Foot-and-Mouth Disease Vaccine, Inactivated

Definition

Foot-and-Mouth Disease Vaccine, Inactivated is a liquid preparation containing one or more types of foot-and-mouth disease virus that have been inactivated in such a manner that its immunogenic activity is retained. Depending on the number of types of viruses incorporated, the vaccine is described as monovalent, bivalent, trivalent or polyvalent.

Production

Preparation of the vaccine

The virus is grown in suitable cell cultures. The virus is separated from cellular material by filtration or other suitable procedures and then the virus is inactivated using binary ethyleneimine (BEI) under suitable conditions. The inactivated virus, also known as antigen, may be concentrated and purified. The antigen is used for the preparation of vaccine. The vaccine contains a suitable adjuvant. Only an inactivated antigen suspension that complies with the requirements mentioned under manufacturer's test may be used in the preparation of the final lot. For a given serotype, the quantity of 146S antigen blended in each batch of the vaccine is not less than that of a batch of the vaccine that has shown to be satisfactory with respect to immunogenicity.

Master seed lot

Choice of vaccine strain. Well characterized strains obtained from an authentic source preferably from national reference laboratory or an authentic source permitted by Government of India shall be used for the vaccine production. The master seed virus shall be tested for identity, screening for mycoplasmas, safety and potency.

Tests on Master seed lot. The seed lot should comply with the tests for identity and inactivation kinetics. A batch of vaccine prepared from the seed lot should comply with entire range of control tests as mentioned below. If the animal tests have been performed with satisfactory results on a representative seed lot, they do not have to be repeated on each batch of the vaccine and, at least 1 in 5 vaccine batches prepared from the same master seed lot and with the same manufacturing process may be subjected for animal testing for routine batch release.

Identification.

When inoculated into seronegative animals, the vaccine stimulates the production of specific neutralizing antibodies against the serotypes incorporated as determined by suitable serological tests. All the master seed lots and the corresponding working seed lots as well as production seed lots of different serotypes shall be identified by using a suitable immunochemical assay such as ELISA. Alternately, identity of the antigen lot by means of molecular method is also acceptable apart from ELISA techniques.

Validation of the inactivation procedure. During inactivation, the virus titer is monitored by a sensitive and reproducible technique in a suitable cell culture system. The inactivation procedure is not satisfactory unless the decrease in virus titer in a time lapsed manner, plotted logarithmically, that is linear and extrapolation indicates that there is less than 1 infectious virus unit per 10000L of liquid preparation at the end of inactivation cycle.

Safety Test. Carry out the test for each route of method of administration to be recommended for the vaccination and in animals of each species for which the vaccine is intended. For each test, use not lesser than 8 animals that are sero negative for foot and mouth disease antibodies. Administer to each animal a double dose of the vaccine. Observe the animals for 14 days and no abnormal systemic or local reaction occurs. The safety test must be demonstrated by the firms at the time of market authorization, and it is not necessary to perform safety test on every batch of the vaccine thereafter unless there is a change of the master seed lot or a major change in production process.

Immunogenicity. PD_{50} challenge test: Use three groups of not less than five cattle per group, not less than 6 months old, which have never been vaccinated and are free from antibodies neutralizing the different types of foot-and-mouth disease virus in the vaccine. Vaccinate the 3 groups of animals by the prescribed route stated on the label. Use different doses of the vaccine for each group without diluting the vaccine. For example, if 3 ml is one dose, a 1/3 dose of vaccine would be obtained by injecting 1 ml, and a 1/9 dose would be obtained by injecting 0.3 ml. Three to four weeks later, challenge all the vaccinated animals and a control group of two cattle susceptible to foot-and-mouth disease, with a suspension of virus that is fully virulent and of the same type as that used for preparation of the vaccine by inoculating 10,000 ID_{50} (50 per cent bovine infectious dose) intradermally into two sites into the tongue (0.1 ml per site). Observe the animals for 8 days and then sacrifice them. Unprotected animals show lesions at sites other than the tongue. Protected animals may display lingual lesions. The test is not valid unless control animals show lesions on at least three feet. From the number of animals protected in each group, calculate the PD_{50} content of the vaccine. The vaccine should contains not less than 3 PD_{50} per dose per each strain incorporated.

PPG test: Alternatively, percentage of protection against generalized foot infection (PPG) test can be carried out. A group of 16 cattle of above six months age which have never been vaccinated and are free from antibodies neutralizing the different types of foot-and-mouth disease virus in the vaccine are vaccinated with a full vaccine dose by the route recommended by the manufacturer. These animals and a control group of two non-vaccinated animals susceptible to foot-and-mouth disease are challenged three to four weeks (For Algel vaccine 3 weeks later and for Oil adjuvanted vaccine 4 weeks later) after vaccination with a suspension of virus that is fully virulent and of the same type as that used for preparation of the vaccine by inoculating 10,000 ID_{50} (50 per cent bovine infectious dose) intradermally into two sites into the tongue (0.1 ml per site). Observe the animals for 8 days and then sacrifice them. Unprotected animals show lesions at sites other than the tongue. Protected animals may display lingual lesions. The test is not valid unless control animals show lesions on at least three feet. The vaccine passes the test if a minimum of 12 animals out of 16 vaccinated are protected. The vaccine passes the test if 75% of vaccinated animals are protected

Indirect Assessment. Test animals shall be bled on day 0 and 21 or 28 days post vaccination (for screening the animals for sero-negative status and for estimation of the antibody titers post vaccination). Indirect tests, including post vaccination measurement of virus neutralizing antibodies in cell culture, may be used to assess the potency of a vaccine provided that a statistical evaluation has established a satisfactory correlation between the results obtained by the test on the relevant vaccine serotype and the potency test in cattle.

The description applies to the testing of a monovalent vaccine. The potency of polyvalent vaccines may be tested by challenging each valency as described above. Immunogenicity tests carried out in cattle species serve the purpose for other ruminant species like sheep and goats. The suggested tests on immunogenicity are carried out at the time of market authorization only.

Manufacturer's tests

Following tests are performed by the manufacturers to ensure in-process quality control during the preparation of each lot of drug substance (final bulk antigen).

Identification. When inoculated into sero negative animals, the antigen stimulates the production of specific neutralizing antibodies against the virus serotypes incorporated as determined, by suitable serological tests**.** Alternately before inactivation, identity of the antigen lot by means of molecular methods is acceptable.

Residual live virus. During inactivation of the virus, samples should be taken at regular intervals for the purpose of monitoring the rate and linearity of the inactivation process. Virus titer in the samples is determined by inoculation into sensitive cell culture. The infectivity of the timed samples is plotted against time, and the inactivation procedure is not considered to be satisfactory unless the extrapolation indicates that there would be

less than one infectious particle per 10^4L of liquid preparation at the end of the inactivation period. A proportion of each batch of bulk inactivated antigen representing at least 200 doses is tested for freedom from infectious virus by inoculation into sensitive cell culture. A sample of inactivated antigen is concentrated to volumes adequate for inoculation into cell cultures and it must show that the concentrated antigen does not interfere with the sensitivity or reading of the assay. The sample is passaged 2 times at an interval of 24 to 48 hours and inoculated cell cultures are examined for the presence of foot and- mouth disease virus by suitable tests. No cytopathic changes attributable to foot-and-mouth disease virus replication should be detected. If infectious foot-and-mouth disease virus is detected, the bulk antigen is rejected.

Antigen content estimation. The 146S antigen content for each batch of bulk inactivated antigens is determined by an *in vitro* method, for example by sucrose density gradient centrifugation and ultraviolet spectrophotometry at 254 or 259 nm.
Any other suitable validated immunochemical method can also be used.

Bacterial and fungal contamination/ Sterility (2.2.11). Complies with the test for sterility.
The bulk should not contain contaminating bacteria and fungi and shall comply with the requirements of the sterility test mentioned under general requirements.

Batch tests

Description

The vaccine may appear as off-white to pinkish liquid suspension containing aluminium hydroxide gel or appear as off-white emulsion, if mineral oil adjuvant is used in the vaccine preparation.

Identification
The serum of a foot-and-mouth disease susceptible animal that has been immunized with the vaccine neutralizes the types of viruses used to prepare the vaccine, when tested by a suitable method.Serology potency test also serves the identification. Alternately before inactivation, identity on the antigen lot by means of molecular methods is acceptable.

Bacterial and fungal contamination/ Sterility (2.2.11). Complies with the test for sterility.

Safety. Use two cattle not less than six months old that do not have antibodies against foot - and - mouth disease virus. Administer to each animal a double dose of the vaccine by the prescribed route of administration stated on the label. Observe the animals daily for at least 14 days. The vaccine complies with the test if no animal shows abnormal local or systemic reactions or dies from causes attributable to the vaccine.
Note: General Requirements shall be referred regarding omission of the batch safety test.

Potency.Complies with the requirements of the test mentioned under section of master seed lot. Where the test is not carried out, an alternative validated method is used, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the test described under master seed lot and has been shown to be satisfactory with respect to immunogenicity in the target species.

Indirect tests, including post vaccination measurement of virus neutralizing antibodies in cell culture, may be used to assess the potency of a vaccine provided that a statistical evaluation has established a satisfactory correlation between the results obtained by the test on the relevant vaccine serotype and the potency test in cattle.

Alternative suitably validated in-vivo method on laboratory animals can be used as potency test for batch release if a correlation is established between the serology data generated in cattle and laboratory animals–with the approval of the National Regulatory Authority.

Vaccines for use in other ruminants. The potency of each batch shall be demonstrated in a suitable validated test. A test carried out in cattle is acceptable for other ruminant species.

Labelling. The label must state that (1) the vaccine is for veterinary use only; (2) the recommended routes of administration; (3) the instructions for use, such as “Shake well before use” (4) the serotypes used in the vaccine (5) do not freeze the liquid preparation (6) storage temperatures; (7) The vaccine is monovalent, bivalent or trivalent or polyvalent ;(8) Total volume and number of doses; (9) expiry date