

Table 7A. Immunization Protocols

Step 1: Immunization of Allotype Donor

Antigen	Protocol No.	Dose and Timing	Bleed
B. pertussis heat killed ***	I	Day 1: Total of 2 x 10 ⁹ bacteria in 0.2 ml saline injected i.p. and in 4 places s.c. Day 3 and 5: same but in 4 places s.c. Day 21 and 37: same as day 1 <i>Do not infect on day 7-21 as the mice will die</i>	Pool bleed Day 25 Day 32 Day 45 Discard
B. pertussis heat killed	III†	Mice supplied with drinking water containing 2 x 10 ¹⁰ bacteria/500 ml for 30 days. (5 mice per cage)	Day 30: bleed. Check titer by bacterial agglutination. Check class of antibody. Pool bleed weekly
H-2	III	1/5 H-2 spleen/animal in isotonic MEM injected i.p. Boost with same monthly.	Bleed 3 weeks after injection then, weekly

Step 2: Immunization for Recipient for Production of Anti-allotype Serum

Dose and Timing	Bleed
Day 1, 3, 5, 22, 26, 50, 57: 10 ⁹ bacteria + 20 µl antiserum from Step 1 in 0.2 ml saline per mouse. Mix at room temperature and inject i.p. Boost as on day 1. *Note: we usually use 2 µl/mouse for injection protocol. If mice are weakly positive on day 25, boosts are changed to 10-20 µl/mouse.	Day 64 and weekly bleed individually and test, or pool bleed depending on circumstances. Boost when titers go down. 10 days after boost, bleed and test.
Day 1, 3, 5, 21, 37 and monthly booster. Incubate serum from Step 1 with number of bacteria just sufficient to completely absorb anti pertussis activity, 3 hrs @ 37° and overnight in cold. Wash 3X with cold saline. Resuspend in saline to 2 x 10 ¹⁰ /ml. Mix with equal volume of FCA*. Inject 0.2 ml. i.p. 4 places s.c.	Day 45 and weekly test individually
Day 1: 20 µl antiserum from Step 1 + 20 µl FCA* injected i.p. and 4 places s.c. Day 21, 28 and monthly booster: 10 µl in 0.2 ml saline i.p.	Day 35 bleed and pool bleed all positive animals weekly

† Protocol established by Dr. Tohru Masuda, this laboratory.

* Freund's Complete Adjuvant

** Phosphate buffered solution.

‡ Incubate antiserum plus B. pertussis, wash and use with sera whose complexes show high levels of XA and low levels of other immunoglobulins.

*** Kindly supplied by Lederle Laboratories. Pearl River, New York 10965

* 20 µl of antiserum + 80 µl saline + 100 µl C.F.A.

40 µl / Injection site ie 4 sc. + 1 I.P.