

# Thermostable Lyophilized Ebola Vaccine Formulations

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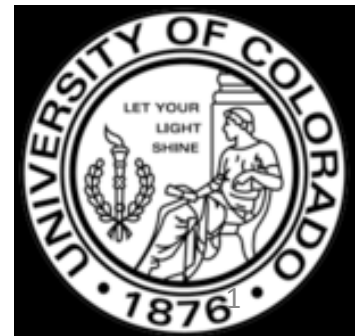
University of Colorado - Boulder

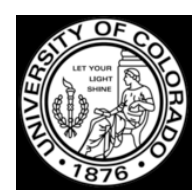
Center for Pharmaceutical Biotechnology

Department of Chemical and Biological Engineering



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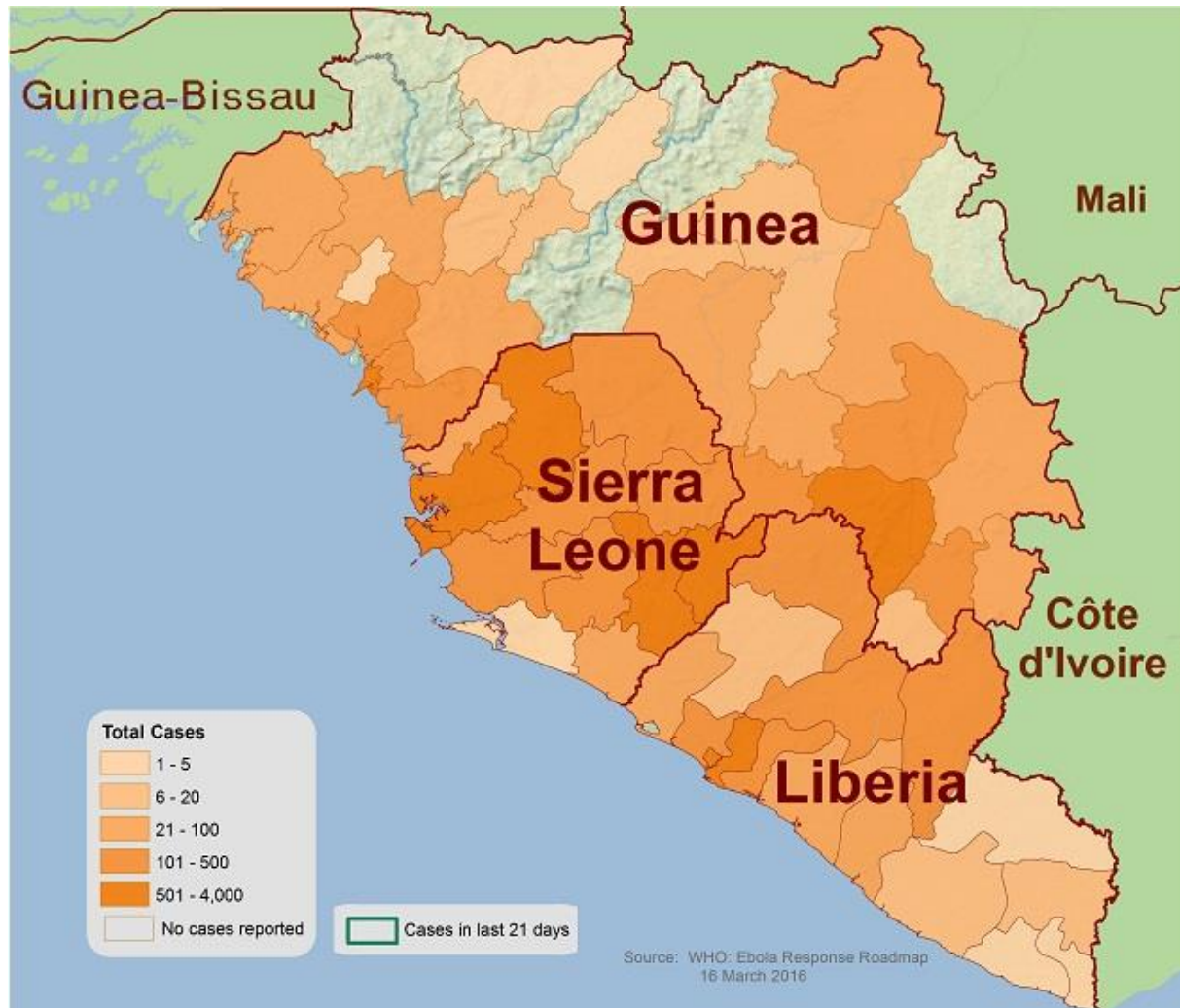
# Cold chain requirements for vaccines



- Current licensed vaccines require transport and storage under a tightly-controlled cold chain
- Cold chain requirements are costly and particularly difficult to maintain in developing countries

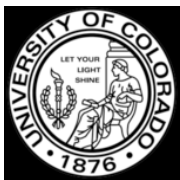


Ebola outbreaks occur in countries where maintaining the cold chain is challenging



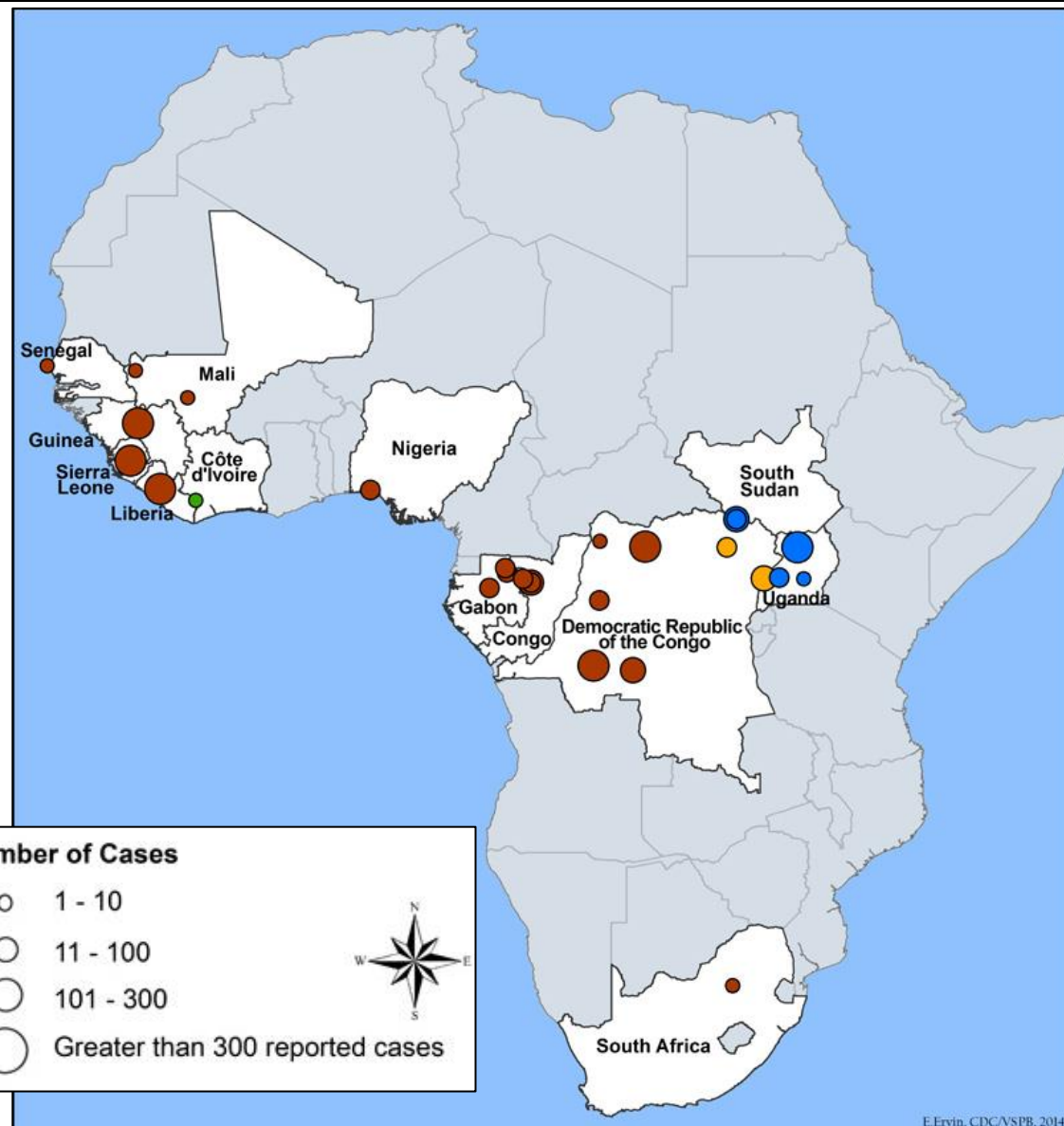
2014 Ebola outbreak in West Africa

Total cases = 28,652   Total deaths= 11,325

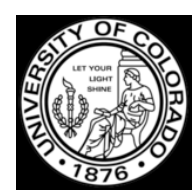


# No licensed vaccines against Ebola virus infections are currently available

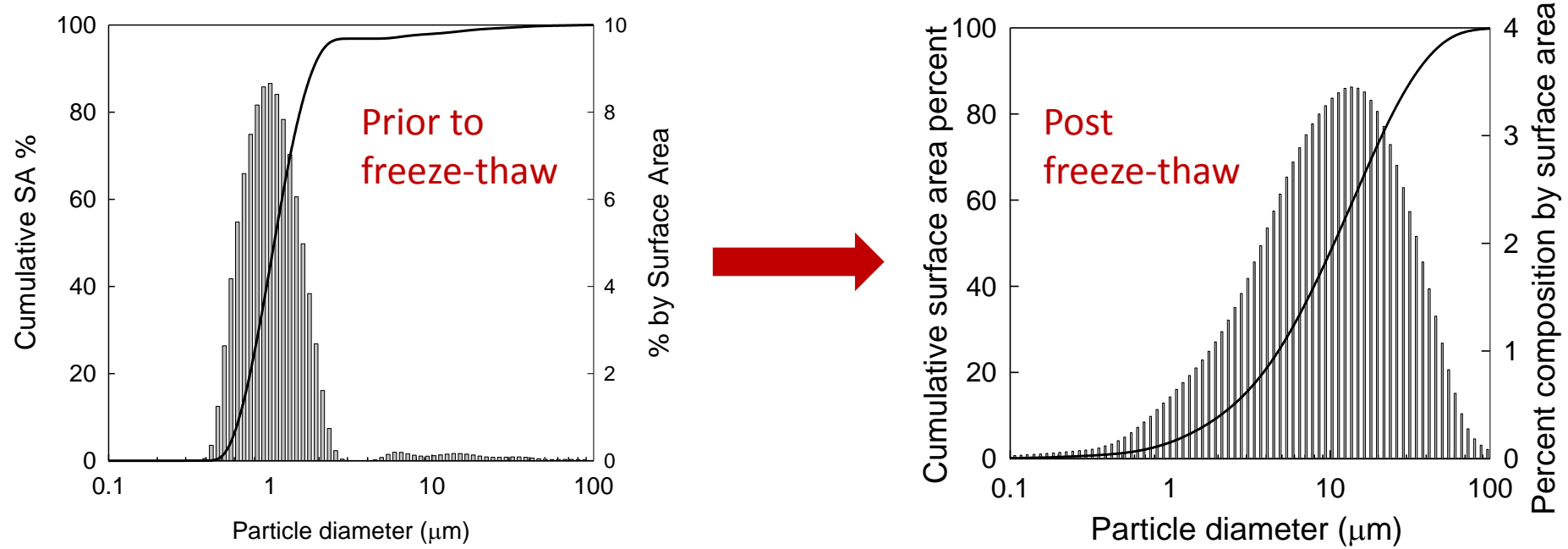
We need a thermally stable vaccine!



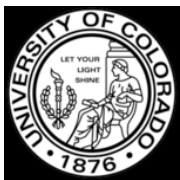
Lyophilization can be used to develop thermostable vaccines and could eliminate the need for a cold chain



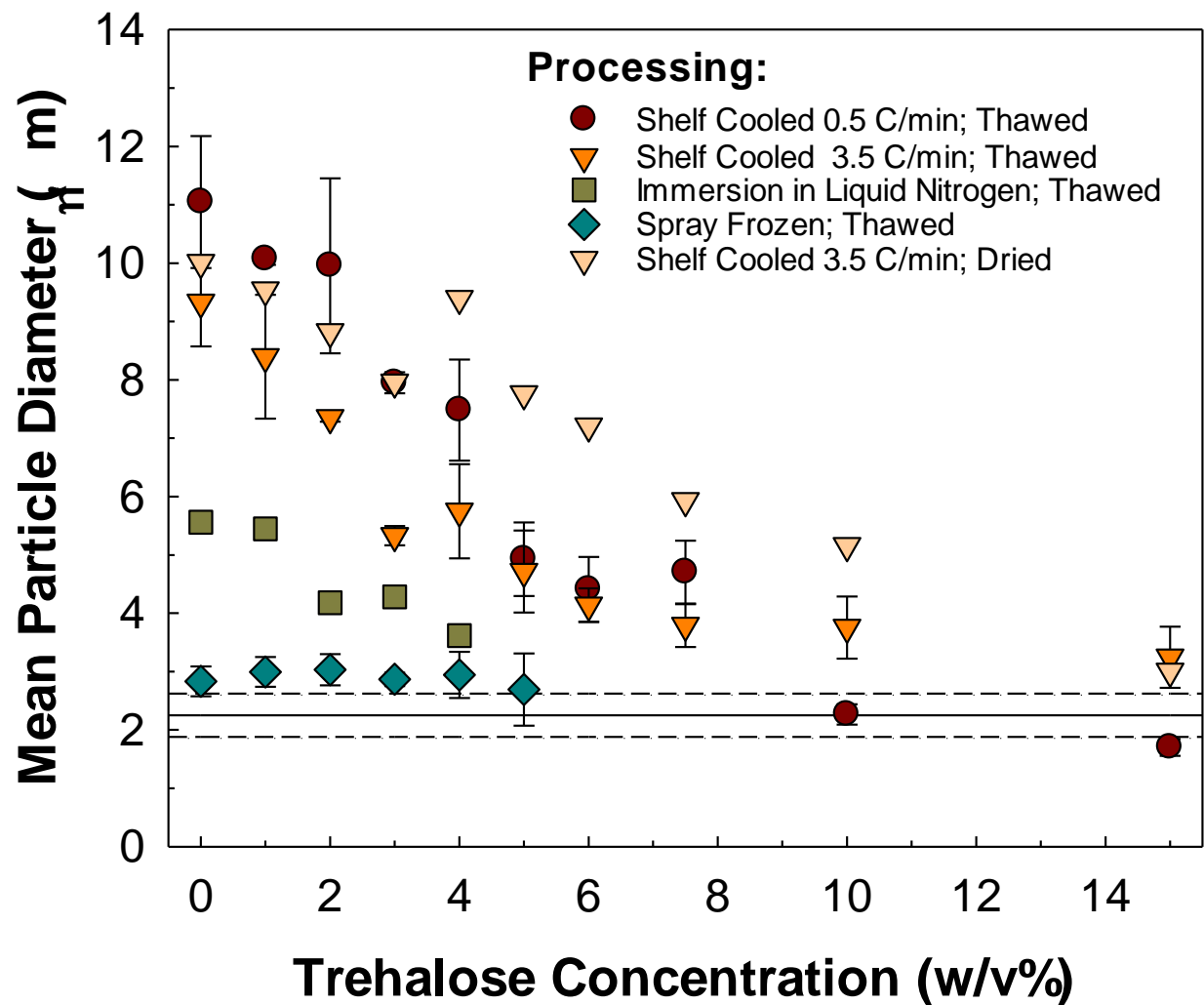
# Freezing-induced aggregation: A challenge for lyophilization of adjuvant-containing vaccines

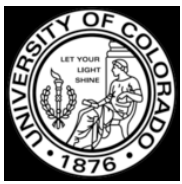


Freezing typically causes aggregation of aluminum salt particles in vaccine formulations and can lead to losses in vaccine efficacy



Aggregation of aluminum salt adjuvants during freezing can be avoided by using high concentrations of glass-forming excipients or faster cooling rates





# Development of a thermostable Ebola vaccine

- Formulations of the key antigen, Ebola glycoprotein, were prepared in liquid and lyophilized forms

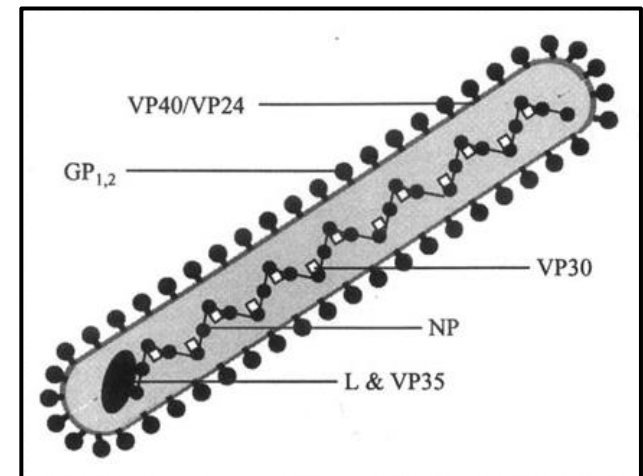
## Vaccine Formulation

0.1 mg/mL Ebola glycoprotein

10 mM ammonium acetate pH 7

9.5% (w/v) trehalose

± 0.5 mg/mL aluminum hydroxide (alum)



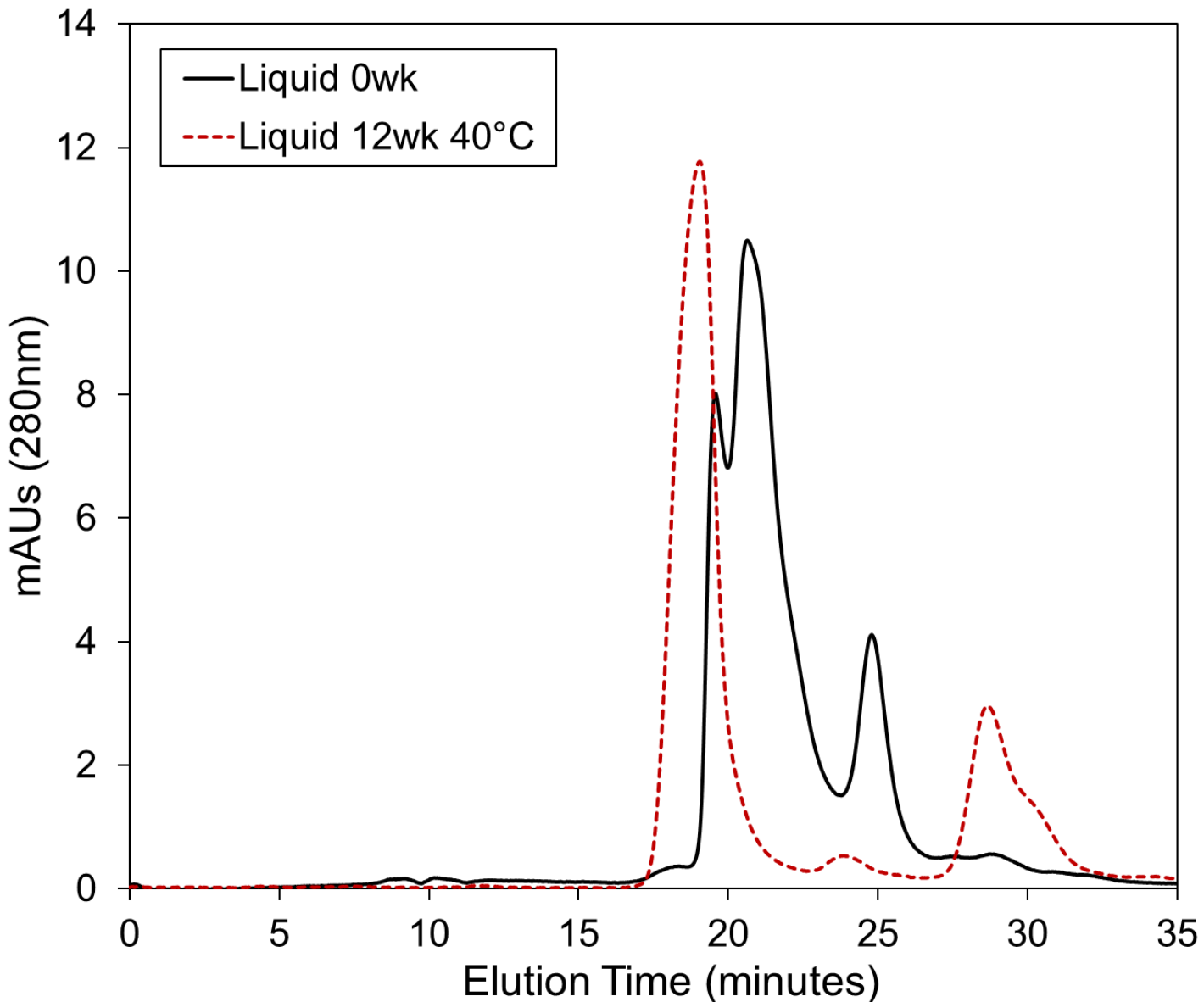
Feldmann et al. 1999

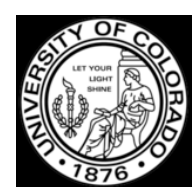
- Lyophilized using fast cooling
- Vaccine stability was tested after 12 weeks of incubation at 40°C
- Vaccine potency was determined by measurement of antibody responses against Ebola glycoprotein in mouse models



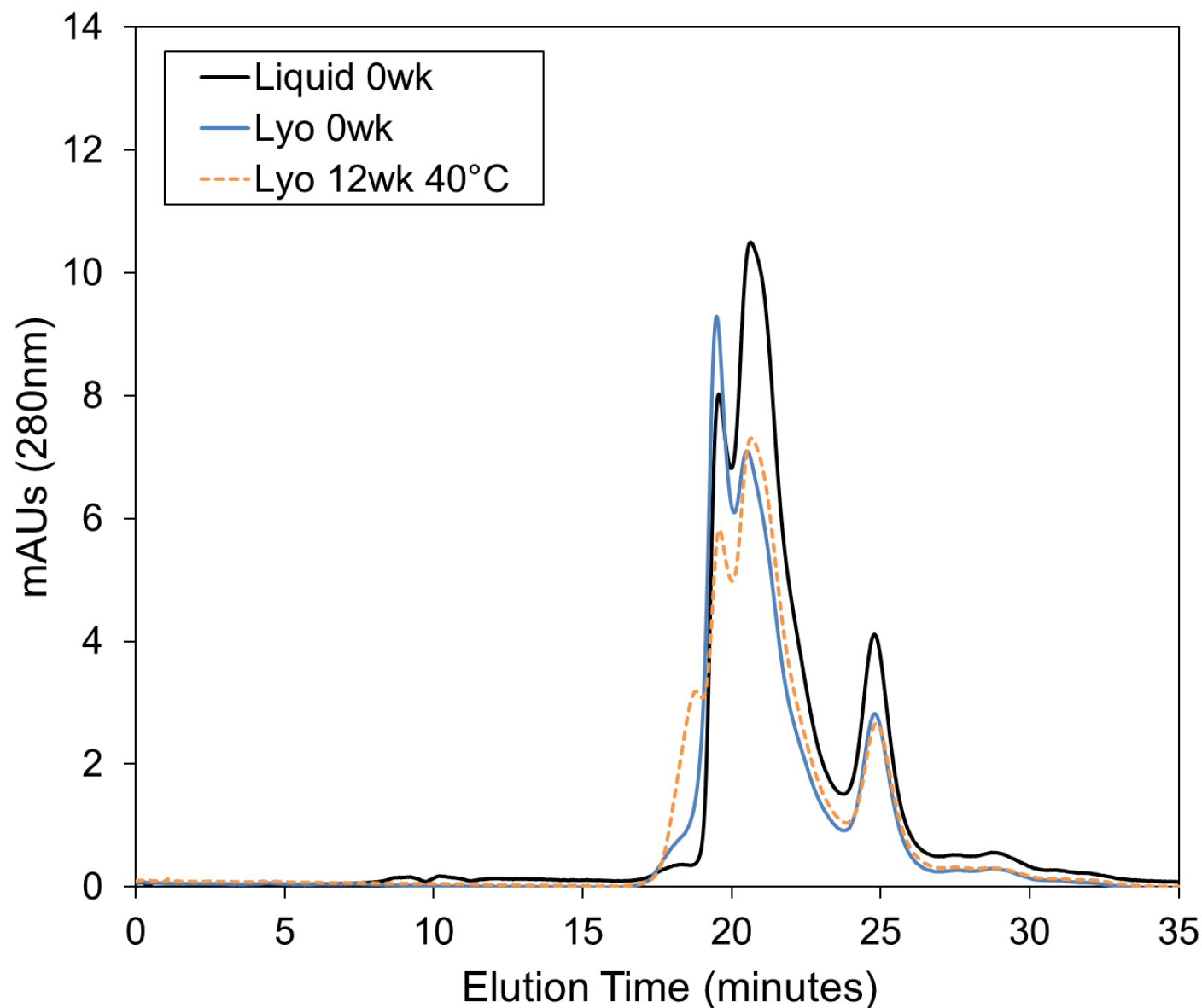


# Ebola glycoprotein is thermally unstable in liquids





# Protein aggregation and degradation was minimized in lyophilized Ebola glycoprotein vaccine formulations





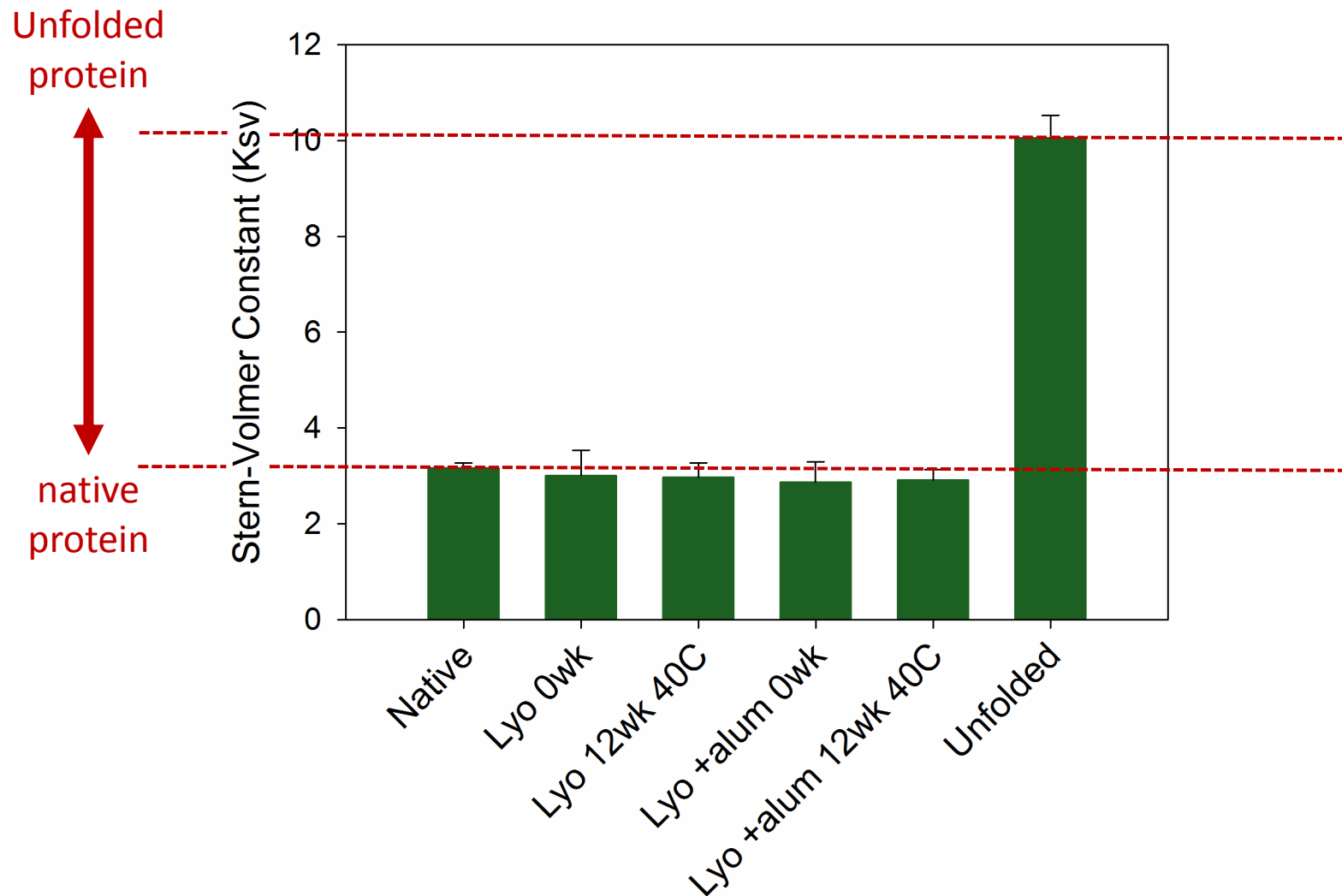
# Minimal aggregation of alum particles was observed after lyophilization of Ebola vaccine formulations

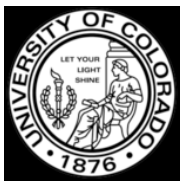
Formulation	Mean particle diameter (ESD)
Liquid E-GP +alum	$2.7 \pm 0.1 \mu\text{m}$
Lyo E-GP +alum	$4.7 \pm 0.1 \mu\text{m}$

\*Particle size was determined using microflow digital imaging with FlowCAM®

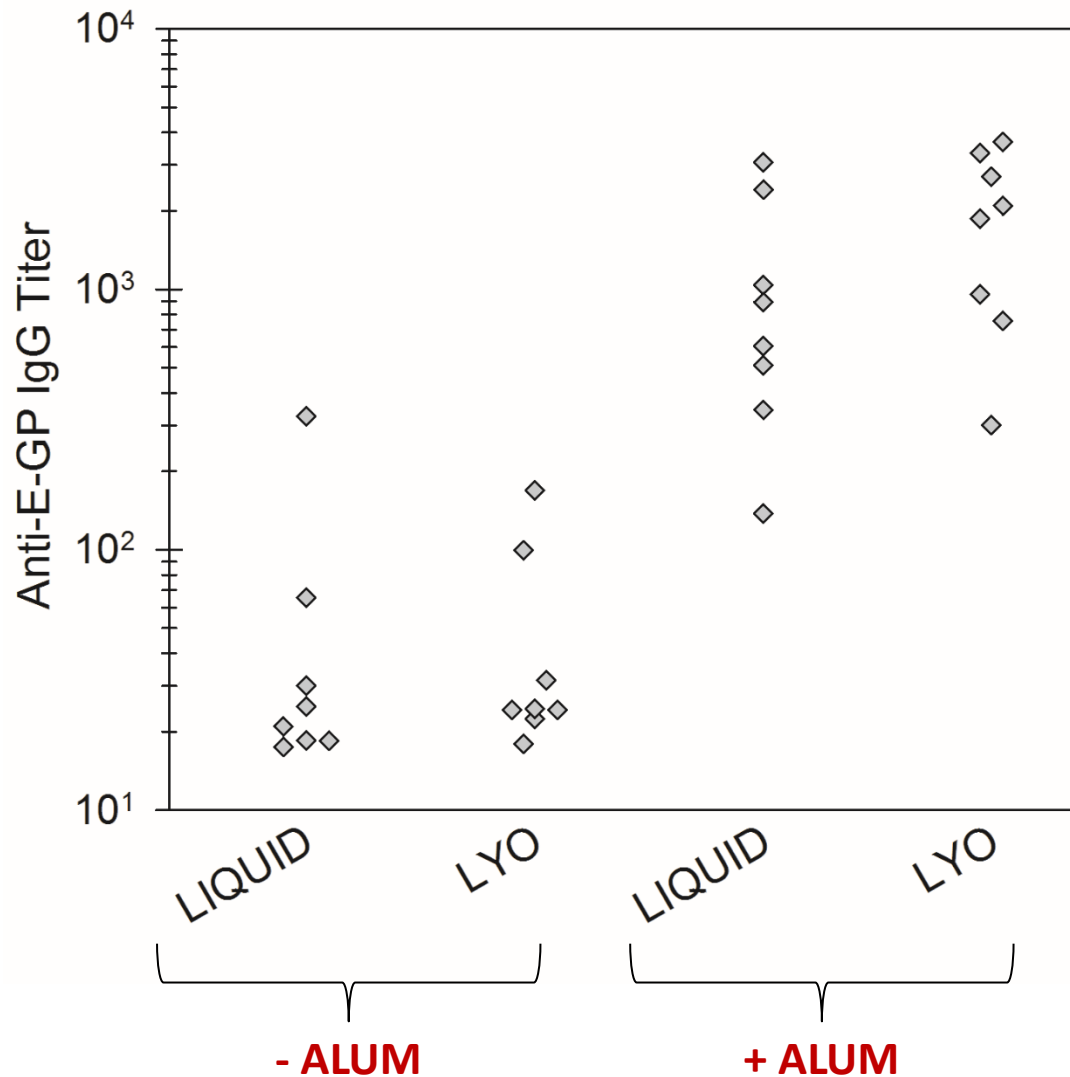


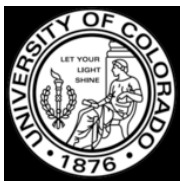
Near-native tertiary structure of Ebola glycoprotein was retained in all lyophilized formulations even after 12 weeks of incubation



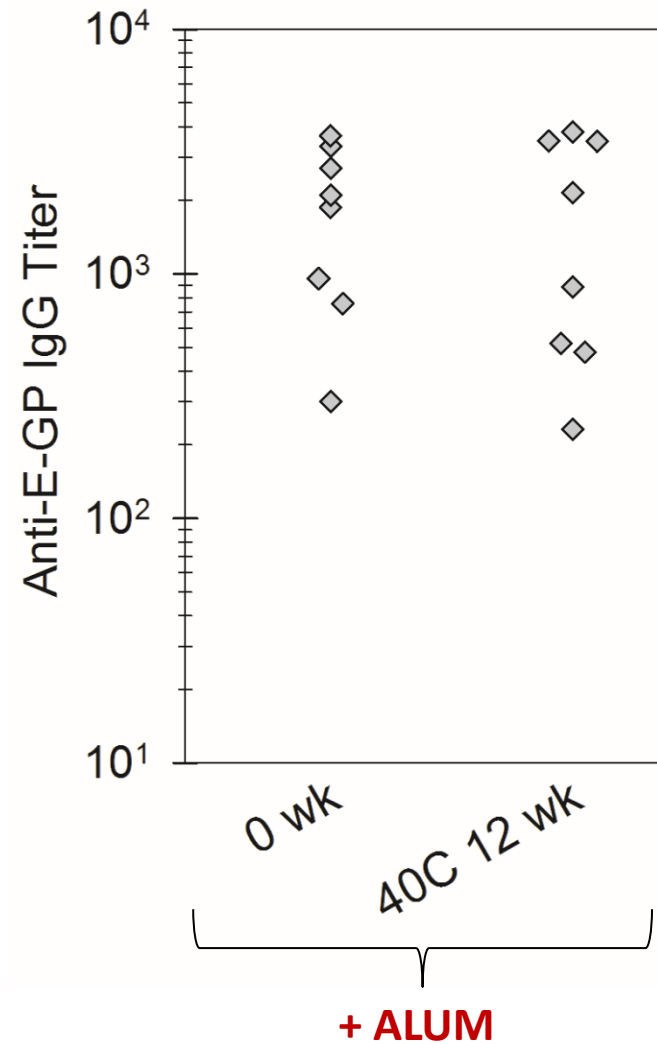
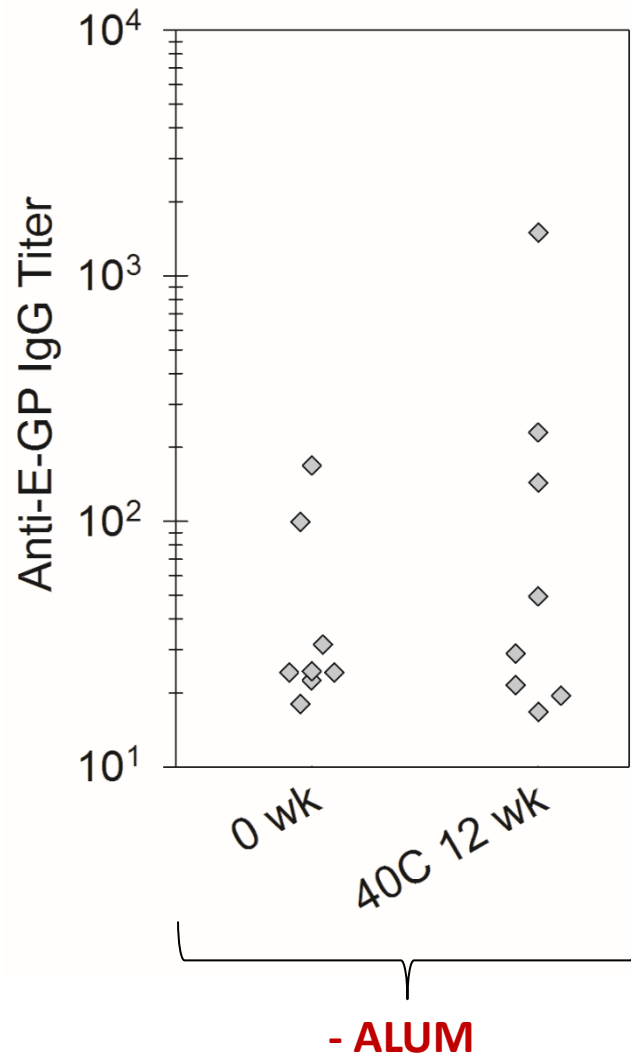


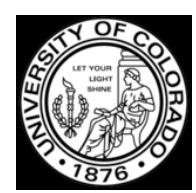
# Ebola vaccines are equally potent in liquid and lyophilized forms





More importantly, lyophilized Ebola glycoprotein vaccines are stable for 12 weeks at 40°C

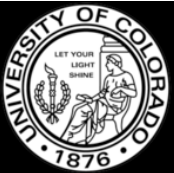




# Conclusions



- Using high trehalose concentrations and fast cooling, aggregation of aluminum salt particles can be avoided in lyophilized Ebola vaccines
- Lyophilized Ebola vaccines are stable at 40°C eliminating the need for a cold chain



# Acknowledgments

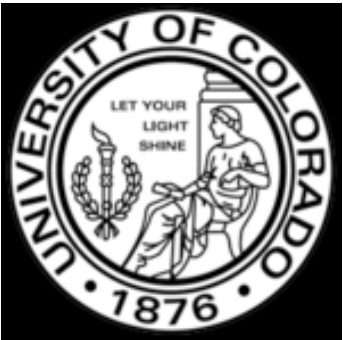
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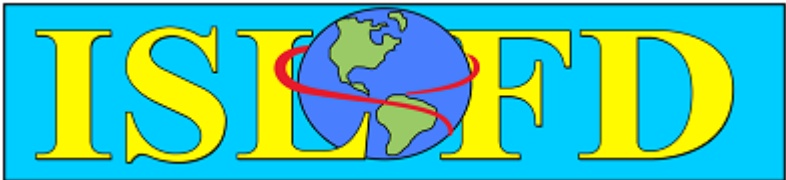
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