Antigen Preparation Prior to Injection

1.) Make 1ml of final emulsion. This is enough for two boosts. Freeze at -20^{o} C after the first boost, and reemulsify before injecting again.

2.) Keep emulsifying needles at 4^{o} C well labeled, and reuse the same one for the same antigen so we don't have to clean each time.

3.) Make antigen/adjuvant etc. to mix in 3ml syringe (luer-lok). Use pipetteman to add antigen into syringe "nose" after adding Freund's adjuvant by sucking up into syringe through a 20g or larger bore needle.

4.) When using Complete Freund's adjuvant (Gibco), warm first and make sure BCG are suspended. When using Incomplete Fruend's, warm it so it sucks well through a needle.

5.) Inject approximately 100µg KLH-linked peptide for each injection. 200 µg aliquots of KLH-linked peptide are stored at -20^{o} C/-80^{o} C in 500 µl size " O-ring " microcentrifuge tubes. 200 µg aliquots have ~ 200-400 µl liquid.

6.) Mix - Antigen (~ 300-400 µl)
PBS up to 500 µl total
Freunds 500 ml or a little more if need to emulsify well

Total ~ 1 ml.

7.) In the past, we have injected ~ 250 µl / site, 2 sites per time. New Emory guidelines say 100 µl of emulsion/site for rabbits at Emory.