

Protective Efficacy of Lyophilized Vesicular Stomatitis Virus–Based Vaccines in Animal Model

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We evaluated the *in vitro* effects of lyophilization for 2 vesicular stomatitis virus–based vaccines by using 3 stabilizing formulations and demonstrated protective immunity of lyophilized/reconstituted vaccine in guinea pigs. Lyophilization increased stability of the vaccines, but specific vesicular stomatitis virus–based vaccines will each require extensive analysis to optimize stabilizing formulations.

Live recombinant vesicular stomatitis virus (VSV) expressing the Ebola virus (EBOV) glycoprotein (VSVΔG/EBOVGP) was evaluated during 2014–2015 as a vaccine to limit the effects of EBOV disease (1). Because of the success and safety of the EBOV vaccine, similar VSV-based vaccines have been proposed for Sudan and Marburg viruses and for other etiologic agents of viral hemorrhagic fever diseases, such as Lassa virus (LASV) (2).

Cold chain maintenance for distributing and storing VSV-based vaccines is a logistical challenge, especially when ultralow temperatures (–60°C to –80°C) are required. The challenge is greater in rural areas, particularly in developing countries, where infrastructure and transport systems are often deficient. We evaluated the effects of lyophilization on the *in vitro* recoverability and *in vivo* protective efficacy of VSV-based vaccines.

We conducted animal studies in accordance with the Canadian Council of Animal Care guidelines; studies received approval from the Canadian Science Centre for Human and Animal Health's institutional Animal Care and Use Committee. We performed work involving infectious Lassa virus

in a Biosafety Level 4 laboratory within the Public Health Agency of Canada. When required, we inactivated materials for subsequent analysis according to approved procedures.

The Study

We conducted propagation and titration (50% tissue culture infectious dose [TCID₅₀]) of VSVΔG/EBOVGP and VSV-based LASV (VSVΔG/LASVGPC) vaccines by using Vero E6 cells as previously described (3). We evaluated 4 excipients as stabilizers: 2.5% lactalbumin hydrolysate (L), 5% sucrose (S), 2.5% trehalose (T), and 0.25% gelatin (G). We prepared 2× concentrations of each solution initially in Hanks' balanced salt solution and then evaluated 3 combinations (LS, LST, or LSTG) (4,5). The control formulation for lyophilization was Dulbecco modified Eagle medium (DMEM) without additives. We mixed each excipient combination 1:1 with VSVΔG/EBOVGP (stock titer 1.26×10^7 TCID₅₀/mL) or VSVΔG/LASVGPC (2.83×10^7 TCID₅₀/mL) and dispensed 200 μL of the mixture into 4 mL sterile glass vials (Electron Microscopy Sciences, <https://www.emsdiasum.com>). We lyophilized the vaccine mixtures by using an automated FreeZone Triad Benchtop Freeze Dryer (Labconco, <https://www.labconco.com>) according to the manufacturer's specifications (Appendix Table, <https://wwwnc.cdc.gov/EID/article/30/5/23-1248-App1.pdf>).

We stored the vials at 4°C, 21°C, or 37°C for 1, 7, 30, and 90 days after lyophilization. At those time points, we reconstituted each vaccine/stabilizer combination in triplicate in 200 μL of 0.85% saline for 1 hour at room temperature by using gentle agitation. We then prepared 10-fold serial dilutions in DMEM and determined virus titers by using standard TCID₅₀ methodologies, as previously described (3). Titrations of formulations conducted immediately before lyophilization indicated that the addition of stabilizers had

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Table 1. Infectious titers of lyophilized vaccines after 90 day storage at different temperatures in study of protective efficacy of lyophilized vesicular stomatitis virus–based vaccines in animal model*

Vaccine	Lyophilization medium			
	DMEM	DMEM + LS	DMEM + LS + T	DMEM + LS + T + G
VSVΔG/LASVGPC				
4°C	6.36 (5.49–7.23)	0.25 (–0.62 to 1.12)	1.25 (0.38–2.12)	0.88 (0.005–1.75)
21°C	NC	3.00 (2.06–3.94)	2.88 (1.94–3.82)	1.50 (0.56–2.44)
37°C	NC	NC	NC	NC
VSVΔG/EBOVGP				
4°C	1.57 (0.83–2.31)	0.43 (–0.32 to 1.17)	1.13 (0.39–1.87)	1.55 (0.81–2.29)
21°C	4.50 (4.22–4.78)	2.00 (1.72–2.28)	6.63 (6.35–6.91)	6.25 (5.97–6.53)
37°C	4.38 (4.20–4.56)	4.50 (4.32–4.68)	4.38 (4.20–4.56)	3.75 (3.57–3.93)

*Values are no. (95% CI), representing the log₁₀ decreases in infectious titers (median 50% tissue culture infectious dose) for vaccines that were lyophilized in the presence of various stabilizers, stored at the indicated temperatures for 90 days, and then reconstituted. Comparisons are between 1 and 90 days after lyophilization. DMEM, Dulbecco modified Eagle medium; G, gelatin; LS, lactalbumin hydrolysate and sucrose; NC, not calculated; T, trehalose; VSVΔG/EBOVGP, vesicular stomatitis virus expressing Ebola virus glycoprotein; VSVΔG/LASVGPC, vesicular stomatitis virus expressing Lassa virus glycoprotein.

no adverse effect on vaccine recovery. We performed mean difference calculations to compare TCID₅₀ data collected on day 1 and day 90 after lyophilization by using 2-way analysis of variance in GraphPad Prism 10 (Graphpad, <https://www.graphpad.com>). For the

VSVΔG/LASVGPC vaccine, the 3 stabilizer formulations provided consistent levels of virus recovery; we observed little variation after lyophilization/reconstitution and only minor decreases in titers when stored at 4°C (Table 1; Figure 1). The VSVΔG/LASVGPC

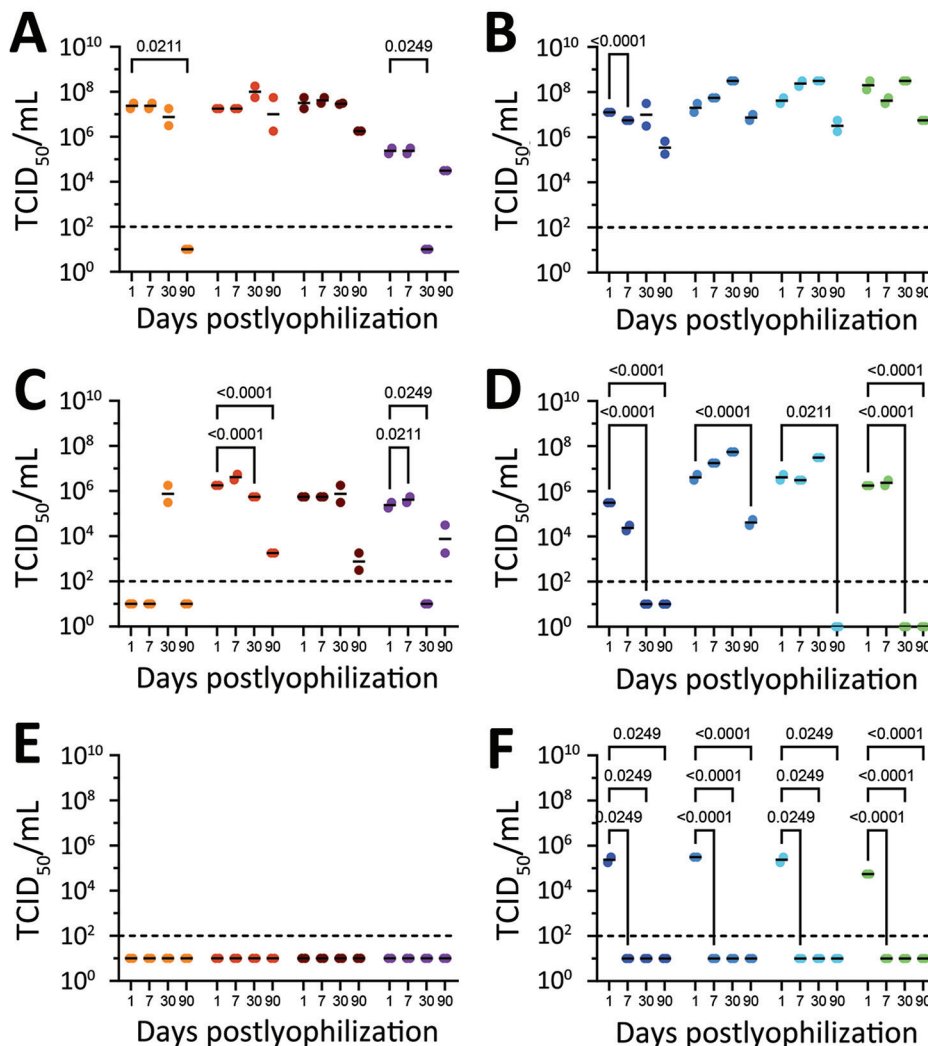


Figure 1. Vaccine recovery after lyophilization in study of protective efficacy of lyophilized vesicular stomatitis virus–based vaccines in animal model. A) VSVΔG/LASVGPC vaccine stored at 4°C; B) VSVΔG/EBOVGP vaccine stored at 4°C; C) VSVΔG/LASVGPC vaccine stored at 21°C; D) VSVΔG/EBOVGP vaccine stored at 21°C; E) VSVΔG/LASVGPC vaccine stored at 21°C; F) VSVΔG/EBOVGP vaccine stored at 21°C. VSVΔG/LASVGPC or VSVΔG/EBOVGP vaccines were lyophilized in DMEM containing no excipients or containing combinations of 5% lactalbumin hydrolysate, 10% sucrose, 5% trehalose, or 0.5% gelatin and stored at different temperatures. At the specified time points, vaccines were resuspended in triplicate in normal saline, titered by using standard tissue culture techniques, and the median TCID₅₀ was calculated for each. p values are indicated above brackets. Errors bars are SDs. DMEM, Dulbecco modified Eagle medium; G, gelatin; LS, lactalbumin hydrolysate and sucrose; NC, not calculated; T, trehalose; TCID₅₀, 50% tissue culture infectious dose; VSV-Lassa, vesicular stomatitis virus expressing Lassa virus glycoprotein; VSV-Zebov, vesicular stomatitis virus expressing Ebola virus glycoprotein.

construct was stable for ≥ 90 days. By comparison, the vaccine was not recoverable after >30 days when stored at 4°C without stabilizers (DMEM only). We observed similar patterns of stability for the VSV ΔG /LASVGPC vaccine when storage temperatures were increased; albeit, even with the addition of stabilizers, vaccine recovery was immediately impaired by $>1 \log_{10}$ when stored at room temperature (21°C), and no recoverable vaccine was observed when formulations were stored at 37°C . The recovery trends for stabilizer formulations and storage temperature were similar for VSV ΔG /EBOVGP and VSV ΔG /LASVGPC. However, in general, the VSV ΔG /EBOVGP vaccine was more stable than the VSV ΔG /LASVGPC vaccine even without stabilizing agents or when stored at increased temperatures (Table 1).

The protective efficacy of VSV ΔG /EBOVGP and VSV ΔG /LASVGPC vaccines against lethal homologous virus challenge is well established (6). To further evaluate lyophilized VSV formulations, we immunized groups of 10 Hartley guinea pigs 1 time with 1×10^6 PFU of either VSV ΔG /LASVGPC or lyophilized/reconstituted VSV ΔG /LASVGPC (Ly-VSV ΔG /LASVGPC) or lyophilized/reconstituted VSV ΔG /EBOVGP (Ly-VSV ΔG /EBOVGP) via intraperitoneal injection as previously described (7). According to in vitro assessments, the lyophilized vaccines contained the LST stabilizer formulation and were stored after

lyophilization for 1 week at 4°C . We collected a blood sample from each of the 30 animals at 28 days postimmunization, after which we challenged them with a previously determined lethal dose (10^4 TCID $_{50}$) or $10\times$ the 50% lethal dose of guinea pig-adapted LASV Josiah strain via intraperitoneal inoculation (8). We monitored 6 animals per group for disease progression and survival; we euthanized the remaining 4 animals per group on postinfection day 13 to analyze virus titers in tissue samples. The first signs of infection developed on postinfection day 8; increased body temperatures near 40°C occurred in most animals (Figure 2, panel A). Body temperatures in animals immunized with VSV ΔG /LASVGPC or Ly-VSV ΔG /LASVGPC returned to normal within 2–3 days, whereas body temperatures in animals that received Ly-VSV ΔG /EBOVGP remained elevated at 40°C – 41°C until death of those animals, which occurred 14–16 days postinfection. We observed weight loss $>12\%$ only in Ly-VSV ΔG /EBOVGP immunized animals (control group); consistent weight losses occurred during 8–10 days postinfection (Figure 2, panel B). One animal immunized with VSV ΔG /LASVGPC experienced an abrupt drop in body weight requiring humane euthanasia on day 13 postinfection. Overall, 100% (6/6) of animals immunized with Ly-VSV ΔG /LASVGPC and 83.3% (5/6) immunized with VSV ΔG /LASVGPC survived the LASV challenge compared with 16.6% (1/6)

Figure 2. Protective efficacy of lyophilized vesicular stomatitis virus–based vaccines in guinea pig model. A) Body temperatures; B) weight changes; C) survival; D) virus titrations in different tissues. Groups of 10 Hartley guinea pigs each were immunized with VSV ΔG /LASVGPC vaccine or lyophilized/reconstituted Ly-VSV ΔG /LASVGPC or Ly-VSV ΔG /EBOVGP. Ly-VSV ΔG /EBOVGP was used as the sham-vaccinated inoculum control group. Animals were challenged 28 days after immunization with a lethal dose of guinea pig–adapted Lassa virus Josiah strain. Disease progression was monitored in 6 animals in each group; the remaining 4 animals per group were euthanized on day 13 postinfection for analysis of infectious Lassa virus in tissues. LOD, limit of detection; Ly-VSV ΔG /EBOVGP, lyophilized vesicular stomatitis virus expressing Ebola virus glycoprotein; Ly-VSV ΔG /LASVGPC, lyophilized vesicular stomatitis virus expressing Lassa virus glycoprotein; TCID $_{50}$, 50% tissue culture infectious dose; VSV ΔG /LASVGPC, vesicular stomatitis virus expressing Lassa virus glycoprotein.

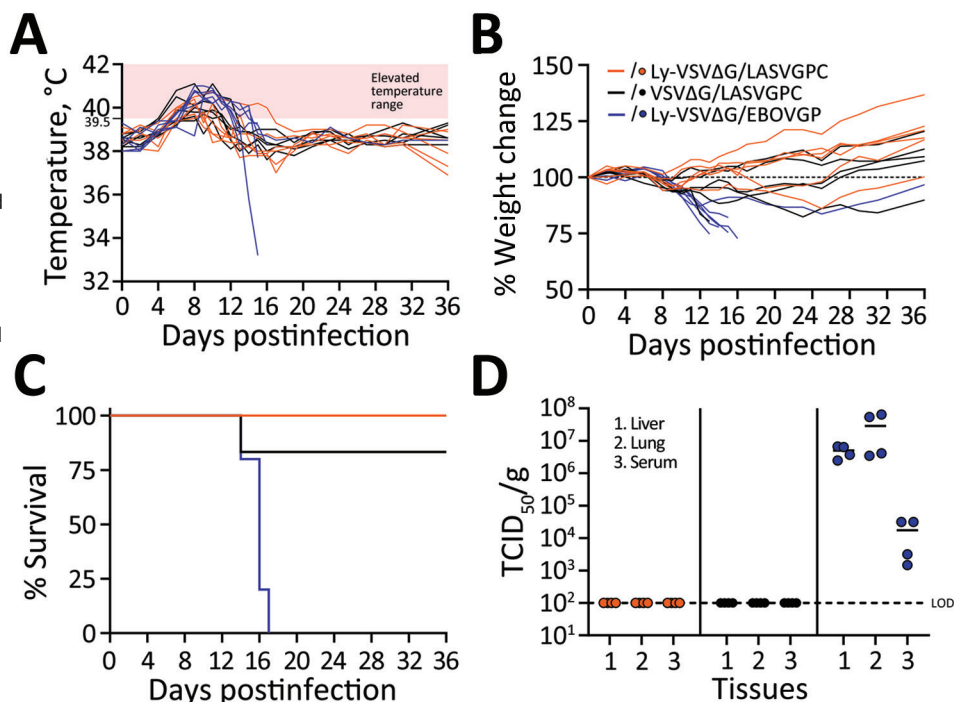


Table 2. Serologic evaluation of Lassa virus antibodies in immunized guinea pigs in study of protective efficacy of lyophilized vesicular stomatitis virus–based vaccines in an animal model*

Animal group, n = 10 each	Median (range)	Average (SEM)	Fold increase†
Preimmunization	1.4 (0.8–3.8)	1.8 (0.4)	NA
Ly-VSVΔG/LASVGPC	100.7 (8–373)	148.6 (44.2)	82
VSVΔG/LASVGPC	160.5 (79–262.8)	172 (17.7)	95
Ly-VSVΔG/EBOVGP	2 (0.7–6.1)	2.4 (0.6)	1.4

*Serum was analyzed for the presence of Lassa glycoprotein-specific antibodies by using an ELISA before challenge with Lassa virus. The standard curve was generated by using human control serum samples; therefore, values should be considered relative and for comparison purposes only. Ly-VSVΔG/EBOVGP, lyophilized vesicular stomatitis virus expressing Ebola virus glycoprotein; Ly-VSVΔG/LASVGPC, lyophilized vesicular stomatitis virus expressing Lassa virus glycoprotein; NA, not applicable; VSVΔG/LASVGPC, vesicular stomatitis virus expressing Lassa virus glycoprotein.

†Fold change of average values from immunized animals compared with randomized preimmunization control specimens.

in the Ly-VSVΔG/EBOVGP control group (Figure 2, panel C). Supporting the survival data, we only found infectious LASV in tissues collected on postinfection day 13 from the Ly-VSVΔG/EBOVGP-immunized control animals (Figure 2, panel D). Although not tested *in vivo*, the *in vitro* data supports similar protective responses from lyophilized VSVΔG/LASVGPC stabilized with LST or LS formulations for ≥ 30 days at 21°C or 90 days at 4°C.

We evaluated vaccine-induced humoral immune responses in serum samples collected immediately before virus challenge (28 days postimmunization) by using LASV and EBOV glycoprotein-specific ELISAs, as previously described (8,9). Animals immunized with Ly-VSVΔG/EBOVGP vaccine all had EBOV-specific ELISA titers $\geq 1:6,400$. Although those animals were not challenged with EBOV to assess the *in vivo* protective efficacy of the Ly-VSVΔG/EBOVGP vaccine, their antibody responses were consistent with a predicted protective response on the basis of findings from other studies, including studies using a similar EBOV guinea pig model (9,10). Instead, we used Ly-VSVΔG/EBOVGP-immunized animals as sham-vaccinated control animals in the lethal LASV challenge experiment to control for non-specific immunity associated with the LST stabilizer formulation. We monitored LASV-specific responses by using a glycoprotein ELISA developed for use in humans (Zalgen Labs, <https://www.zalgen.com>), which impedes direct determination of antibody concentrations in guinea pig samples. Nevertheless, we observed >75-fold increases in seroreactivity according to optical densities and average calculated concentrations in animals immunized with the Ly-VSVΔG/LASVGPC or VSVΔG/LASVGPC vaccines compared with preimmunization samples or serum samples collected from animals immunized with Ly-VSVΔG/EBOVGP (Table 2). Furthermore, the similar average values calculated for animals immunized with Ly-VSVΔG/LASVGPC and VSVΔG/LASVGPC indicates the lyophilization process did not appear to deleteriously effect the overall immunogenicity of the VSV-LASV vaccine.

Conclusions

We show that lyophilization can increase stability of VSV-based vaccines, potentially enhancing infrastructure and transport systems in rural areas and developing countries where cold chain management is challenging. Although the 2 VSV-based vaccines evaluated in this study only varied in their glycoproteins, *in vitro* recoverability efficiencies between them using different stabilizers, particularly gelatin, imply that a universal lyophilization method for all VSV-based vaccines might not be achievable. Therefore, each VSV-based vaccine will require in-depth experimentation to optimize formulations.

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About the Author

Mr. Salawudeen is a graduate student in the Department of Medical Microbiology and Infectious Diseases, University of Manitoba, and a pharmacist. His research interests focus on vaccination strategies, especially prophylactic vaccines for emerging pathogens in sub-Saharan Africa.

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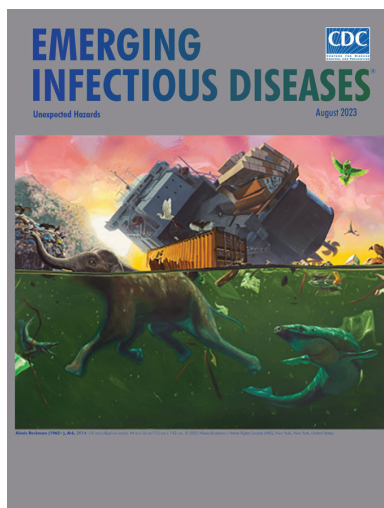
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- Elimination of *Dirofilaria immitis* Infection in Dogs, Linosa Island, Italy, 2020–2022
- Prospecting for Zoonotic Pathogens by Using Targeted DNA Enrichment
- Predicting COVID-19 Incidence Using Wastewater Surveillance Data, Denmark, October 2021–June 2022



- Chromosome-Borne CTX-M-65 Extended-Spectrum β -Lactamase–Producing *Salmonella enterica* Serovar Infantis, Taiwan
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Appendix

Appendix Table. Lyophilization program*

Cycle	Duration, min	Shelf temperature, °C	Vacuum pressure, mbar
Freezing	230	–40	0.53
Primary drying	60	–35	0.53
	60	–30	0.53
	60	–25	0.53
	60	–20	0.53
	60	–15	0.53
	60	–10	0.53
	60	–5	0.53
	60	0	0.53
	40	5	0.53
	20	10	0.53
	20	15	0.53
	20	20	0.53
	20	25	0.53
	25	30	0.53
Secondary drying	30	30	0.13

*Vaccine mixtures were lyophilized by using an automated FreeZone Triad Benchtop Freeze Dryer (Labconco, <https://www.labconco.com>).