ASEAN STANDARD REQUIREMENTS FOR AVIAN INFLUENZA VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

The master and working seed viruses are produced in Specific-Pathogen-Free (SPF) embryonated chicken eggs in a seed lot system. The seed viruses must satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seed viruses are lyophilized and kept at 2 to 8°C. Seed viruses in liquid shall be stored at -50^{o}C or lower.

2. PRODUCTION SUBSTRATE

Embryonated chicken eggs used throughout production of the vaccine must be derived from SPF flocks complying with tests which appear as *Appendix 1* or healthy flocks.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, fungi, Salmonella and Mycoplasma by the methods which appear as *Appendix 2*. However, tests for Salmonella and Mycoplasma may be carried out on bulk samples. The test for Mycoplasma may be omitted if it can be demonstrated that the inactivating agent inactivates Mycoplasma.

2. PURITY TEST

Bulk production samples should be tested for absence of extraneous viruses by the egg inoculation test or the chicken inoculation test or the tissue culture inoculation test, and the test for Avian Leucosis virus using the methods which appear as Appendix 3. This test may be omitted if it can be demonstrated that the inactivating agent inactivates avian leucosis viruses.

3. INACTIVATION

At least ten 9-11 days old embryonated chicken eggs susceptible to avian influenza are each inoculated with 0.2 ml of the inactivated product by the allantoic sac

route. The eggs are incubated in 37^{o}C for a minimum of 7 days. Three passages are carried out. There should be no evidence of avian influenza virus.

4. SAFETY

At least 10 susceptible chickens of the minimum age for which the vaccine is intended are each vaccinated with 2 doses of vaccine by the recommended route. and observed for a minimum of 14 days. No abnormal local or systemic reactions attributable to the vaccine should occur in any of the chickens.

5. POTENCY

Bulk or final container samples should be tested by one or more of the following methods:

a. Three groups of 20 susceptible chickens aged 21-28 days are inoculated intramuscularly with volumes of vaccine equivalent to 1/25, 1/50 and 1/100 of a dose. At least 14 days post-vaccination, the vaccinates together with 10 unvaccinated controls are challenged by intramuscular inoculation with 10^{4} LD50 of virulent AI virus and observed for 21 days for death and clinical signs. Detection of virus replication in respiratory (oropharyngeal or tracheal) and intestinal (cloaca) tracts should be carried out for at least 7 days post-challenge. There should be no evidence of virus replication. The vaccine should contain at least 50 PD_{50}. (Remarks: This test should be performed under Biosafety Level 3).

b. Potency of avian influenza vaccine can be evaluated by testing the ability of the vaccine to induce a significant HI titre in susceptible birds. Protective immune response must be correlated to an efficacy test with live challenge (Appendix 4).

III. OTHER REQUIREMENTS

The vaccine should comply with the General Requirements for Veterinary Vaccines that appear as *Appendix 4.*