

ADDRESSING VACCINE STABILITY AND COLD CHAIN CHALLENGES WITH RECOMBINANT HUMAN SERUM ALBUMIN TO ENABLE GLOBAL ADMINISTRATION

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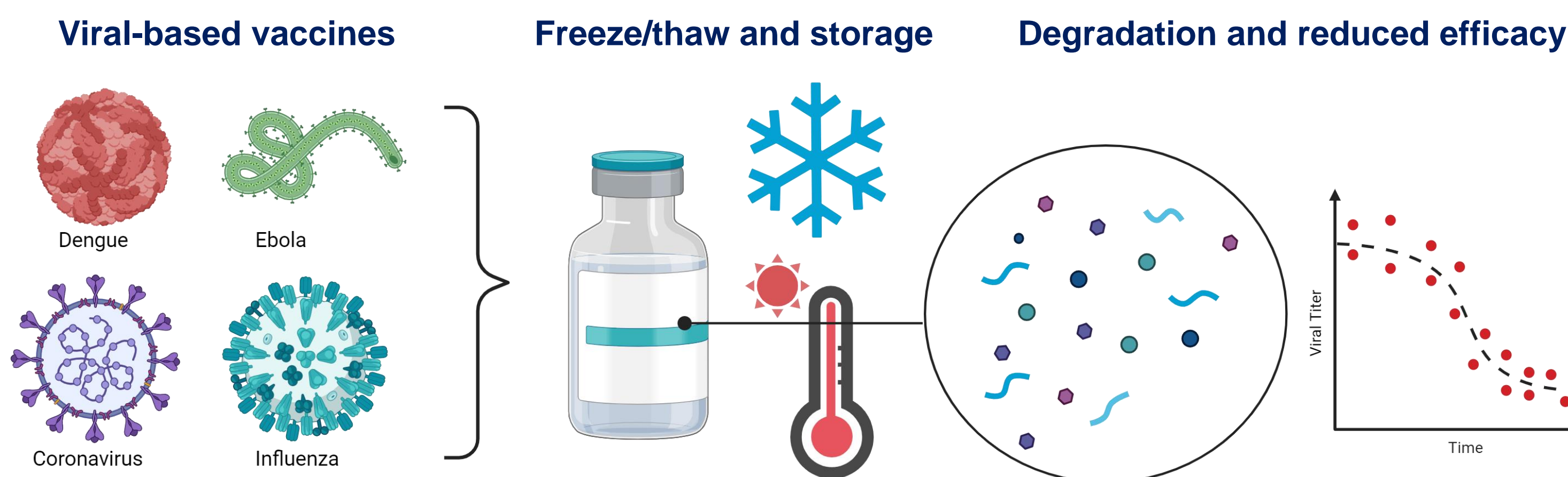
INTRODUCTION



Viral-based vaccines are effective in the prevention of infection and reducing disease severity caused by several viral pathogens, including **COVID-19, Ebola, and influenza**.¹⁻³



However, these vaccines are **vulnerable to freeze-thaw cycles** during processing, shipping, storage, and administration, which can reduce their efficacy.

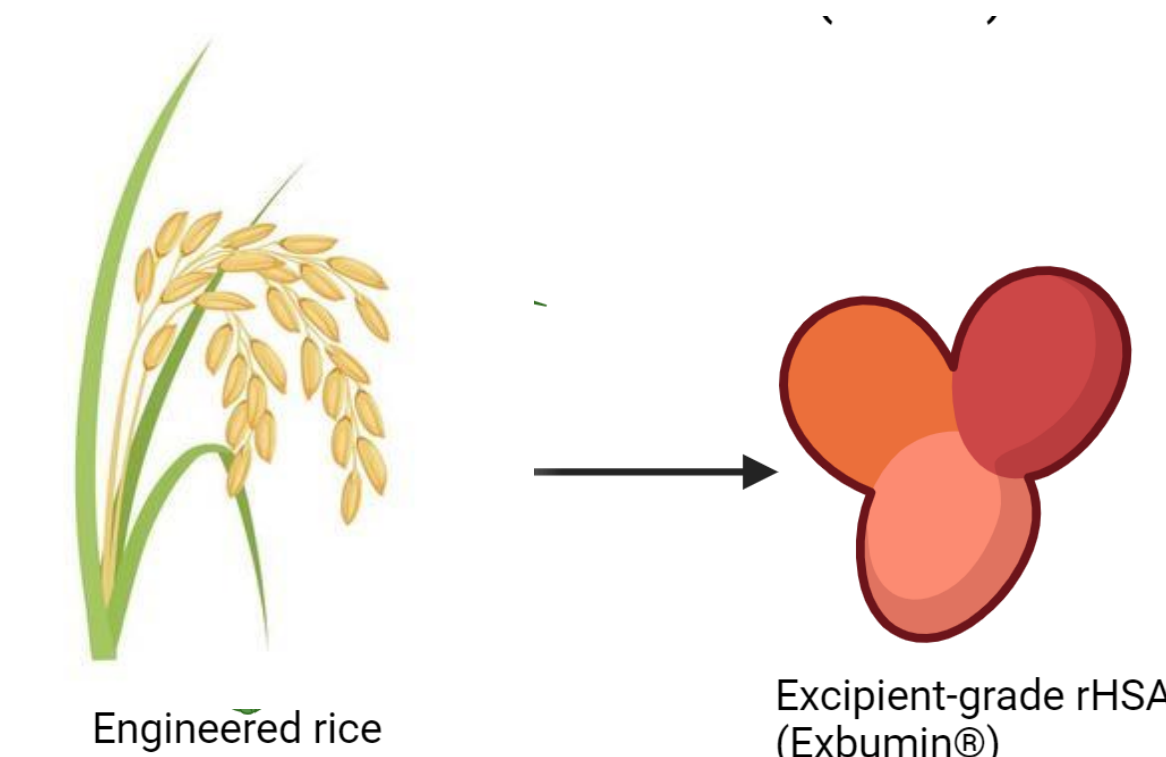


AIM

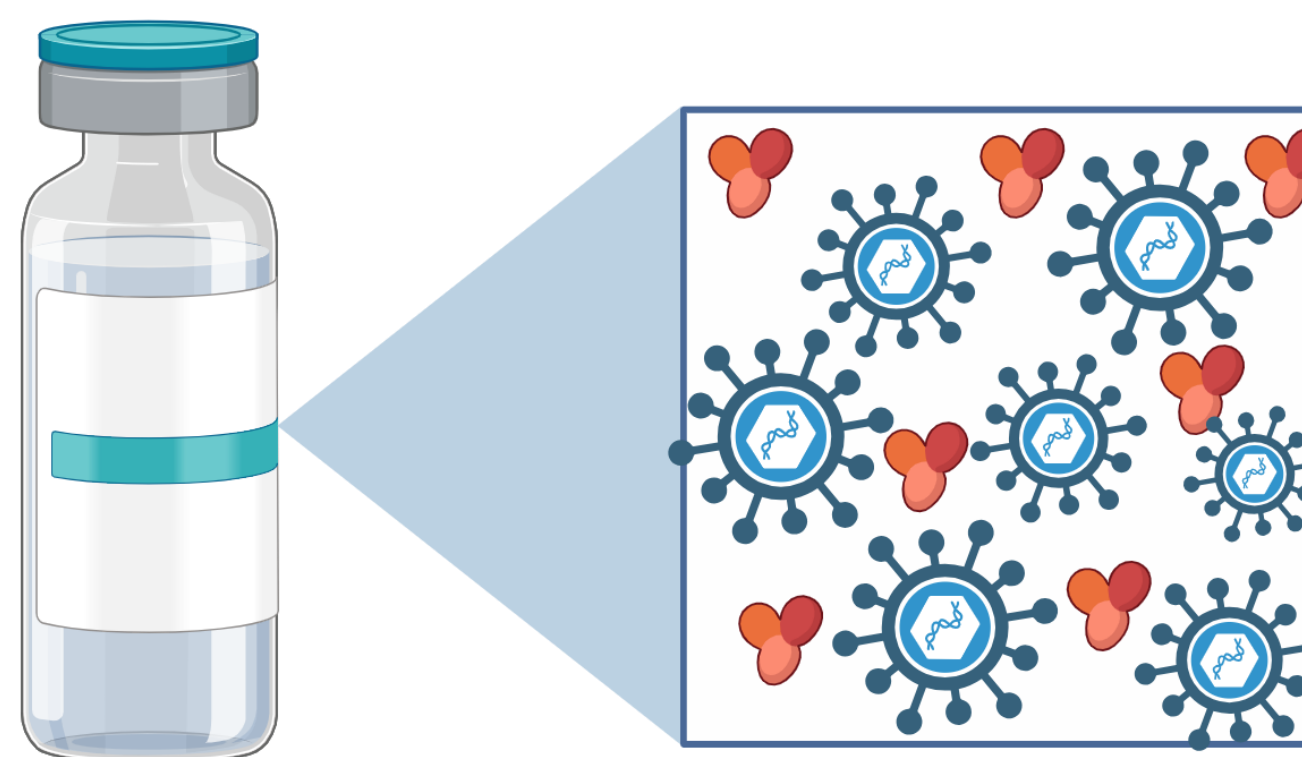


To address this challenge, InVitria's team has developed **Exbumin®**, a recombinant Human Serum Albumin ("rHSA") reagent designed for formulation in virus storage buffers.

Rice-derived recombinant human serum albumin (rHSA)



Exbumin® storage buffer formulations for viral vaccine stabilization



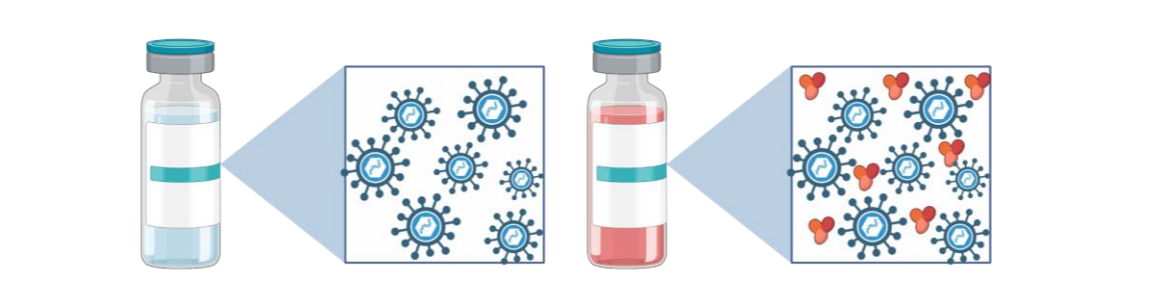
METHODS

- In collaboration with an industry partner, we explored the efficacy of Exbumin® when used as an additive in storage buffers for a new influenza viral-based vaccine.

Tested three influenza subtypes

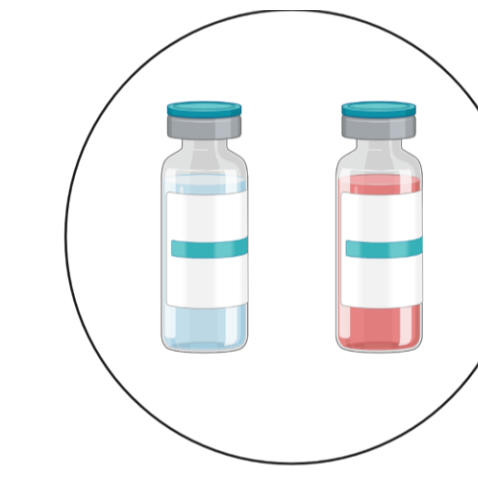


Formulated with or without Exbumin®



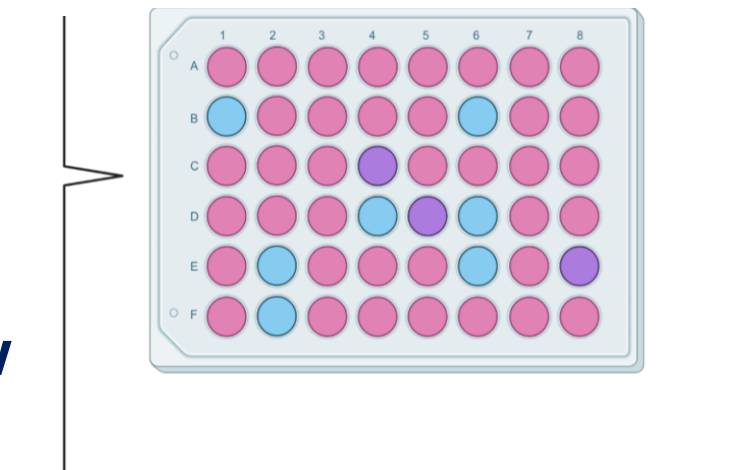
- Three influenza virus subtypes (H1N1, H3N2, and Yam)** were evaluated, stored at 4°C for 360 days either with or without Exbumin®. Titer was assessed by TCID50 assay at several timepoints

Varied storage conditions



- Additionally, we examined Exbumin®'s ability to safeguard these influenza viral subtypes from multiple freeze-thaw cycles (16 cycles in total). Again, TCID50 assay was used to characterize the titer.

Assess virus stability with TCID assay



RESULTS 1

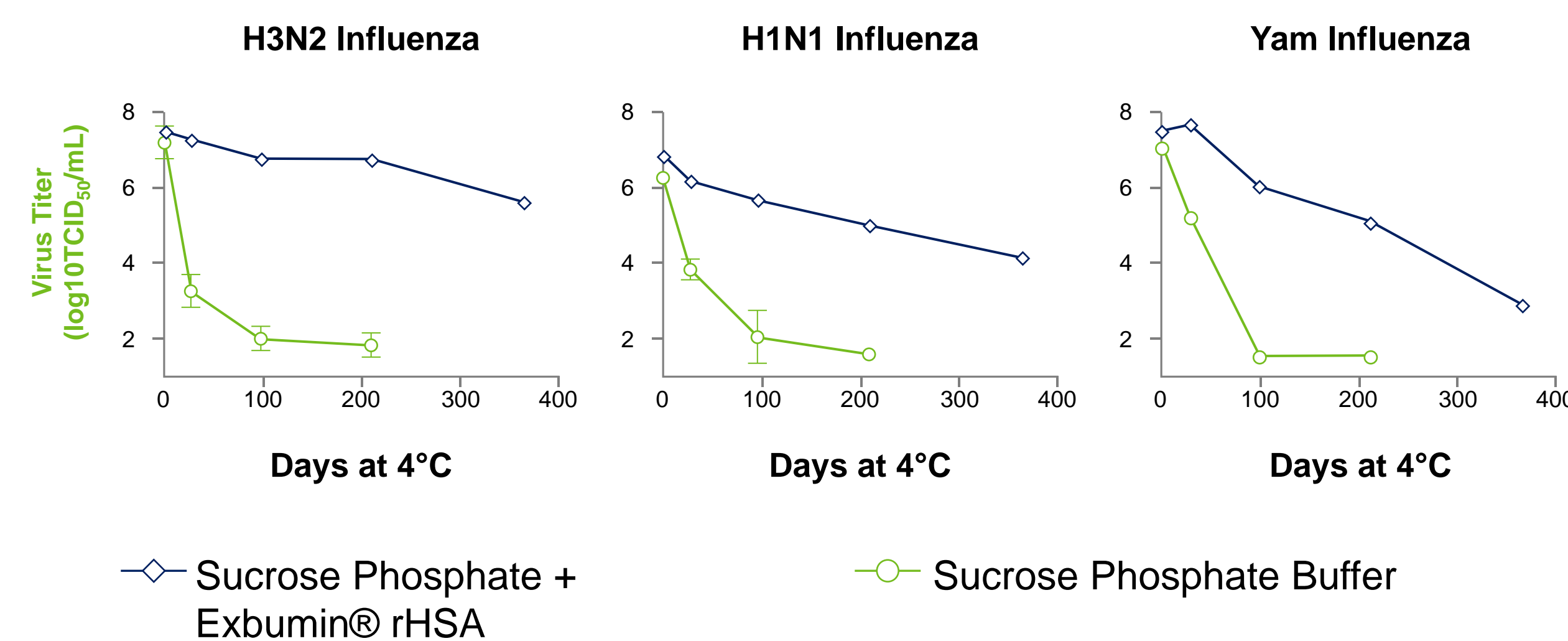


Figure 1. Stability of Influenza subtypes H3N2, H1N1, and Yam in a sucrose phosphate buffer with and without Exbumin® rHSA at 4°C

- After 360 days of storage at 4 °C, **no viral titer was detected in buffers lacking Exbumin®**.
- Conversely, **in the presence of Exbumin®**, infectious titers of approximately **5.50x10⁵ TCID₅₀/mL for Flu A H3N2, 3.80x10⁴ TCID₅₀/mL for Flu A H1N1, and 2.85x10² TCID₅₀/mL for Yam Flu** viral subtypes were recorded.
- Furthermore, **titer improvements of up to 4 logs** were observed after as little as **20 days** in samples including Exbumin® rHSA compared to buffer alone

RESULTS 2

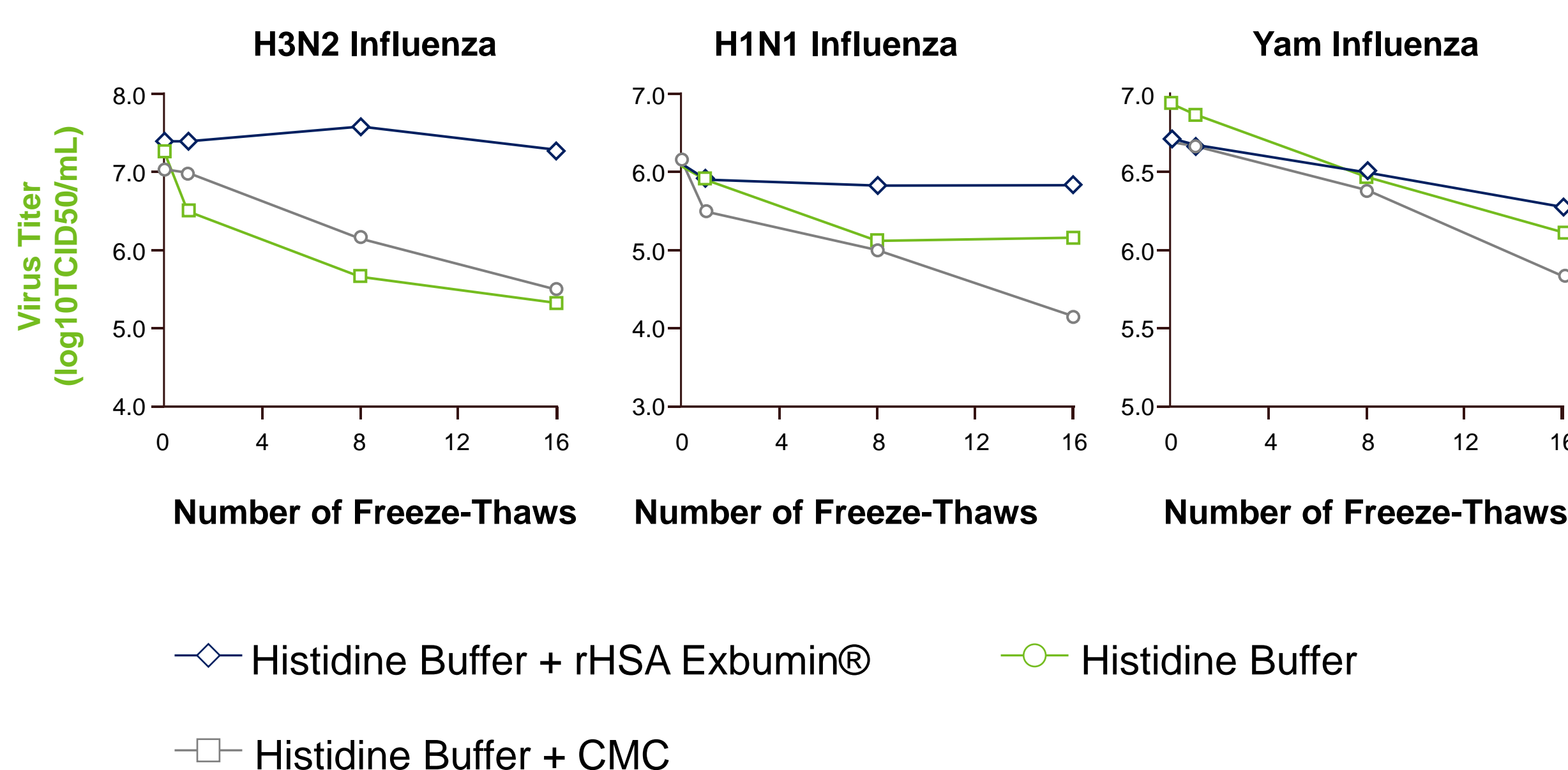


Figure 2. Stability of Influenza subtypes H3N2, H1N1 and Yam after repeated freeze thaw cycles

- Our analysis of up to 16 freeze-thaw cycles revealed **significant improvements in viral stability in the presence of Exbumin®** compared to buffer alone.
- Exbumin® out-performed carboxymethyl-cellulose (CMC)**
- The titer of Flu A H1N1 increased by 2 logs, Flu A H3N1 by approximately 1.8 logs, and Yam Flu by 0.5 log when stored in a histidine buffer supplemented with Exbumin®.

RESULTS 3

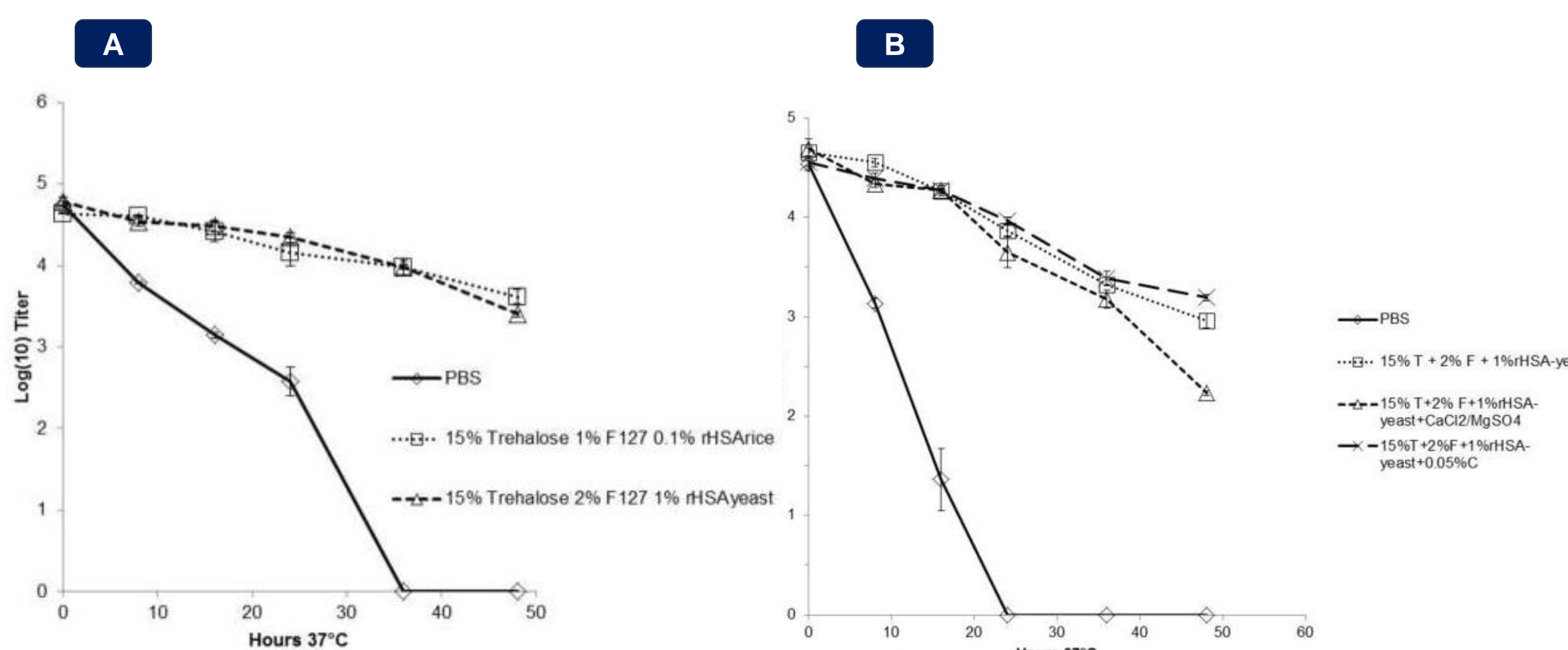


Figure 3. Kinetics of flavivirus inactivation at 37°C

- A study by Wiggan et al. showcased Exbumin®'s potential by stabilizing a live attenuated dengue viral vaccine, resulting in a remarkable >10-fold improvement in titer.⁵
- Triplicate samples of virus were incubated with FTA (F127 (F), Trehalose (T), and albumin (A) (derived from rice or yeast) in PBS formulations for 48 hours at 37°C. Graphs depict mean values ± s.d.
 - (A). Kinetics of DEN-2 PDK-53-V viral inactivation at 37°C in FTA containing rice or yeast rHSA.
 - (B). Kinetics of DEN-2/WN viral inactivation at 37°C in FTA. Two formulations (in B) also contained 0.9 mM CaCl₂ and 0.5 mM MgSO₄, or 0.05% chitosan (C).
- Titers shown at zero represent undetectable titers, with the detection limit being 1.7 log₁₀pfu.

RESULTS 4

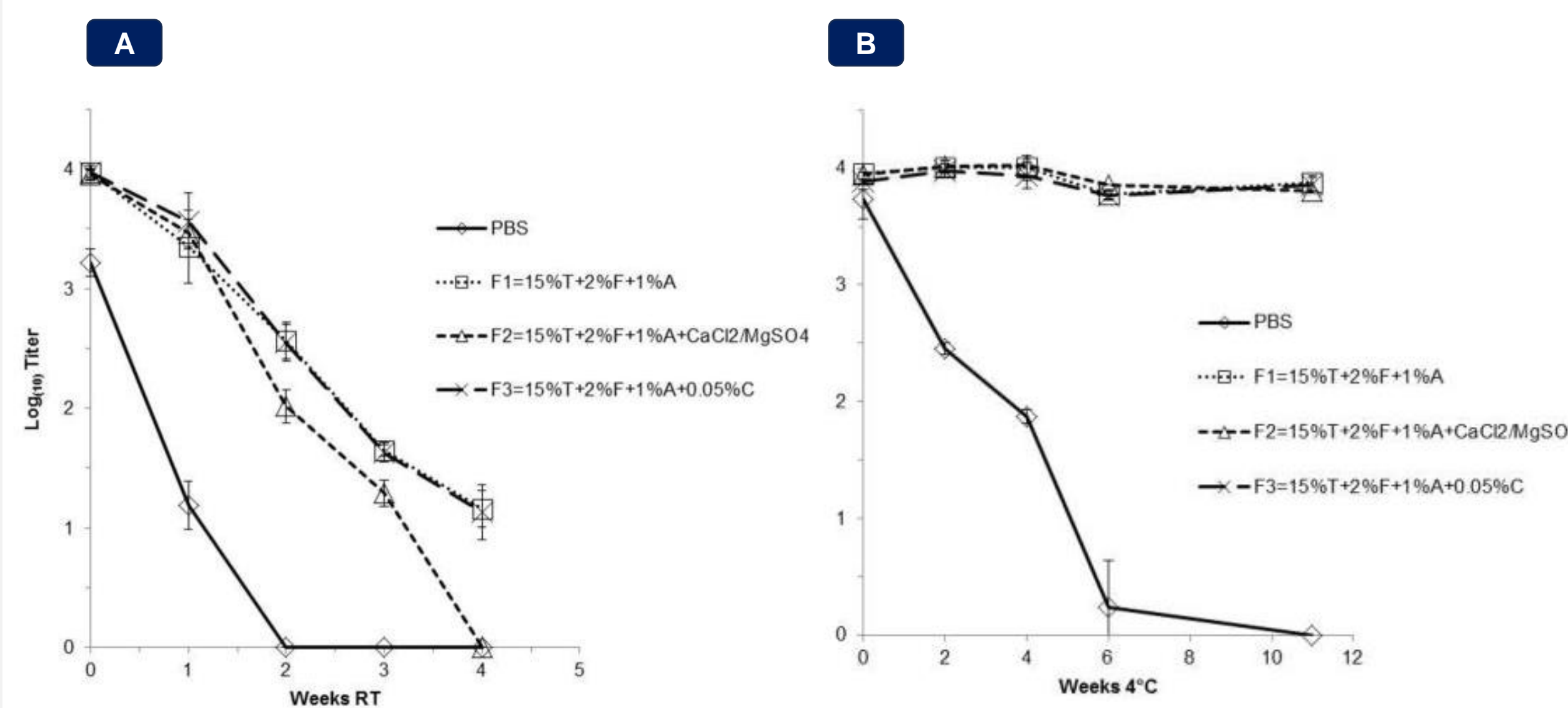


Figure 4. Kinetics of flavivirus virus inactivation at various temperatures

- DEN-2/WN virus was mixed with the indicated FTA formulations and stored for (A) 4 weeks at room temperature (~25°C), and (B) for 11 weeks at 4°C.
- Triplicate aliquots were taken at the indicated time points.
- Graphs depict mean values ± s.d. FTA formulations contain 15% trehalose (T), 2% F127 (F), 1% rHSA-yeast (A) in PBS.
- Two of the formulations also contained either 0.9 mM CaCl₂ and 0.5 mM MgSO₄, or 0.05% chitosan (C). Titers shown at zero represent undetectable titers, with the detection limit being 1.7 log₁₀pfu.

CONCLUSIONS



Recombinant human serum albumin (rHSA) was shown to stabilize a variety of virus types both in cold storage over time and across numerous freeze-thaw cycles
Wiggan et al demonstrated that rice-derived rHSA was shown to be a more potent stabilizer of Dengue Virus than yeast-derived rHSA



ACKNOWLEDGEMENTS

- Customer collaboration
- Wiggan et al.
- Diagrams created using BioRender.com
- Cell Culture Lab & Product Applications, InVitria Inc., Cambridge, MA

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