



ASEAN STANDARD REQUIREMENT FOR PORCINE MYCOPLASMA HYOPNEUMONIAE VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED MYCOPLASMA

The master and working seeds of *Mycoplasma hyopneumoniae* 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 should be kept at 2 to 8°C. The liquid form of seed should be stored at -70 °C or lower.

2. PRODUCTION SUBSTRATE

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

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

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

2. PURITY TEST

Bulk Production samples should be tested as follows:

- a. Live cultures are examined for morphological characteristics using Diene's stain and Gram stain or other appropriate staining methods. Only *M. hyopneumoniae* should be present.
- b. Live cultures should be streaked onto appropriate solid media, incubated under appropriate conditions allowing pure growth of *M. hyopneumoniae* to be observed.

3. INACTIVATION TEST

The bulk or final product is tested for inactivation by culturing in a medium known to support growth of *M. hyopneumoniae*. Incubation under suitable conditions is carried out at 35°C – 37°C for 14 days. After 14 days transfer a suitable aliquot from the first sample to fresh media and incubate under the same condition for a further 14 days. No *M. hyopneumoniae* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested as follows:

At least 2 healthy, susceptible 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 21 days. No abnormal local or systemic reaction attributable to the product should occur in any of the pigs.

5. POTENCY TEST

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

At least 20 healthy, 6 to 7 weeks old mice per group are each inoculated with not more than 1/10 dose of the Test Vaccine and the Reference Standard Vaccine by the recommended route. 3 to 4 weeks later, the mice may be re-inoculated as above. At least 14 days after the last inoculation the vaccinated mice together with 10 unvaccinated controls are each serologically tested for antibodies against each antigenic component in the vaccine by using an ELISA Procedure. The control should remain negative. The test will be considered satisfactory if a Test Vaccine demonstrates an average mean optical density value equal to, or greater than, the Reference. Or using a one-tailed Student's T test or other appropriate statistical methods, the Test Vaccine must not be significantly ($p \leq 0.05$) lower than the Reference Standard Vaccine.

III. OTHER REQUIREMENTS

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

