Inactivated Rabies Virus-Based Ebola Vaccine Preserved by Vaporization Is Heat-Stable and Immunogenic Against Ebola and Protects Against Rabies Challenge.

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Inactivated Rabies Virus–Based Ebola Vaccine Preserved by Vaporization Is Heat-Stable and Immunogenic Against Ebola and Protects Against Rabies Challenge

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Background. Ebola virus (EBOV) is a highly lethal member of the Filoviridae family associated with human hemorrhagic disease. Despite being a sporadic disease, it caused a large outbreak in 2014–2016 in West Africa and another outbreak recently in the Democratic Republic of Congo. Several vaccine candidates are currently in preclinical and clinical studies but none are stable without cold chain storage.

Methods. We used preservation by vaporization (PBV), a novel processing technology to heat-stabilize FiloRab1 (inactivated rabies-based Ebola vaccine), a candidate Ebola vaccine, and stored the vials at temperatures ranging from 4°C to 50°C for 10 days to 12 months. We immunized Syrian hamsters with the best long-term stable FiloRab1 PBV vaccines and challenged them with rabies virus (RABV).

Results. Syrian hamsters immunized with FiloRab1 PBV–processed vaccines stored at temperatures of 4°C and 37°C for 6 months, and at 50°C for 2 weeks, seroconverted against both RABV-G and EBOV-GP. Notably, all of the FiloRab1 PBV vaccines proved to be 100% effective in a RABV challenge model.

Conclusions. We successfully demonstrated that the FiloRab1 PBV vaccines are stable and efficacious for up to 6 months when stored at temperatures ranging from 4°C to 37°C and for up to 2 weeks at 50°C.

Keywords. Ebola; rabies; vaccine; preservation by vaporization; stable; protection; challenge model.

The 2014–2016 Ebola virus (EBOV) outbreak in West Africa ended with >28 600 cases and 11 325 deaths [1]. More recently, an EBOV outbreak began in August 2018 in the Democratic Republic of Congo. Several vaccine candidates are currently in preclinical and clinical studies [8, 9]. Independent of its potential efficacy, the vaccine group received better medical care than the control group [7]. However, its efficacy was recently challenged by reports that the vaccine group received better medical care than the control group [8, 9]. Independent of its potential efficacy, the vaccine utilization drawback is that it requires –70°C storage. Such a requirement represents a significant logistical challenge, especially in outbreak countries with inadequate cold chain capacity. Furthermore, the rVSV virus may be affected by pH, temperature, and protein concentration [10]. The vaccine can suffer loss in potency due accidental heating or freezing, with studies showing that the stability of the rVSV-ZEBOV vaccine is limited to 24 hours at 25°C and to 1 week at 4°C, and storage at 40°C caused a significant reduction in the vaccine titer [11].

Lyophilized rabies vaccines have been shown to be the most temperature- and heat-stable of all vaccines in that it remains effective when stored for 24–48 months at 4°C [12]. However, maintaining a constant temperature of 4°C requires...
a refrigeration structure, and methods need to be implemented that are very expensive and difficult to manage in emerging countries and/or rural areas.

Preservation by vaporization (PBV) is a novel technology that can reduce the need for such methodical refrigeration. It is a cost-effective and efficient industrial scale stabilization of proteins, viruses, bacteria, and other sensitive biologicals (US patent number 9469835) [13]. Smith et al reported that live attenuated rabies virus (ERAg333) was successfully formulated into a stable, dry foam using the PBV processing technology. The vaccine remained stable for 23 months at 22°C, 2 months at 37°C, and 3 hours at 80°C. More importantly, both the inactivated RABV PBV (inactivated by irradiation) and the live RABV PBV vaccines effectively induced rabies virus neutralizing antibody (VNA) titers similar to the commercial rabies vaccine in vaccinated animals and protected all mice from an intramuscular (IM) RABV challenge [14, 15].

This data indicated that PBV processing could possibly render similar potency to the inactivated rabies-based Ebola vaccine (FiloRab1), a candidate Ebola vaccine that has proven efficacious in several nonhuman primate (NHP) studies [16–18]. FiloRab1 consists of the recombinant RABV that expresses the rabies glycoprotein (RABV-G) and the Ebola glycoprotein (EBOV-GP) on its virion surface. The efficacy of this vaccine was enhanced to 100% protection when adjuvanted with glycopranoxy lipid A in stable emulsion (GLA-SE) in NHP studies [18], providing protection against both RABV and EBOV.

In this study, we present data on the PBV process that was used to generate the FiloRab1 PBV vaccines prior to storage at 4°C, 25°C, 37°C, and 50°C for 10 days to 12 months. We assessed the antigenic stability of RABV-G and EBOV-GP on the virion of FiloRab1 PBV vaccines by antigen-specific enzyme-linked immunosorbent assay (ELISA). The best long-term stable FiloRab1 PBV candidates were tested for immunogenicity in a Syrian hamster model. As Ebola challenge requires a Biosafety Level 4 (BSL-4) facility and appropriate small animal models are not available, we chose to test the efficacy of our FiloRab1 PBV vaccines using a well-established Syrian hamster model for RABV [19]. Here we report the immunogenicity of the FiloRab1 PBV vaccines stored at 4°C and 37°C for 6 months and at 50°C for 2 weeks, against both RABV-G and EBOV-GP. Notably, all of the FiloRab1 PBV vaccines were 100% efficacious in a RABV challenge model. Thereby, we successfully demonstrated that the FiloRab1 PBV vaccines are stable and efficacious for up to 6 months when stored at temperatures ranging from 4°C to 37°C and for up to 2 weeks at 50°C.

**METHODS**

**Generation of FiloRab1 Vaccine**

The vaccine vector BNSP333-coZGP (FiloRab1) was constructed, recovered, purified with sucrose, inactivated with β-propiolactone (BPL), and characterized as previously described [16–18, 20]. The vaccine was stored at −80°C.

**Preservation by Vaporization of FiloRab1**

Liquid, BPL-inactivated FiloRab1 (1 mg/mL) vaccine was mixed with 4 parts preservation solution comprised of 20% (w/w) sucrose, 10% α-methylglucoside, 1% monosodium glutamate, and 0.3% sodium hexametaphosphate in deionized water, pH 7. This vaccine preservation solution mixture was distributed by 250-μL portions into 3-mL borosilicate glass serum vials. Vials were dried using the PBV foam drying process in a conventional Virtis Genesis freeze dryer. After 2 hours of primary drying, the material formed a solid dry foam that was stable under vacuum. Subsequent desorption drying was performed under vacuum with stepwise temperature raises from 25°C to 45°C. Desorption drying at 45°C continued for 24 hours to ensure stability of the vaccine at ambient temperatures. The vials were sealed under vacuum and crimped with aluminum seals. Samples were stored with Drierite in incubators for stability storage under refrigeration (4°C ± 3°C), room temperature (22°C ± 3°C), 37°C, and 50°C. Samples were retrieved from storage temperatures at specific time points of 10 days, 2 weeks, 1 month, 3 months, 6 months, and 12 months, and tested for antigenicity as compared to liquid control vaccine that was stored at −80°C. Each PBV vial contained 0.087 mL (87 μg) of the original FiloRab1 −80°C vaccine (1 mg/mL).

**Antigenicity ELISA**

Vials of FiloRab1 PBV vaccines were resuspended in 500 μL of cell culture–grade water (HyClone Water, Cell Culture Grade [Endotoxin-Free]) and incubated at room temperature for 10 minutes, shaking gently. Resuspended vaccines were subsequently stored at 4°C or on ice during experiments. FiloRab1 PBV Vaccine and the FiloRab1 −80°C stocks were diluted in 1× carbonate coating buffer, at a starting concentration of 0.02 mg/mL (Pierce BCA assay) and further serially diluted 3-fold within the 96-well plates (Nunc, Immunol 4 HBX), with 100-μL final volume per well. Plates were washed (1× PBST = 1× PBS-Tween20; 1× phosphate buffered saline, 0.05% Tween-20), blocked (5% milk in 1× PBST), and washed (1× PBST) before being probed with either 1C5 anti-RABV-G (Abcam) or 15H10 anti-EBOV-GP (Hybridoma, BEI resources, NR-12184) mouse antibodies at 2 mg/mL or 4 mg/mL, respectively. Goat antimouse immunoglobulin G (IgG) (Heavy and Light chain [H+L]) horseradish peroxidase (HRP) (Jackson ImmunoResearch, code 115-035-146, 0.8 mg/mL, 1:10 000) secondary antibody was used to detect RABV-G and EBOV-GP content. The later steps of the ELISA were done as previously described [16].

**Animals Ethics Statement**

Animal use protocols were approved by the Centers for Disease Control and Prevention’s Institutional Animal Care and Use Committee under protocol 2694WUHMC. In brief, 6-week-old, female Syrian hamsters (strain Hsd:Han Aura, Envigo) were assigned to groups of 12 animals. Animals were induced and
maintained under anesthesia with 1%-5% inhalation isoflurane and were monitored postprocedure until fully recovered.

Animal Study
Microchip identifiers (Bio Medic Data Systems, Seaford, Delaware) were implanted subcutaneously in the animals' backs. The PBV vaccines were reconstituted in 0.5 mL of sterile water. All animals were vaccinated IM with 0.05 mL of the vaccine in the hind limb according to the group, on day 0 and boosted with the same vaccine dose at day 28. Three control groups of 12 Syrian hamsters each received either 1/20th dose of the human rabies vaccine RabAvert (group 1) or a high dose of the liquid FiloRab1 vaccine of 50 μg (group 2); or they were not vaccinated to serve as a control group for the RABV challenge (group 6). The experimental groups of 12 Syrian hamsters each were immunized with 8.7 μg of the FiloRab1 PBV vaccines stored at 4°C for 6 months (group 3), 37°C for 6 months (group 4), or 50°C for 2 weeks (group 5). Blood was collected on days 0, 14, 28, and 56 using the subclavicle method: 0.2 mL of blood was collected from the vena cava using a 25 g needle and 1-mL syringe. Four animals (one group 1 and one group 4 on day 14; one group 2 on day 28 and one group 5 on day 56) did not recover from blood collection. On day 56, all animals were challenged with 10^3.5 mice intracerebral Lethal Dose 50% (MICLD_{50}) RABV TX coyote 323R (canine variant, Mexico) IM in the left hind leg. For the first 6 days postinfection, animals were monitored daily and twice daily from days 7 to 21. Animals were euthanized by isoflurane overdose at the first specific clinical signs of rabies, according to euthanasia criteria including a pain score, or as deemed necessary by the responsible veterinarian. All surviving animals were euthanized on day 45 postinfection and terminal sera were collected. A cross-section of the brain stem was collected at necropsy.

Direct Fluorescent Antibody Test
The brain stems collected at necropsy were tested for rabies diagnosis by the direct fluorescent antibody (DFA) test [21].

Rapid Fluorescent-Focus Inhibition Test
Serum separated from collected blood was used to determine the titer of rabies virus neutralizing antibody by the rapid fluorescent-focus inhibition test (RFFIT) [22].

Analysis of Humoral Responses by ELISA
Individual Syrian hamster sera and control sera were tested for the presence of EBOV-GP IgG by ELISA, as previously described [16]. Goat anti-Syrian hamster IgG(H+L) HRP (Jackson ImmunoResearch, code 107-035-142, 0.8 mg/mL, 1:20 000) secondary antibody was used for detection of EBOV-GP–specific responses in sera.

Statistical Analysis
All statistical analysis was performed by using GraphPad Prism 7 software. For the antigenicity ELISA, log half maximal effective concentration (EC_{50}) for samples within each incubation period (10 days to 12 months) was normalized and compared to -80°C using the Kruskal–Wallis multiple comparisons test. For the RFFIT assays and the immunogenicity ELISA, one-way analysis of variance test was performed on log-transformed data (for RFFIT, IU/mL; for ELISA, EC_{50}) and a post hoc analysis using Tukey honest significant difference test with a 95% confidence interval to test significance within groups. The Kaplan–Meier survival curves postchallenge were compared by performing the log-rank (Mantel–Cox) test.

RESULTS

Heat Stabilization of FiloRab1
To stabilize the FiloRab1 vaccine, we used the PBV stabilization process. We then stored the samples at 4°C, 25°C, 37°C, and 50°C. Samples were removed after 10 days, 2 weeks, and 1, 3, 6, and 12 months, stored at 4°C, and analyzed accordingly. Liquid samples from the initial inactivated vaccines stocks were stored at -80°C as standards.

Antigen Stability of FiloRab1 Vaccine by ELISA
After storage at the different temperatures, the content of the 2 viral glycoproteins, RABV-G and EBOV-GP, on the surface of FiloRab1 virions (inactivated vaccine) were determined by EBOV-GP– and RABV-G–specific ELISAs. We found that the EBOV-GP and RABV-G content of FiloRab1 PBV vaccines stored at 4°C, 25°C, and 37°C were not affected when stored for 10 days to 12 months in comparison to the FiloRab1 vaccine stored at -80°C (Figure 1). The FiloRab1 PBV sample kept at 50°C showed no effect on the EBOV-GP and the RABV-G contents for up to 2 weeks, but small decreases in antibody binding to both the RABV-G and EBOV-GP were seen for FiloRab1 PBV 50°C samples at the 1-month time period. Therefore, only the lower temperatures of 25°C and 37°C were studied for the whole period of 12 months. The results of these experiments indicated that the PBV process is an excellent method to heat-stabilize the FiloRab1 vaccine with respect to the conservation of antigens within the virions.

Immunogenicity of Antigenically Stable Vaccines in Syrian Hamsters
While the ELISA was used for the initial screening of the stability of the vaccine, we wanted to analyze whether FiloRab1 PBV vaccine was still immunogenic when stored at different temperatures. For this study, we chose the samples of FiloRab1 PBV stored at 4°C or 37°C for 6 months and FiloRab1 PBV stored at 50°C for 2 weeks (Figure 2A). The liquid FiloRab1 vaccine stored at -80°C served as the positive control since previous experiments in NHPs and mice indicated that the liquid vaccine stored at –80°C served as the positive control since pre-

RABV Neutralizing Antibodies Induced by FiloRab1 and Its Stabilized Derivates
The rabies-specific potencies of the FiloRab1 PBV vaccines were analyzed by

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titers against EBOV-GP prior to immunization (Figure 3 and Supplementary Figure 1). However, as early as the first analyzed timepoint at day 14, all 4 groups developed GP-specific IgG responses, which were not significantly different between the groups immunized with FiloRab1 –80°C and the FiloRab1 PBV–vaccinated groups. Similar findings were made for the RABV VNAs, the PBV-vaccinated animals mounted similar IgG titers, indicating that the heat stabilization was successful for both of the antigens of interest.

### Efficacy Test in Vaccinated Animals

There is no challenge system available for EBOV (wild type) in Syrian hamsters and a modest predictable EBOV challenge system with a mouse-adapted variant of EBOV. However, the efficacy of the FiloRab1 vaccine can be analyzed for effectiveness to protect against RABV by challenging the animals with RABV. Therefore, all animals (Syrian hamster model) were challenged IM in the hind leg with 10^{–5.5} MICLD_{50} TX coyote 323R (canine RABV variant from Mexico), a highly pathogenic primary isolate of RABV. As shown in the Kaplan–Meier curves in Figure 4B, all of the FiloRab1 PBV–vaccinated animals, the –80°C FiloRab1–vaccinated animals, and hamsters immunized with RabAvert survived the RABV challenge, whereas the unvaccinated control animals died by day 18 postchallenge. The survival curves for vaccination groups were significantly different from the control group (log-rank \( P < .0001 \)). Survival curves for FiloRab1 groups were not significantly different from the RabAvert group (log-rank \( P = 1.0 \)) (Figure 4B).

### EBOV-GP–Specific Immune Responses

Because the FiloRab1 vaccine is a dual vaccine against EBOV and RABV, we also analyzed the EBOV-GP–specific antibody titers by an EBOV-GP–specific ELISA. We found that all groups of immunized hamsters had only background level of antibody titers against EBOV-GP prior to immunization (Figure 3 and Supplementary Figure 1). However, as early as the first analyzed timepoint at day 14, all 4 groups developed GP-specific IgG responses, which were not significantly different between the groups immunized with FiloRab1 –80°C and the FiloRab1 PBV–vaccinated groups. Similar findings were made for the RABV VNAs, the PBV-vaccinated animals mounted similar IgG titers, indicating that the heat stabilization was successful for both of the antigens of interest.

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Figure 2. Syrian hamsters vaccinated with inactivated rabies-based Ebola (FiloRab1) preservation by vaporization (PBV) vaccine and controls. Groups of 12 Syrian hamsters each were immunized with either 1/20th dose of RabAvert (commercial rabies vaccine), 50 μg of FiloRab1 –80°C, 8.7 μg of FiloRab1 PBV 4°C, FiloRab1 PBV 25°C, FiloRab1 PBV 37°C, or FiloRab1 PBV 50°C. Sera were collected on days 0, 14, 28, and 56 for analysis. A, Syrian hamster immunization/challenge schedule. Syringes represent immunizations, the lightning bolt represents challenge, and droplets represent blood draws. B, Sera analyzed for rabies virus (RABV) neutralizing antibody (VNA) titers by rapid fluorescent-focus inhibition test; ordinary one-way analysis of variance statistical analysis was performed on log-transformed data and a post hoc analysis using Tukey honest significant difference test with a 95% confidence interval to test significance within groups. *P = .01–.05; **P = .001–.01; ***P = .0001–.001; ns, not significant. Dotted line represents the WHO accepted threshold of 0.5 IU/mL.

Figure 3. Ebola glycoprotein (EBOV-GP)–specific humoral responses (half maximal effective concentration [EC₅₀]) seen in inactivated rabies-based Ebola (FiloRab1) preservation by vaporization (PBV)–vaccinated animals. Sera from vaccinated Syrian hamsters analyzed for EBOV-GP immunoglobulin G responses analyzed by enzyme-linked immunosorbent assay. All FiloRab1 PBV–vaccinated animals (PBV 4°C: green triangles; PBV 37°C: purple inverted triangles; PBV 50°C: orange diamonds) developed similar EBOV-GP–specific titers as the FiloRab1 –80°C–vaccinated animals. Ordinary one-way analysis of variance statistical analysis was performed on log-transformed data and a post hoc analysis using Tukey honest significant difference test with a 95% confidence interval to test significance within groups. ****P < .0001; ns, not significant.
was performed. To compare the groups, a log-rank (Mantel–Cox) test analysis of survival curves for FiloRab1 groups are not significantly different from the RabAvert group (log-rank P < .0001). Survival curves for FiloRab1 groups are not significantly different from the RabAvert group (log-rank P = 1.0). To compare the groups, a log-rank (Mantel–Cox) test analysis was performed.

### DISCUSSION

Heat-stable vaccines are needed in developing countries where it can be difficult to maintain a constant cold temperature. Heat stabilization has been a significant challenge for several vaccines against EBOV [11, 24]. For example, the VSV-based live vaccine against EBOV must be stored at −70°C and is highly unstable at ambient temperature [11]. By contrast, the lyophilized rabies vaccine is stable at 4°C for 24 months [12], which is a significant improvement from frozen storage. However, even storage at 4°C is not always available due to breaks in the cold chain during transport to affected areas. Therefore, an efficacious vaccine that can withstand temperature ranges from 4°C to 50°C is needed.

The second-generation RABV-based Ebola vaccine (FiloRab1) adjuvanted with GLA-SE has shown 100% efficacy in an Ebola challenge in 2 different NHP studies [16, 18]. In these studies, the unadjuvanted FiloRab1 had similar rabies VNA titers to the GLA-SE adjuvanted group, ranging from 4 IU/mL to 45 IU/mL, well above the WHO-recognized threshold of 0.5 IU/mL [23]. These results clearly demonstrate that FiloRab1 adjuvanted with GLA-SE can provide protection against both Ebola and rabies. In addition, the inactivated FiloRab1 vaccine builds on the excellent safety profile of the inactivated RABV vaccine that can be safely administered to pregnant women, children, and immunocompromised individuals, unlike many of the virally vectored EBOV vaccine candidates.

Here we used the cost-effective and efficient industrial-scale PBV stabilization system to stabilize the FiloRab1 vaccine. We observed that in FiloRab1 PBV vaccines, EBOV-GP and RABV-G are antigenic stable from 4°C to 37°C for up to 12 months. The FiloRab1 PBV 50°C samples were stable for 2 weeks but showed slight degradation (P = .0329) after 1–3 months (Figure 1).

The long-term stable FiloRab1 PBV vaccines (stored at 4°C or 37°C for 6 months and at 50°C for 2 weeks) were used to immunize Syrian hamsters (Figure 2A). All FiloRab1 PBV–vaccinated animals except the 50°C group developed RABV VNA titers similar to the −80°C FiloRab1 control by day 56 (Figure 2B and Supplementary Table 1). Similarly, all FiloRab1 PBV–vaccinated immunized animals seroconverted against EBOV-GP, similar to the control −80°C FiloRab1–vaccinated animals (Figure 3 and Supplementary Figure 1). This shows that the antigenic stability analyzed by ELISA was correlative to the immunogenicity seen in the PBV-vaccinated animals. Therefore, the PBV process successfully stabilized both the RABV-G and the EBOV-GP on the virion surface.

We next tested the efficacy of our vaccine using the well-characterized RABV challenge model. All of the FiloRab1 PBV–, FiloRab1 −80°C–, and RabAvert control–vaccinated animals

<table>
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<tr>
<th>Group</th>
<th>Survival (%)</th>
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<tr>
<td>1. RabAvert</td>
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<td>NA</td>
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<tr>
<td>2. FiloRab1 −80°C</td>
<td>100</td>
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<tr>
<td>3. FiloRab1 PBV 4°C</td>
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<td>4. FiloRab1 PBV 37°C</td>
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<td>5. FiloRab1 PBV 50°C</td>
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<td>6. Control</td>
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Figure 4. Inactivated rabies-based Ebola (FiloRab1) preservation by vaporization (PBV)–vaccinated animals survive rabies challenge. The FiloRab1 PBV (4°C, 37°C, 50°C), FiloRab1 −80°C, RabAvert (commercial rabies vaccine), and control groups of vaccinated animals were challenged with 103.5 Mice Intracerebral Lethal Dose 50% rabies virus TX coyote 323R (canine variant, Mexico) intramuscularly in the left hind leg. Animals vaccinated with FiloRab1 PBV (4°C, 37°C, 50°C), FiloRab1 −80°C, or the RabAvert group survived lethal rabies challenge, whereas the control group (group 6) succumbed to challenge by day 18. A, Table showing the percentage survival for all groups. NA, not applicable. B Kaplan–Meier survival curves post–rabies challenge. Survival curves for vaccination groups are significantly different from control group (log-rank P < .0001).

To analyze the level of protection as well as to diagnose the cause of death of the control animals, the presence of RABV antigen in the brain stem of challenged animals was tested by DFA for groups 1–6 (Supplementary Table 2). Among the 12 survivors in groups 1–4, 50% of the animals were randomly chosen to test for the presence of RABV antigen. All of these animals were diagnosed negative for RABV antigen, indicating the efficient prevention of viral spread. Because of the slightly lower VNA in the FiloRab1 PBV 50°C–vaccinated group, all 12 animals were tested for RABV antigen, and all 12 were negative. All 12 control unvaccinated animals tested positive for the presence of RABV antigen by DFA.
survived lethal RABV challenge (Figure 4), with no presence of RABV antigen in their brains at necropsy (Supplementary Table 2), whereas the control unvaccinated animals succumbed to RABV by day 18. In summary, 2 doses of the FiloRab1 PBV vaccines demonstrated excellent efficacy when stored at 4°C and 37°C for 6 months and at 50°C for 2 weeks. EBOV challenge necessitated the use of a BSL-4 facility, which was out of the scope of this study.

In all, we demonstrated stability, immunogenicity, and efficacy of the FiloRab1 PBV vaccines stored at temperatures ranging from 4°C to 50°C in comparison to the FiloRab1 –80°C vaccine. While this study addressed the vaccine stability at single temperatures, further studies are warranted to address the effect of temperature fluctuations on vaccine stability. Our laboratory has recently received a National Institute of Allergy and Infectious Diseases contract (project number 272201700082C) to test the immunogenicity and efficacy of all our rabies-based hemorrhagic fever vaccines (FiloRab1-Ebola; FiloRab2-Sudan; FiloRab3-Marburg; and LassRab-Lassa fever) in NHPs. Taken together, these stability data may be useful for logistical planning of vaccine delivery for future Ebola outbreaks. Hence, the FiloRab1 PBV vaccine offers bivalency against 2 common pathogens (RABV and EBOV) and improved stability for long-term storage in comparison to other Ebola vaccine candidates in preclinical and clinical studies.

SUPPLEMENTARY DATA

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. M. J. S. is an inventor on the US Provisional Patent Application Title “Multivalent vaccines for rabies virus and filoviruses.” M. J. S. also serves on the scientific advisory board of IDT Biologika, Dessau, Germany. V. B. is an inventor on US patent number 9469835B2. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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