ASEAN STANDARD REQUIREMENTS FOR FOOT-AND-MOUTH DISEASE VACCINE FOR PIGS, INACTIVATED

I. SEED AND PRODUCTION SUBSTRATE REQUIREMENTS

1. SEED VIRUS

Master seed virus should be selected based on ease of growth in cell culture, virus yield, stability, broad antigenic spectrum and in accordance with the epidemiological importance of each variant. It should be characterized and distributed by the official control laboratories in regions where such laboratories exist. The virus must satisfy sterility, purity, safety, and potency tests before they are used for vaccine production. The master and working seed viruses are produced in primary cells or cell lines in a seed lot system. Seed viruses must be stored at low temperature (eg. -70°C) or freeze dried.

2. PRODUCTION SUBSTRATE

Suspension or monolayer cells used throughout the production of the vaccine should be free from cytopathogenic and haemadsorbing agents.

II. QUALITY CONTROL REQUIREMENTS

1. STERILITY TEST

Final container samples should be tested for absence of bacteria, Mycoplasma and fungi by methods that appear as Appendix 2. Test for Mycoplasma may be carried out in bulk samples.

2. PURITY TEST

Bulk container samples should be tested for absence of cytopathogenic and haemadsorbing agents and antibody against nonstructural proteins.

3. INACTIVATION TEST

The test for inactivation is an in-process test that should be carried out for every batch of antigen. Following inactivation, a sample of each batch of inactivated antigen representing at least 200 doses should be tested for freedom from infectious virus by inoculation of sensitive monolayer cell cultures, preferably of the same origin as those used for the production of antigen. It may

be preferable to concentrate the antigen to do this, in which case it must be shown that the concentrated material does not interfere with the sensitivity or reading of the assay. The cell sheets are examined daily over a period of 2-3 days, after which the spent medium is transferred to fresh monolayers and the original monolayers are replenished with fresh medium. Using this method, traces of live virus can be amplified by the passage procedure and detected to the basis of CPE observed. At least three passages of the original virus preparation are commonly used. A variant on this method is to freeze-thaw the old monolayers to release intracellular virus, which can be detected by further passage.

4. SAFETY TEST

Final container samples should be tested as follows:

4.1. For registration of vaccine

At least eight pigs from FMD-free areas of the minimum age for which the vaccine is intended should be used. If not available from FMD-free areas, FMD-seronegative pigs can be used. Each pig is inoculated by the recommended route of administration with one dose and 14 days after first injection repeat with 2 doses of the vaccines by recommended route. The repeat dose test should correspond to the primary vaccination schedule (e.g. two injections) plus the first vaccination (i.e a total of three injections). The pigs are observed for clinical signs for local and systemic reactions to vaccination for at least 14 days after each injection. The vaccine is considered satisfactory if no animal develops clinical signs for abnormal local and systemic reactions attributable to the vaccine.

4.2. For final product batch release

For the purposes of batch release, at least two healthy pigs from FMD free areas should be used. If not available from FMD-free areas, FMD sero-negative pigs can be used. Target animals are inoculated by the recommended route of administration with at least two doses of vaccine. The animals are observed for local and systemic reaction of vaccination for no fewer than 14 days. The vaccine complies with the test if no animal shows notable signs of disease or dies from causes attributable to the vaccine.

5. POTENCY TEST

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

5.1. PD_{50} test: FMD-seronegative pigs of at least 2 months of age, obtained from areas free from FMD that have not previously been vaccinated against FMD, should be used. If not available from FMD free areas, FMD-seronegative pigs can be used. Three groups of no fewer than five pigs per group should be vaccinated by the route recommended by the manufacturer. The vaccine should be administered at different doses per group by injecting different volumes of the vaccine. For example, if the label states that the injection of 2 ml corresponds to the administration of 1 dose of vaccine, a 1/4 dose of vaccine would be obtained by injection 0.5 ml, and a 1/10 dose would be obtained by injection 0.2 ml. These animals and a control group of two non vaccinated animals are challenged either 3 weeks (aqueous) or up to 4 week (oil) after vaccination with a suspension of pig virus that is fully virulent and appropriate to the virus types in the vaccine under test by inoculating the equivalent of a total of 10,000 PID_{50}, intramuscular route or by intradermal injection into the heel bulb of one foot. Animals are observed daily for 10 days. Control animals should develop clinical signs on more than one foot. From the number of animals protected in each group, the PD_{50} content of the vaccine is calculated. There are a variety of methods for calculating PD_{50}, but procedures based on the Kärber method are generally preferred when interpreting PD_{50} estimates calculated in this way. The vaccine should contain at least 3 PD_{50} per dose for pigs.

5.2. PGP test (percentage of protection against generalized foot infection): For this method, a group of 16 FMD seronegative pigs of at least 2 months of age, with the same characteristics described for the PD_{50} test, are vaccinated with a pig dose by the route and in the volume recommended by the manufacturer. These animals and a control group of two non-vaccinated animals are challenged 4 weeks or more after vaccination with the challenge strain, which is a suspension of pig virus that is fully virulent and appropriate to the virus types in the vaccine under test by inoculating a total of 10,000 PID_{50}, intramuscularly or by intradermal injection into the heel bulb of one foot. Unprotected animals show lesions at sites other than the foot within 10 days after inoculation. Control animals must develop lesions on at least one foot; the vaccine should protect at least 12 animals out of 16 vaccinated. Animals are observed at 10 days after challenge.

5.3. The potency testing standard is the challenge test. However, for batch release indirect tests can be used for animal welfare considerations, as long as correlation has been validated to percentage of protection in the target animal. Frequently indirect potency tests include antibody titration after vaccination of target species. Ideally, indirect tests are carried out for each

strain for one species and each formulation of vaccine to establish correlation between the indirect test results and the vaccine efficacy.

III. OTHER REQUIREMENTS

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