



ASEAN STANDARD REQUIREMENT FOR AVIAN E.COLI VACCINE, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENT

1. SEED BACTERIA

The master and working seeds of *Escherichia coli* 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 -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:

a. Gram stained smears of live cultures are examined for morphological characteristics. Only *E. coli* shall be present.

b. The appropriate strain of *E. coli* is confirmed by suitable serological or immunochemical methods. Only vaccine strains of *E. coli* 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 *E.coli*. No *E.coli* or any other bacteria should be detected.

4. SAFETY TEST

Final container samples should be tested as follows:

At least 10 susceptible chickens of the minimum age for which the vaccine is intended, are each vaccinated with at least two doses of the vaccine by the recommended route and observed for at least 21 days. No abnormal local and systemic reaction attributable to the product should occur in any of the chickens.

5. POTENCY TEST

Final container samples should be tested as follows:

At least 20 susceptible chickens of the minimum age for which the vaccine is intended, are each vaccinated with 1 dose of vaccine by the recommended route. At least 21 days post vaccination, the vaccinated chickens together with 10 unvaccinated controls are each challenged with an appropriately selected virulent *E. coli* and observed for at least 14 days. In a valid test at least 70% of vaccinated chickens should be protected and at least 70% of the control should die.

III. OTHER REQUIREMENTS

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

