepared and 100 µL of each virus dilution was transferred to the respective well keeping cell control and gently swirled. Plate was incubated at 37°C and 5% CO2 for 66 hrs and indirect FAT was performed to detect viral protein in infected cells (Dhar *et al.*, 2022).

Briefly, after completion of incubation cells were fixed. Then fixed cells were washed and quenched to wane off auto fluorescence due to used fixative. After that addition of detergent acted as porogen helping Mab to reach viral protein inside cells.

Each cover slip was then incubated with diluted Mab in blocking buffer and keeping at room temperature inside moist chamber. After washing cover slips were incubated with anti-mouse FITC conjugate. Finally stained cells were mounted on slides after washing to remove unbound conjugate.

Results and Discussion

Freeze drying and freeze drying loss

The presence of virus in the cell cytoplasm can be confirmed by presence of bright apple green fluorescence while nucleus and uninfected cells appear red (Fig.1). After freeze drying, FAT titer of the freeze dried vials were estimated and it was found that there was loss of minimum of $0.2Log_{10}TCID_{50}$/mL and maximum of 1 $Log_{10}TCID_{50}$/mL.

Reproducibility of lyophilization and batch to batch variation

Lyophilization is a turn based method and hence batch to batch variation may occur. The observed fluctuation was negligible. Hence, over all freeze drying consistency was acceptable.

Virus titration after exposure

Freeze dried vials stored at -20℃ was stable up to 1.5 years and there was 1 Log_{10} titer loss in another next six months (Fig.2). The comparison of the different freeze-dried batches (Fig. 3) stored at elevated temperatures were summarized in the following table -

Assessment of coefficient of variation

Coefficient of variation was assessed using Graph Pad Prism Version 7 (Fig. 4) and summarized. Freeze dried vials exposed to 4℃ for 1 month period showed 8.25% coefficient of variation whereas at 25℃ it showed 12.56% coefficient of variation.

The most common way used for vaccine stability enhancement is to convert them into a dry, solid and stable form by lyophilization (Adams *et al.*, 2015)For live vaccines, stability is equivalent to the preservation of the infectious titres and for inactivated and subunit vaccines, the preservation of the antigenic structure and the correct steric presentation of the relevant epitopes. In general, the following factors may have a negative effect on stability: temperature, pH outside the physiological limits, organic solvents, repeated freezing and thawing, inactivating agents, and light. Approaches to stabilization of most vaccines are based on the elimination or neutralization of the negative factors. Storage in the refrigerator for long periods is harmless for certain very stable vaccines such as influenza, rubella, Newcastle disease virus, Marek's and other vaccines, such as IBV, show a significant loss in titer after 2 years. But classical swine fever virus is not very stable and infectious titre drops in presence of light and higher temperature.

Table.1

Step	Temperature	Time (hours)	Vacuum
1	-45	4	No vacuum
2	-45	4	Vacuum
3	-30	2	Vacuum
4	-30	2	Vacuum
5	-25	2	Vacuum
6	-20	2	Vacuum
7	-10	2	Vacuum
8	-5	2	Vacuum
9	+10	2	Vacuum
10	+25	2	Vacuum

Table.2

Temperature (℃)	Days	Titre
4	10	7.5
	20	6.5
	30	6.5
25	10	6.5
	20	6.5
	30	5.5

Fig.1 Photomicrograph of FAT stained cells. (A) Bright green apple fluorescence visible in the infected cell cytoplasm (B) Red stained control or uninfected cells

A B

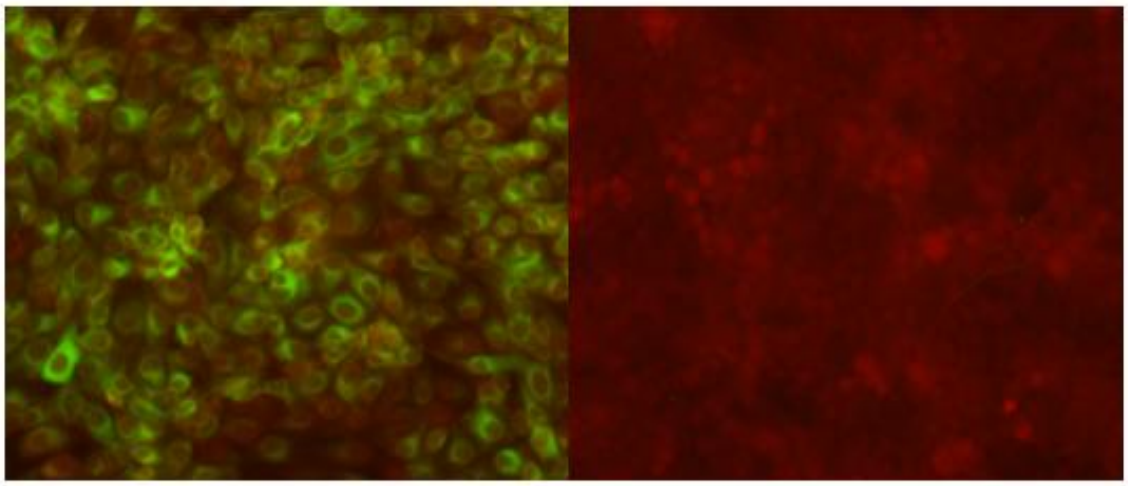


Fig.2 Storage stability study of freeze dried vials at -20°C. Freeze dried vials were stored at -20°C and titer was determined by FAT and plotted.

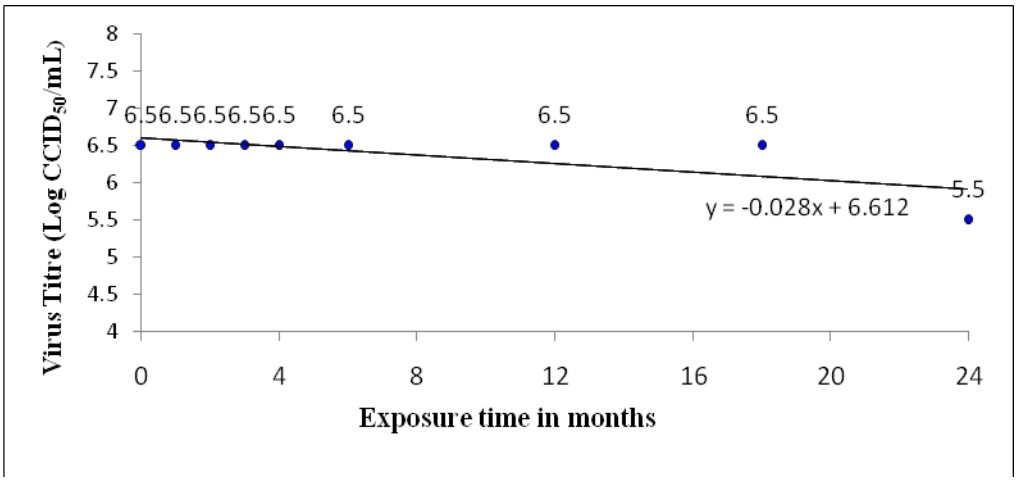


Fig.3 Storage loss of freeze dried vials stored at (A) 4°C and (B) 25°C over a period of one month

A……B

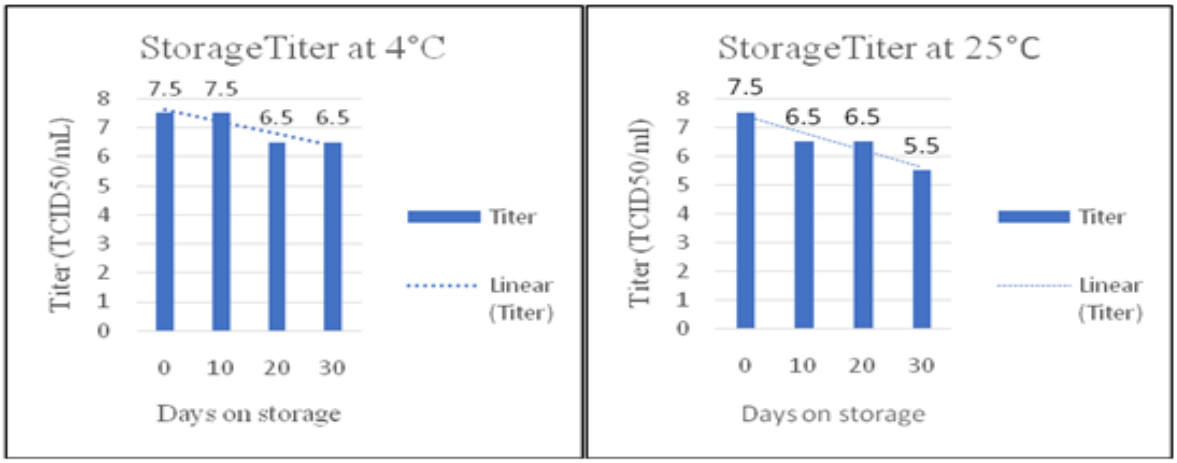
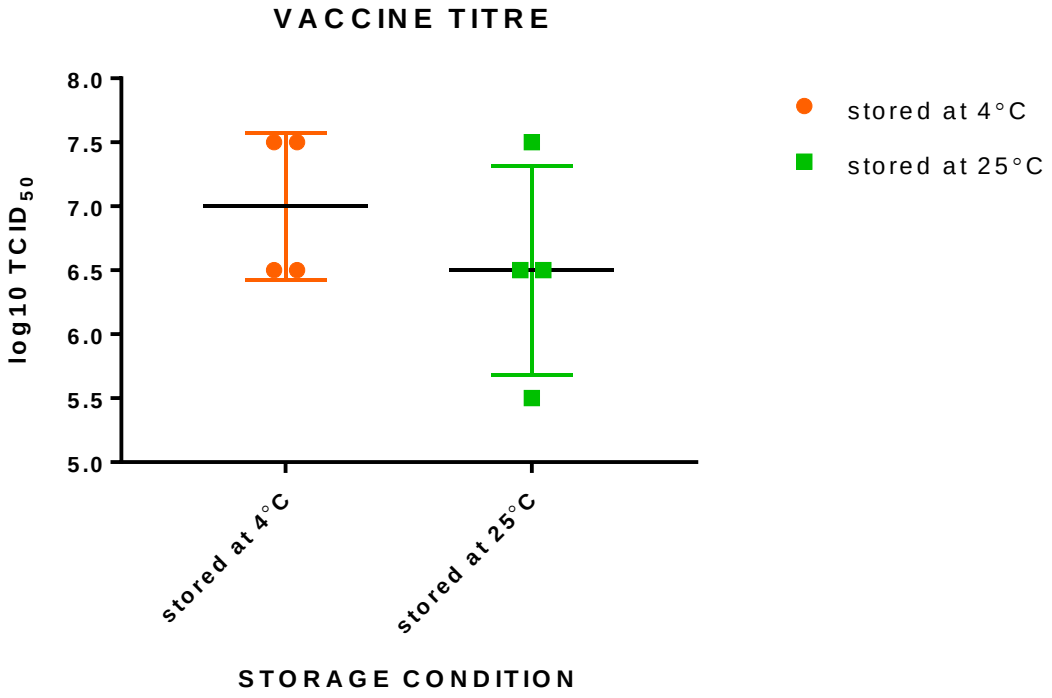


Fig.4 Assessment of coefficient of variation of freeze dried vaccine vials stored at 4°C and 25°C



In general the loss during the accelerated stability test is higher than the loss after 24 months storage in the refrigerator. Sucrose stabilizes the native state of globular proteins against both chemical denaturants and temperature (Graziano, 2012). Another similar study, where stabilizer containing trehalose, glycine, thiourea and PBS showed 0.5 Log_{10} reduction of viral activity during storage at 2-8 ℃ over the period of 24 months and 1Log_{10} loss after storage for six months at 25℃ (Zuo *et al.*, 2020).

Transport of vaccine can be done at 4 ℃or 25 ℃as transporting vaccine vials by keeping at -20℃or below temperature accounts huge cost to maintain that temperature. Adopting this vaccine delivery in the field level can be assured as there is not much titre loss or dosage can be calculated accordingly keeping the potency and efficacy of the vaccine uncompromising.

Conflict of interest

Authors declare no conflict of interest

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References

Adams, G. D., and Ramsay, J. R.1996. Optimizing the lyophilization cycle and the consequences of collapse on the pharmaceutical acceptability of Erwinia L-asparaginase. Journal of Pharmaceutical *S*ciences85(12): 1301-1305.

Adams, G. D., Cook, I., and Ward, K. R. 2015. The principles of freeze-drying. In Cryopreservation and freeze-drying protocols (pp. 121-143). Springer, New York, NY.

Becher, P., Ramirez, R. A., Orlich, M., Rosales, S. C., König, M., Schweizer, M., Stalder, H., Schirrmeier, H., and Thiel, H. J. 2003.

Genetic and antigenic characterization of novel pestivirus genotypes: implications for classification. Virology311(1): 96-104.

Bett, B., Deka, R., Padmakumar, V., and Rajasekhar M. 2012. Classical swine fever in northeast India: prevention and control measures, ILRI Policy Brief, 1-4.

Bovarnick, M. R., Miller, J. C., and Snyder, J. C. 1950. The influence of certain salts, amino acids, sugars, and proteins on the stability of rickettsiae. Journal of Bacteriology 59(4): 509-522.

Cardoso, F. M. C., and Petrovajová, D. Horňáková, T. 2017. Viral vaccine stabilizers: status and trends. Acta Virologica, 61(3): 231-239.

Chen, D., and Kristensen, D. 2009. Opportunities and challenges of developing thermostable vaccines. Expert Review of Vaccines, 8(5): 547-557.

Dhar, P., Das, S. C., Manu, M., Mahapatra, C. S. Lateef, S. K. 2022. Development and validation of an in vitro titrimetric method for determination of classical swine fever viruses in PK-15 cells. Journal of Immunological Methods113321.

Dhar, P., Rai, A. Verma, R. 2008. Adaptation of classical swine fever virus (lapinized strain) in PK-15 cells and confirmation by reverse transcription-polymerase chain reaction (RT-PCR) and fluorescent antibody technique (FAT). Indian Journal of Animal Sciences *(*India*)*.

Graziano, G., 2012. How does sucrose stabilize the native state of globular proteins?. International Journal of Biological Macromolecules, 50(1): 230-235.

Morgan, C. A., Herman, N., White, P. A., and Vesey, G., 2006. Preservation of micro-organisms by drying; a review. Journal of Microbiological Methods, 66(2): 183-193.

Zhang, J., Pritchard, E., Hu, X., Valentin, T., Panilaitis, B., Omenetto, F. G., and Kaplan, D. L., 2016. Erratum: Stabilization of vaccines and antibiotics in silk and eliminating the cold chain. Proceedings of the National Academy of Sciences of the United States of America 113(26): p.E3810.

Zuo, X. X., Zhao, Y. H., Zhou, M. X., Deng, B. H., Hu, L. G., Lv, F., Lu, Y., and Hou, J. B. 2020. Live vaccine preserved at room temperature: Preparation and characterization of a freeze-dried classical swine fever virus vaccine. Vaccine38(52): 8371-8378.

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