REVISED
ASEAN STANDAR REQUIREMENT FOR
PORCINE ACTINOBACILLUS PLEUROPNEUMONIAE BACTERIN

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED BACTERIA

The master and working seeds of *Actinobacillus pleuropneumoniae* should be produced in suitable media in a seed lot system. The seeds shall satisfy sterility, purity, safety and potency tests before they are used for vaccine production. The seeds are preserved and stored under suitable validated systems, such as the lyophilized seed kept at 2 to 8°C and the liquid form of seed bacteria stored at -20 °C or lower.

2. PRODUCTION SUBSTRATE

The medium should contain appropriate nutrients to allow optimal growth of the bacteria.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, fungi and mycoplasma by methods that appear as *Appendix 2*.

2. PURITY TEST

Bulk product samples should be tested as follows:
Gram stained smears of live cultures are examined for morphological characteristics. Only *A. pleuropneumoniae* shall be present.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *A. pleuropneumoniae*. No *A. pleuropneumoniae* or any other bacteria should be detected.

4. SAFETY TEST

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

a) At least 8 mice between 18-22 g are each inoculated with 0.5 ml of the vaccine by the intraperitoneal or subcutaneous route and observed for at least 7 days. No unfavorable reaction attributable to the product should occur in any of the mice.

b) At least 2 guinea pigs are each inoculated with 2 ml of the vaccine by the intramuscular or subcutaneous route and observed for at least 7 days. No unfavorable reaction attributable to the product should occur in any of the guinea pigs.

c) At least 2 healthy, susceptible seronegative pigs of the minimum age for which the product is intended are each inoculated with two doses of the vaccine by the recommended route. Observe the animals for at least 14 days. Record body temperature the day before vaccination, at vaccination, 2 h, 4 h and 6 h later and then daily for 2 days. No abnormal local or systemic reaction attributable to the product should occur; a transient temperature increase not exceeding 2°C may occur.

The bulk product samples should be tested for bacterial endotoxin by suitable methods such as the following:

A test for bacterial endotoxins is carried out on the final bulk or, where the nature of the adjuvant prevents performance of a satisfactory test, on the bulk antigen or mixture of bulk antigens immediately before addition of the adjuvant. The maximum acceptable amount of bacterial endotoxins is that found for a batch of vaccine that has been shown satisfactory in the following test: Ten healthy, susceptible seronegative pigs of the minimum age for which the product is intended, are each inoculated with two doses of the vaccine by the recommended route. Observe the animals for at least 14 days. Record body temperature the day before vaccination, at vaccination, 2 h, 4 h and 6 h later and then daily for 2 days. No abnormal or local systemic reaction occurs; the average temperature increase for all animals does not exceed 1.5°C and no animal shows a rise above 2°C. The method chosen for determining the amount of bacterial endotoxin present in the vaccine batch used in the safety test for determining the maximum acceptable level of endotoxin is used subsequently for batch testing.

.

5. POTENCY TEST

Bulk or final container samples should be tested by an appropriately validated potency test such as that which follows:

a) Healthy mice are each inoculated with not more than 1/40 of the pig dose of the bacterin by the intraperitoneal route. At least 13 days later the mice may be re-inoculated as above. At least 10 days after the final inoculation, the mice are divided into groups of 20 vaccinates and 20 unvaccinated controls per group for each serotype of *A. pleuropneumoniae* contained in the vaccine. Each group is challenged with a virulent homologous serotype of *A. pleuropneumoniae* and observed for a minimum of 10 days. At least 80% of the control should die and at least 75% of the vaccinates should survive. This test shall only be valid if a study had been carried out to establish the correlation between the mouse-dose and the pig-dose.

b) One mouse dose, equivalent to 1/6 of the pig dose of the test vaccine and a Standard Reference vaccine (Standard) is prepared. At least 16 mice per group are each inoculated by the intraperitoneal route with a decreasing mouse dose of the vaccine and the Standard, respectively.

At least 14 days later the mice are re-inoculated as above. At least 6 days after the final inoculation the vaccinates together with 10 unvaccinated controls are challenged with virulent *A. pleuropneumoniae* and observed for a minimum of 10 days. The relative potency of the test vaccine is compared with the Standard used. This test shall only be valid if a study had been carried out to correlate relative potency value to pig protective doses.

c) At least 7 seronegative pigs of the minimum age for which the product is intended, are each inoculated with the recommended schedule. At least 7 unvaccinated pigs of the same age are used as controls. 3 weeks after the last vaccination, the pigs are challenged by a suitable route and quantity of a serotype of *A. pleuropneumoniae.* The animals are observed for at least 7 days; to avoid unnecessary suffering, severely ill control animals are killed and are then considered to have died from the disease. Kill all surviving animals at the end of the observation period. Carry out a post-mortem examination on all animals. Examine the lungs, the tracheobronchial lymph nodes and the tonsils for the presence of *A. pleuropneumoniae*. Evaluate the extent of lung lesions at post-mortem examination. Each of the 7 lobes of the lungs is allotted a maximum possible lesion score of 5. The area showing pneumonia and/or pleuritis of each lobe is assessed and expressed on a scale of 0 to 5 to give the pneumonic score per lobe (the maximum total score possible for each complete lung is 35). Calculate separately for the vaccinated and the control animals the total score (the maximum score per group is 245, if 7 pigs are used per group).

The vaccine complies with the test if the vaccinated animals, when compared with controls, show lower incidence of: mortality; typical clinical signs (dyspnoea, coughing and vomiting); typical lung lesions; re-isolation of *A. pleuropneumoniae* from the lungs, the tracheobronchial lymph nodes and the tonsils. Where possible, the incidence is analysed statistically and shown to be significantly lower for vaccinates.

III. OTHER REQUIREMENTS

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