

## Mouse CIA – A Visual Guide

For Research Use Only - Not Human or Therapeutic Use

### MICE: CHOOSE 1 STRAIN

DBA/1 (DBA) mice **OR** C57BL/6 (B6) mice

- 7-8 weeks old
- SPF housing conditions
- High fat diet

### RECOMMENDED ADJUVANTS & COLLAGENS: CHOOSE 1 ADJUVANT + 1 COLLAGEN

Catalog #	Adjuvants	Catalog #	Collagens
7002	Incomplete Freund's Adjuvant (IFA) (DBA - Booster only)	20011	Chick Type II Collagen, Lyophilized (DBA or B6)
7008	Complete Freund's Adjuvant (CFA), 1 mg/ml (DBA)	20012	Chick Type II Collagen, 2 mg/ml Solution (DBA or B6)
7001	Complete Freund's Adjuvant (CFA), 4 mg/ml (DBA)	20021	Bovine Type II Collagen, Lyophilized (DBA)
7023	Complete Freund's Adjuvant (CFA), 5 mg/ml (B6)	20022	Bovine Type II Collagen, 2 mg/ml Solution (DBA)

### IMMUNIZATION SCHEDULE: CHOOSE 1 OPTION

#### OPTION 1: Single Immunization Protocol (DBA mice)

**Day 0:** Subcutaneous injection: Collagen emulsion with CFA: 4 mg/ml *M. tuberculosis*

**Day 0 - 28:** Score arthritis every 7 days

**Day 28 - 56:** Score arthritis every 2-3 days: Arthritis incidence 90 - 100%

**Day 56:** End of experiment.

**OR**

#### OPTION 2: Booster Immunization Protocol

**Day 0:** Subcutaneous injection: Collagen emulsion with CFA: 1 mg/ml (DBA) or 5 mg/ml *M. tuberculosis* (B6)

**Day 0 - 28:** Score arthritis every 7 days

**Day 21:** Subcutaneous injection (booster): Collagen emulsion with IFA (DBA) or CFA: 5 mg/ml *M. tuberculosis* (B6)

**Day 28 - 56:** Score arthritis every 2-3 days: Arthritis incidence 90 - 100% (DBA), 50-70% (B6)

**Day 56:** End of experiment

**PREPARE EMULSION WITH A HOMOGENIZER: DO NOT USE THE DOUBLE-SYRINGE METHOD!**

Figure 1

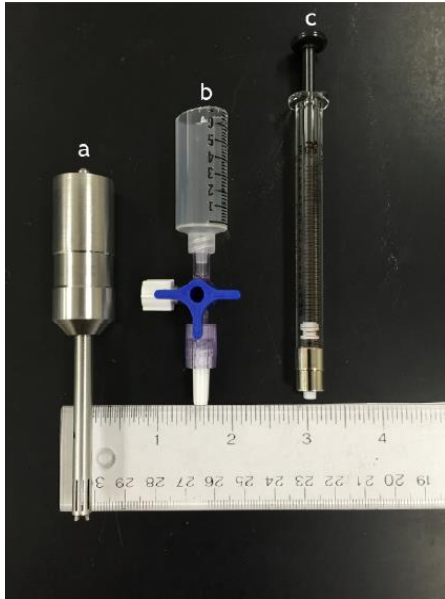
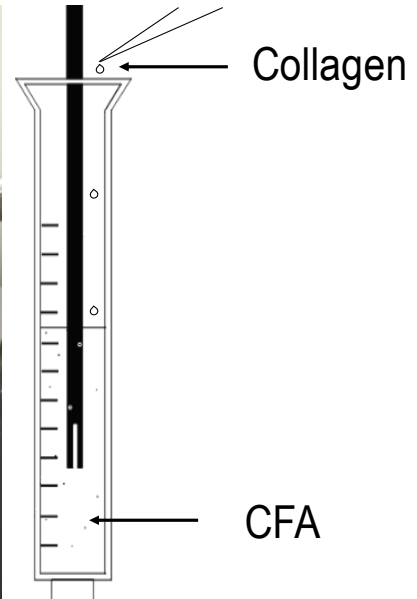


Figure 2



Figure 3

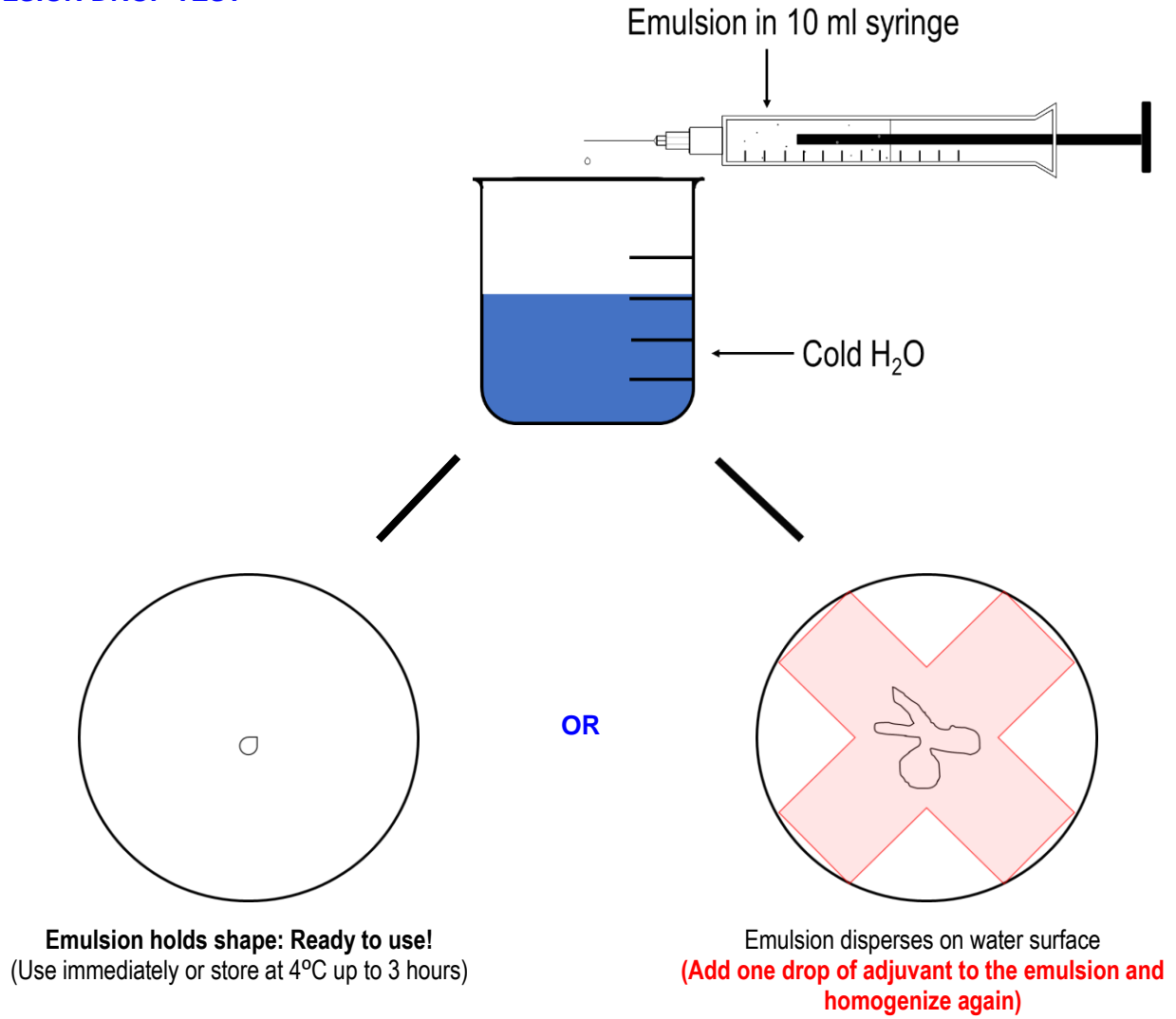


## Procedure\*:

1. Gather materials (Figure 1. Homogenizer blade (a), 10 ml syringe with a 3-way stopcock (b), and 1 ml Hamilton syringe (c)).
2. Lock stopcock.
3. Keep setup on ice (Figure 2).
4. Add CFA to syringe.
5. Add an equal volume of collagen solution, drop-wise (Figure 3).
6. Mix at 10,000 - 30,000 rpm for 2 minutes. Move the syringe up and down while mixing.
7. Cool syringe on ice for 5 minutes.
8. Mix at 10,000 - 30,000 rpm for 2 minutes. Move the syringe up and down while mixing.
9. Evaluate emulsion with the drop test (next page).
10. Transfer the emulsion to a Hamilton syringe using the 3-way stopcock.

\*For more information, please see Chondrex, Inc's [Mouse CIA Protocol](#)

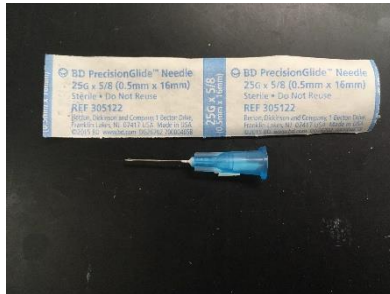
**EMULSION DROP TEST**



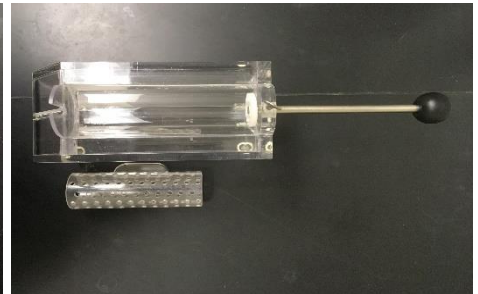
**IMMUNIZATION**



(a) Emulsion in Hamilton Syringe  
**(no air bubbles!)**

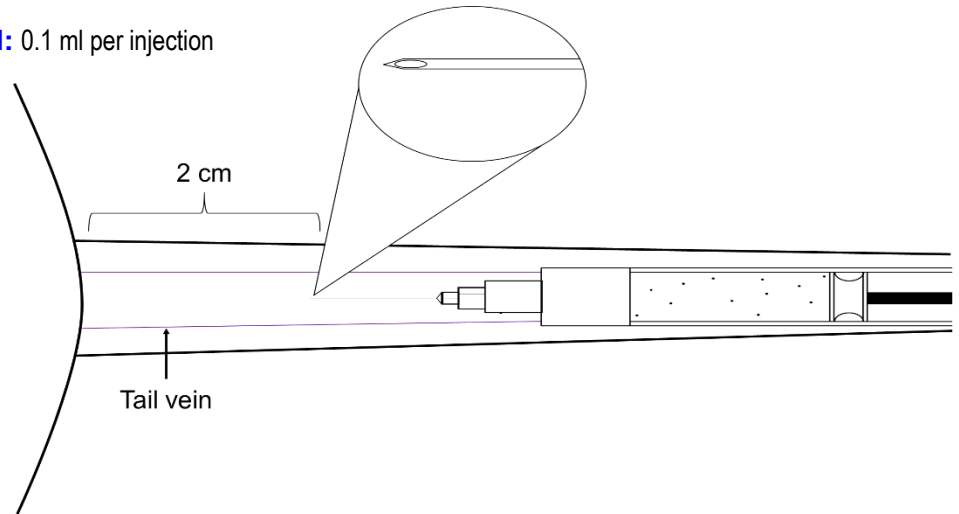


(b) Needle (25 or 27-gauge x 5/8")

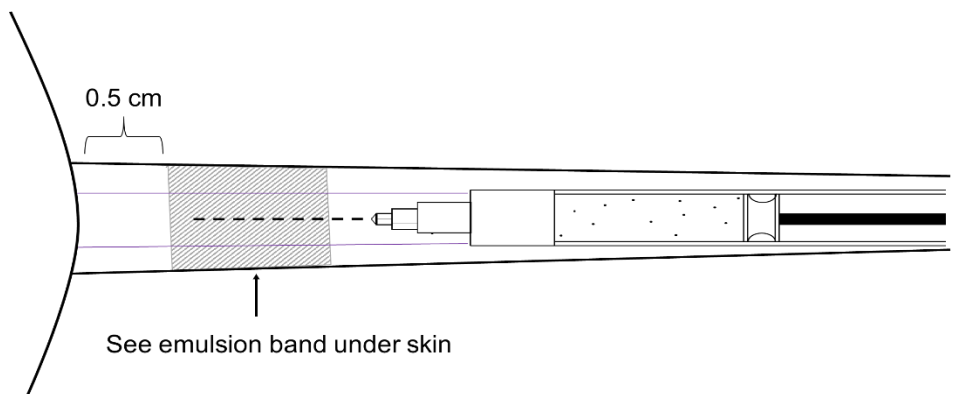


(c) Restraining Device

**SUBCUTANEUS INJECTION:** 0.1 ml per injection



**AFTER INJECTION:**



**DO NOT USE  
IP (a) OR IV (b)  
INJECTIONS TO  
IMMUNIZE MICE!**

(a)

