Stability and Reconstitution Testing of Investigational Syphilis Vaccine Adjuvant

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Adjuvants are important components of complete vaccines designed to stimulate the immune system to mount a robust response to the main antigen. The adjuvant studied here is PAI-sRI TLR 1/2,4, an emulsion adjuvant that, paired with antigens from the syphilis-causing bacterium *Treponema pallidum*, is being tested in a potential syphilis vaccine. Adjuvants are lyophilized, essentially freeze dried, to increase stability for transport and storage. In order to be combined with the antigen, lyophilized PAI-sRI TLR 1/2,4 must be reconstituted back into liquid form. Reconstitution of PAI-sRI TLR 1/2,4 yields a product much larger than originally manufactured and with a particle size too variable for effective use. We hypothesized that vigorous mixing, called vortexing, would likely be needed to dissociate the adjuvant material and return it to an average particle size and range (PDI) resembling the original (pre-lyophilization) product. PAI-sRI TLR 1/2,4 was reconstituted and passed through a series of experiments, testing multiple vortexing time points and rest periods in order to ensure desirable PDI and particle size. It was found that a 5 minute vortexing time produced the best results, closest to the manufactured product pre-lyophilization. It was also found that the adjuvant was stable for at least 6 hours at room temperature with a high likelihood of remaining stable even longer. This research has shown that PAI-sRI TLR 1/2,4 will be properly sized for an extended period of time post reconstitution as a part of this investigational syphilis vaccine.

A Vaccine for Syphilis

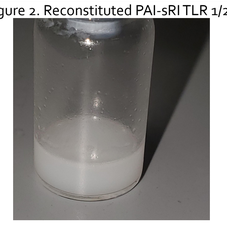
- **Syphilis currently has no approved vaccine available**
- **Vaccines are primarily two parts**
  - Immune response inducing antigen
  - Immune response enhancing adjuvant
- **New antigen derived from *Treponema pallidum* is being researched to be used as a syphilis vaccine**
  - *T. pallidum* is the bacteria that causes syphilis
- **PAI-sRI TLR 1/2,4** is a specialized adjuvant that produces the strongest immune response when combined with this investigational antigen
- In order to be stored properly the adjuvant needs to be lyophilized, what is essentially dry freezing so that it can be stored safely

Adjuvants

- Adjuvants are a component of vaccines which contain immune system stimulants.
- These include compounds that bind Toll-like receptors (TLRs) on immune cells and activate them to produce antibodies or cytokines (Zhao).
- Different TLRs activate different parts of the immune system to have an increased response to vaccine antigens (Zhao).
- **PAI-sRI TLR1/2,4**
  - Hydrophobic
  - Emulsion
  - Key component of investigational syphilis vaccine
- **Important Ingredients**
  - Squalene-Oil that is used for emulsion
  - Polysorbate 80-Detergent for emulsion formation
  - L18-MDP –NOD2 agonist
  - Pam3CSK4 –TLR1,2 agonist
  - TDB-Immune system stimulator
  - 3D-(6-acyl)-PHAD-TLR 4 agonist

Figure 1. Lyophilized PAI-sRI TLR 1/2,4

Figure 2. Reconstituted PAI-sRI TLR 1/2,4



Intro and Methods

- Using a Malvern Zetasizer to take dynamic light scattering measurements of PAI-sRI TLR1/2,4 taken after vortexing to determine optimal vortexing time and if resting the product is necessary or causes quality decline.
- D.nm (diameter in nanometers) and polydispersity index (PDI), the measure of the range of particle sizes, will be used as metrics.
- Anything under 250 d.nm and 0.200 PDI is small enough to not be filtered out when purification occurs.
- We hypothesized that vortexing will eventually lead to an average particle size and range that is similar to the original product. This desirable size will persist without further vortexing for at least two hours.
  1. **Dilution factor (1:500 in dH_2O)**
  2. **Consecutive vortexing, samples taken after each timepoint**
     - 0 seconds, 15s, 1 minute, 2 m, 5m, 30m
  3. **Single vortexing event**
- 5 minutes with 0, 1, 2, 3, 4, 5, and 6 hours of rest

Figure 3. Skin lesions from secondary syphilis (Wikimedia)

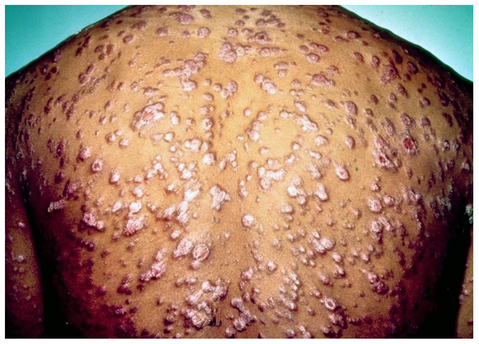


Figure 4. Example micelle and liposome (Wikimedia)

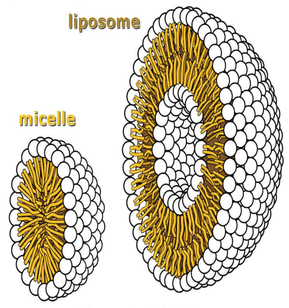
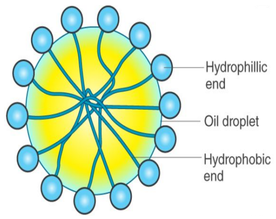


Figure 5. Example emulsion (Wikimedia)



Results

Figure 5. Z average of PAI-sRI TLR 1/2,4 after consecutive vortexing events

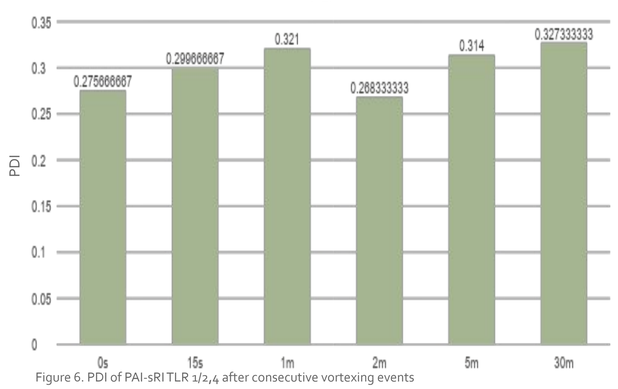


Figure 6. PDI of PAI-sRI TLR 1/2,4 after consecutive vortexing events

Figure 7. *T. pallidum* Micrograph (Wikimedia)

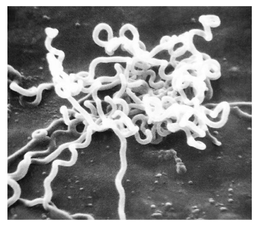


Figure 8. TLR4 Agonist SyMPL

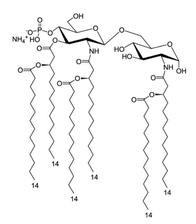


Figure 9. Consecutive vortexing data

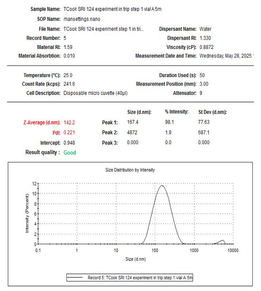


Figure 10. Malvern Zetasizer

Conclusions

- After 5 minutes of vortexing, z average returns almost to post production size with very desirable PDI.
- Longer vortexing potentially causes emulsion to break down into larger and smaller droplets which is undesirable.
- Rest is unnecessary to achieve proper particle size or distribution.
- Product is shelf stable for at least 6 hours post vortexing. Further testing needs to be done to find end of stability.
- Further testing is also required to find an optimal vortexing speed, as speed was kept constant.

Lipid Vesicles for Drug Delivery

- **Micro fluidization and sonication**
- **Micelles**
  - Monolayer
  - Hydrophobic drug delivery
- **Vesicles**
  - Bilayer
  - Hydrophilic drug delivery
- **Emulsion**
  - Monolayer
  - Hydrophobic drug delivery
  - Requires a detergent to form

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