Preliminary Stability Assays for a Thermostable, Trivalent Filovirus Vaccine

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Motivation

Zaire ebolavirus (EBOV), *Marburg marburgvirus* (MARV) and *Sudan ebolavirus* (SUDV) are the most prevalent and pathogenic species of filovirus. Our previous work has shown that it is possible to thermostabilize subunit vaccines with glycoproteins from each virus individually through lyophilization. But cross-protection is not expected from a monovalent vaccine, so developing a lyophilized thermostable, trivalent filovirus vaccine is important.

Subunit vaccines are generally safer than other vaccine types because they are non-replicating. However, because subunit vaccines only consist of part of the pathogen, they often require an adjuvant to boost the immune response. Adjuvants are added post-reconstitution of a lyophilized vaccine and require cold-chain storage. Therefore, it would be desirable to have the adjuvant lyophilized within the trivalent vaccine. A single vial thermostable vaccine formulation consisting of all three glycoproteins and adjuvant together will help to potentially eliminate cold-chain requirements to make transportation and administration of the vaccine to remote areas easier.

Methods

Trivalent vaccines were formulated with varying protein levels and ratios, and lyophilized with or without adjuvant in a trehalose (at 9.5% w/v) and ammonium acetate buffer (10mM, pH7). The adjuvant used in this study was CoVaccine HT, which consists of sucrose fatty acid sulfate ester immobilized on oil droplets of a nanoemulsion of squalane-in-water. The formulations studied are outlined below, where the letter C after a formulation number denotes that it was co-lyophilized with CoVaccine HT:

	Formulation	EBOV-GP (µg/mL)	SUDV-GP (µg/mL)	MARV-GP (µg/mL)	CoVaccine (mg/mL)
Monovalent EBOV	1	10			---
	1C	10			3
	2	30			---
Monovalent SUDV	3		10		---
	3C		10		3
	4		30		---
Monovalent MARV	5			10	---
	5C			10	3
	6			30	---
Trivalent equal mass	7	10	10	10	---
	7C	10	10	10	3
	8	30	30	30	---
Trivalent reduced SUDV	9	10	4	10	---
	9C	10	4	10	3
	10	30	12	30	---
Trivalent reduced EBOV & SUDV	11	5	5	10	---
	11C	5	5	10	3
	12	15	15	30	---

To determine vaccine stability, we investigated various stability-indicating assays that focus on tracking protein and adjuvant degradation. Assays to monitor adjuvant degradation included particle size distributions as the submicron size is important for its adjuvanticity and zeta potential to track colloidal stability. Protein stability was observed using size-exclusion chromatography, gel electrophoresis, and flow imaging microscopy to observe any protein aggregation.

Acknowledgements

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Size Exclusion Chromatography

- Previous work showed higher molecular weight species (approx. 9-14 minutes elution time) are more immunogenic and effective at eliciting specific antibodies than monomer (14-17 minutes)
- High R^2 values in Fig 2B indicate compatibility of lyophilizing three proteins together with little to no aggregation
- All trivalent formulations have assembly states with >90% HMW species. This relative area distribution was maintained even when GP concentration increased

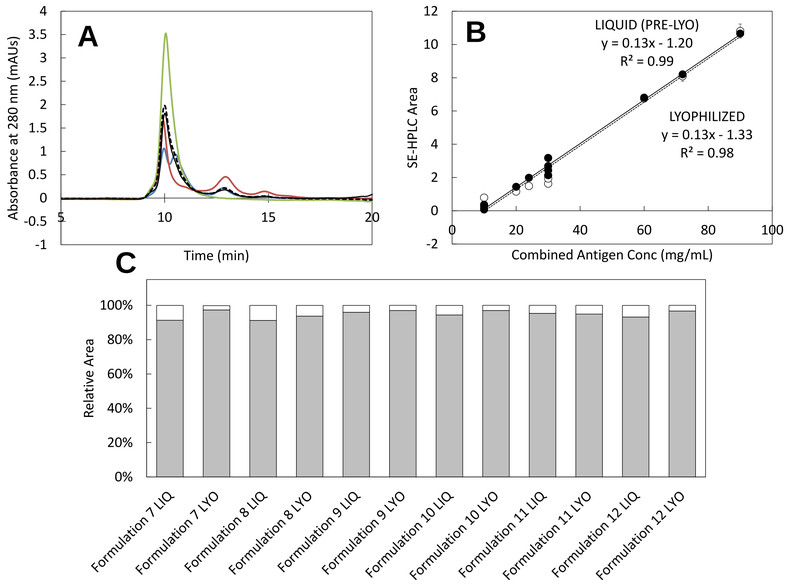

Figure 1. (a) Representative size-exclusion chromatogram of lyophilized monovalent and trivalent formulations for Formulations 2 (blue), 4 (red), 6 (green), and 7 (solid black). The average chromatogram of Formulations 2, 4, and 6 is shown in dotted black line to indicate the proximity of the predicted chromatogram to the actual chromatogram. (b) Area under the entire chromatogram was calculated, with pre-lyophilized liquid formulations shown in solid dots and lyophilized formulations shown in open dots as a function of concentration. (c) Total soluble protein area was divided into HMW species and monomer species and is shown as the percent of the total area for trivalent liquid, pre-lyophilized formulations (LIQ) and lyophilized formulations (LYO).

Gel Electrophoresis

- Main band at ~100 kDa (marked by arrow) present in all samples with or without adjuvant, though weaker in lower concentration formulations

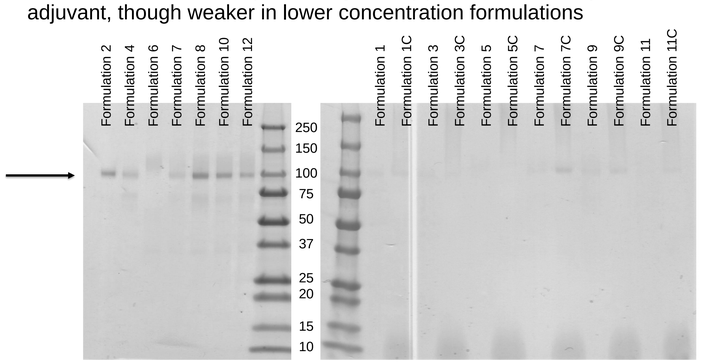

Figure 2. SDS-PAGE with Coomassie staining for all lyophilized formulations, with higher concentration groups on the left gel and lower concentration groups with or without adjuvant.

Flow Imaging Microscopy

- Measurements taken using a FlowCam® VS1 system, which measures particles between 2-2000 um
- Average particle diameter was between 4-5um regardless of formulation
- Particles/mL maintained below 2500, confirming little to no aggregation seen in Fig 1B

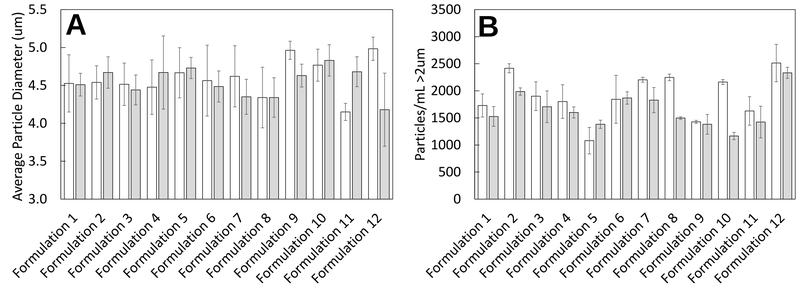

Figure 3. Flow imaging microscopy of trivalent formulations without adjuvant. Liquid samples before lyophilization are shown in white bars and lyophilized formulations are shown in gray. (a) Average particle diameter of particles measured between 2-2000um. (b) Particle count normalized to sample volume.

Adjuvant Characterization

- Zeta potential and particle size distributions were measured using Anton Paar Litesizer 500
- After lyophilization, zeta potential was maintained around -80 mV, which indicates strong colloidal stability
- Measured hydrodynamic diameter was around 110 nm for each adjuvanted formulation and control CoVaccine HT sample. Target manufacturing size of CoVaccine HT is around 100 nm
- Polydispersity index (breadth of particle size distribution) was between 10-20%, though lyophilized formulations were slightly higher than the liquid CoVaccine HT control

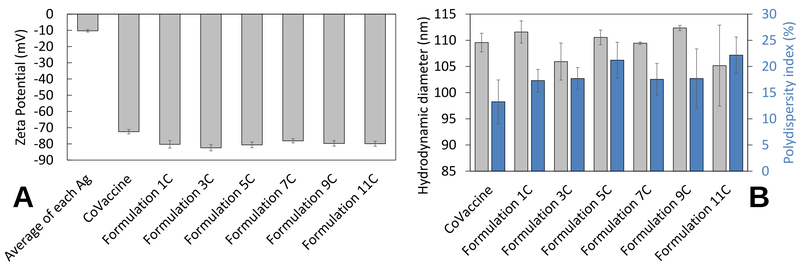

Figure 4. (a) Zeta potential of co-lyophilized adjuvanted formulations compared to the protein on its own (the average of each GP) and CoVaccine as a liquid nanoemulsion. (b) Particle size distributions were measured for lyophilized adjuvanted formulations compared with liquid CoVaccine. Hydrodynamic diameter is shown in gray bars on the left axis and polydispersity index in blue bars on the right axis.

Conclusions and Future Work

- No immediate protein or adjuvant degradation was detectable after freeze-drying
- Work is ongoing on determining immunogenicity of all formulations
  - Top formulations will move forward with accelerated stability studies with incubations for 12 weeks at 25°C and 40°C
- Method optimization to more accurately evaluate lower concentration formulations