MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY
OFFICE OF THE MINISTER

NORMATIVE INSTRUCTION NO. 11 DATED 18 JANUARY 2018

MINISTRY OF AGRICULTURE LIVESTOCK AND FOOD SUPPLY

OFFICE OF THE MINISTER

Official Federal Government Gazette (DOU) of 21/01/2018 (no. 15, Section 1, page 4)

THE ACTING MINISTER OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY, using the powers conferred upon him by Article 87, sole paragraph, item II of the Constitution, and pursuant to Decree-Law no. 467, enacted February 13, 1969, Decree no. 5,053, enacted April 22, 2004, and Case File no. 21000.054662/-26, declares that:

Article 1. Approves the Technical Regulation for the Production, Quality Control, Sale and Use of Foot and Mouth Disease (FMD) Vaccines, in accordance with the aforementioned Normative Instruction.

CHAPTER I
DEFINITIONS

Article 2. For the purposes of the present Normative Instruction, the following definitions apply:
I - antigens: purified, standardized, inactivated, specific, sensitive biological components that are capable of inducing an immune response;
II - presentation: type of packaging, and volume and number of doses;
III - 0 DPV: Zero days post-vaccination;
IV - 28 DPV: Twenty-eight days post-vaccination;
V - 28 DPR: Twenty-eight days post-revaccination;
VI - 56 DPV: fifty-six days post-revaccination;
VII - 84 DPV: Eighty-four days post-vaccination;
VIII - 168 DPV: One hundred and sixty-eight days post-vaccination;
IX - EPP - Expected Percentage of Protection;
X - NSP - Nonstructural Proteins;
XI - batch: the quantity of the product that is produced in a final cycle of manufacturing (formulation and filling), the essential characteristic of which is homogeneity and identification by a single alpha-numerical code;
XII - master seed: each and every sample of the initial seed of a virus, cells, or other substrate intended for the manufacturing of a work seed, which is multiplied or replicated, maintaining given conditions of safety, purity, immunogenicity and potency; and
XIII - work seed: each and every sample of virus derived from the master seed, intended for the manufacture of antigens, which is multiplied or replicated in accordance with the same methods of multiplication as the master seed, maintaining given conditions of safety, purity, immunogenicity and potency.

CHAPTER II
VACCINE PRODUCTION

Article 3. The substrates and ingredients used in producing and in controlling the quality of vaccines, addressed in the present regulation must be in accordance with standards of purity and quality pre-established in the pharmacopoeia or internationally acknowledged technical/scientific literature.
Sole paragraph. The combinations and abstracts used in the formulation must not change specific substances in the vaccine, or reduce the minimum demanded potency within the vaccine's shelf life, or the immune response during the established period of immunity.
Article 4. Batches of FMD vaccine sold in Brazil must be produced from FMD virus strains duly authorized by the Ministry of Agriculture, Livestock and Food Supply (MAPA).
Article 5. FMD vaccine manufacturers may only handle and maintain strains or samples of foot and mouth disease virus that have been authorized by the Ministry of Agriculture, Livestock and Food Supply.
Article 6. Master seeds and work seeds must contain only the identified specific agent, and must be free of other pathogens and contaminants.
Article 7. The manufacturing or importing establishment must notify the corresponding Inspection Service of the Federal Agriculture Superintendent's Office (SFA) in the jurisdiction of which it lies, of its annual manufacturing or importing schedule within thirty days of receipt of communication by MAPA of the official demand for vaccines.
Article 8. The stages of production and quality control of batches of vaccines must be carried out in accordance with the technical report of the registration of the product as approved by MAPA and as have been registered in specific protocols, complying with the rules of this regulation and the current good manufacturing practice standards in force in Brazil.

CHAPTER III
QUALITY CONTROL IN THE VACCINE PRODUCTION PROCESS

Article 9. Foot and mouth disease vaccine manufacturing establishments must perform quality control during the manufacturing process for the vaccine, encompassing at least the following aspects:
I - quality control of raw materials;

II - typification, purity and titration tests of the master seed and work seed;
III - control of contaminating agents in the biological substrates;
IV - testing for active residual virus at the end of the inactivation step for each batch of monovalent antigen, for a sample equivalent to at least two hundred doses of vaccine; and
V - inactivation kinetics.

CHAPTER IV
QUALITY CONTROL IN THE FINISHED PRODUCT

Article 10 - Only batches of FMD vaccine that have undergone test processes for sterility control, active residual virus, tolerance, potency, NSP antibodies, and emulsion stability and have been approved by the manufacturer may be solved.
Sole paragraph. MAPA is responsible for authorizing sale of each batch after official testing or acceptance of the manufacturer's tests by the Coordination Office for Inspection of Veterinary Products of the Department of Inspection of Livestock Inputs (CPV/DFIP/SDA/MAPA).

CHAPTER V
STERILITY TESTING

Article 11. The sterility and efficiency of the culture media that are used must be evaluated by the manufacturer and by the official laboratory before the start of the test, or simultaneously with performance of the product sterility assay.
Article 12. Product sterility tests must be carried out using the membrane filtration, or direct inoculation, methods, or yet another methodology accepted by MAPA.

CHAPTER VI
ACTIVE VIRUS TESTING

Article 13. To test for active virus in the finished product, either use a methodology accepted by MAPA, or adopt the sensitive cell inoculation methodology where cells have been submitted in advance to sensitivity tests, with samples equivalent to at least two hundred doses of vaccine.
Article 14. To test for active residual virus in the finished product, each manufacturer must state its methodology for emulsion breaking (antigen elution) in its formulation.

CHAPTER VII
TOLERANCE TESTING

Article 15. Tolerance testing must be carried out with 18 (eighteen) bovines aged from 18 (eighteen) to 24 (twenty-four) months, in good sanitary and nutritional condition, applying to each animal at a single vaccination site the dose recommended by the manufacturer, exclusively subcutaneously at 0 DPV, following good clinical practices.
Paragraph 1. When administered, the vaccine must not produce clinical signs of foot and mouth disease or any unwanted local or systemic reaction in the target species.
Paragraph 2. The animals must be observed at least twice, the first observation being on the day of vaccination, and the second at 28 DPV to check for signs that may be attributed to vaccine reaction.
Paragraph 3. If nodules appear at the vaccination site, they must be measured to calculate the average of the area(s) of this lesion in the group of tested animals.
Paragraph 4. Should one or more animals present such signs as death, torsion, neck stiffness, ambulatory problems, or if there are nodules for which the average to which paragraph 3 refers is above 45 cm^2 (forty-five square centimeters), a second staging of the test will be performed using the same number of animals.
Paragraph 5. If in the second step of the test the findings described in the previous paragraph occur again, that batch of vaccine will be deemed to have failed.
Paragraph 6. To test for tolerance, the same animals used in the NSP antibody test may be used.

CHAPTER VIII
POTENCY TESTING

Article 16. To test for potency of the finished product, Elisa CFL or another test accepted by MAPA will be used, with bleeding at 56 DPV, or the Protection against Podal Generalization (PPG) test with challenge at 84 DPV.
Article 17. Potency testing using Elisa CFL follows the methodology below:
I - use homogeneous, healthy, well-nourished bovines aged from 18 to 24 months, weighing a minimum of 200 kg, not previously vaccinated against foot and mouth disease, not previously infected with foot and mouth disease, and the sera of which do not contain antibodies against structural and nonstructural FMDV proteins;
II - the animals addressed in item I must have acclimated for at least ten days prior to being vaccinated;
III - animals younger than those defined in item I may be tested, after authorization from DFIP, provided that the methodology used to conduct the test has an established correlation to the benchmark methodology defined by MAPA;
IV - the bovines will be selected by Elisa-Screening laboratory test using the methodology described by the supplier that has been accepted by MAPA;
V - vaccinate either subcutaneously or intramuscularly in accordance with the procedure defined by the official control laboratory: eighteen bovines per tested batch, with 1 (one) dose of foot and mouth disease vaccine;
VI - at least one control vaccine with known results, obtained by the same methodology, must be included in each official test;

VII - after each test, whatever the number of batches evaluated in per test, at least two unvaccinated bovines must be added as witnesses to validate the test;
VIII - the blood of the bovines will be harvested at the moment of vaccination on day zero and at 56 DPV;
IX - microplates with 96 (ninety-six) wells will be used to distribute and inoculate the test and control sera, then dilutions and test layout will be standardized and defined by MAPA;
X - each microplate will contain antigen controls (all the reagents, minus the sera) and blank (all the reagents, minus the antigen and the sera);
XI - the plates will be read by spectrophotometer with a 492 nm (four hundred and ninety-two nanometer) filter, subtracting the average of optical densities (OD) from the blank for all readings;
XII - the 50% (fifty per cent) titer of Elisa-CFL with polyclonal or monoclonal antibodies of the serum challenged by FMD virus is defined by the reciprocal of the dilutions of this serum, expressed as a base 10 log, which gives an OD equal to 50% (fifty per cent) of the average of the ODs obtained in the antigen control;
XIII - the test will be deemed valid when it complies with the acceptance parameters indicated by the serological method defined and made available by MAPA;
XIV - the antibody titers in Elisa-CFL of the bovine test sera will be transformed and expressed as an expected percentage of protection (EPP), in accordance with the correlation table for levels of antibodies versus protection against podal generalization (PPG) approved by MAPA; for each batch of vaccine, calculate the average EPP excluding the sera with the greatest and least titer;
XV - a batch of vaccine is deemed to be approved when the average EPP is equal or superior to 80% (eighty per cent) for each one of the antigens tested;
XVI - a batch of vaccine is deemed to have failed when the average EPP is less than 80% (eighty per cent) for at least one of the antigens tested; and
XVII - when requested, the B sample test must test the antigen(s) against which the batch was not approved in the first test, using a new group of eighteen animals, without ruling out the possibility of using the remaining antigens.
Sole Paragraph - Bovines whose day zero sera show FMDV antibody titers above those that will be stipulated by MAPA will be excluded from the test.
Article 18 - The potency test using Protection against Podal Generalization in Bovines (PPG) must only be performed by MAPA using the following methodology:
I - use bovines with the characteristics described in items I and II of Article 17 of the present Regulation;
II - the bovines will be selected by Elisa-Screening laboratory test using the methodology described by the supplier that has been accepted by MAPA;
III - select 18 (eighteen) bovines and vaccinate sixteen of them with a dose of the vaccine, by the route recommended by the manufacturer, keeping two bovines as unvaccinated sentinels;
IV - at 84 DPV inoculate 0.2 mL (zero point two milliliters) of a suspension of challenge virus containing 10,000 (ten thousand) infecting dose (ID) 50% bovines, intradermolingually at two sites, with a volume of 0.1 mL (zero point one milliliter) per inoculation site, into the bovines addressed in item II, including the sentinel animals;
V - use bovine origin challenge virus coming from the lingual epithelium, preserved at or below -70ºC (minus seventy degrees centigrade), in the form of fractionated monovalent glycerinated virulent suspensions;
VI - the suspensions must be typified, titered and sub-typified by complement fixation, titered in bovines and cell cultures or lactating mice, presenting a bovine infectious dose of 10,000 (ten thousand) ID 50%;
VII - the bovines must be bled at the moment of vaccination (day zero) and at intervals of four weeks up until the end of the test;
VIII - the sera must be kept frozen until the moment of performing the serological tests, when necessary;
XI - the reading will be taken between seven and eight days after inoculation of the challenge virus; all the bovines will be examined, and lingual and podal lesions noted; a bovine is deemed protected when not presenting foot and mouth disease lesions on any of its feet;
X - a batch of vaccine will be deemed to have been approved when it obtains at least 75% (seventy-five per cent) animals protected out of the sixteen that were vaccinated;
XI - a batch will be deemed to have failed when it protects fewer than 75% (seventy-five per cent) animals out of the sixteen that were vaccinated;
XII - in the A or B sample tests, a control vaccine may be included;
XIII - the A or B sample test will be deemed valid if the two unvaccinated sentinel bovines present podal lesions on one or more feet, within seven or eight days after inoculation; and
XIV - if death occurs but cannot be attributed to the act of the challenge in at most four animals, except for the two sentinels, the test will be deemed valid, and the calculation will be performed on the basis of the remaining animals.

CHAPTER IX
SNP ANTIBODY TESTING

Article 19 - ELISA 3ABC/EITB must be used to test for NSP antibodies as follows:
I - use a group of bovines with the characteristics described in items I and II of Article 17 of the present Regulation;

II - the animals will be bled on selection and at day 0 (zero) of the test, prior to vaccination, and the sera that undergo testing must not show any reactivity deemed positive;
III - after bleeding on day 0 (zero), these bovines will be vaccinated and bled at 28 DPV;
IV - the same 18 (eighteen) animals will be revaccinated between 28 DPV and 42 DPV, bled at 28 DPR, and the sera will be tested;
V - all batches of vaccine not inducing a reaction deemed positive in any of the bovines used in the test at 28 DPV and at 28 DPR will be deemed to have been approved;
VI - any batch of vaccine that induces a reaction deemed positive in one or more of the bovines used in the test at 28 DPV or at 28 DPR will be deemed to have failed; and
VII - only ELISA kits authorized by MAPA may be used to perform this test.
Sole Paragraph - Another methodology that has been validated and accepted by MAPA may be used.

CHAPTER X
EMULSION STABILITY TESTING

Article 20 - Batches of vaccines must be tested for heat stability to assess the emulsion quality, using samples of at least one vial for each presentation, as follows:
I - incubate in an oven at 36ºC +/- 2ºC (thirty-six degrees centigrade plus or minus two degrees centigrade), for fifteen days; and
II - keep another set of vials in a refrigerator at a temperature ranging from 2ºC (two degrees centigrade) to 8ºC (eight degrees centigrade), for at least thirty days.
Paragraph 1. If the aqueous phase appears in the bottom of the vial during the tests described in items I or II, test the vial by centrifuging.
Paragraph 2. The centrifuging test addressed in Paragraph 1 consists of transferring part of the contents of the vial to a centrifuge tube, centrifuging for 1 (one) hour at 2,500 xg and measuring the volume of the aqueous phase at the bottom of the tube after centrifuging.
Paragraph 3. After performing the procedures described in Paragraph 2, if an aqueous phase volume above 5% (five per cent) of the volume centrifuged is found, the batch must be deemed to have failed.

CHAPTER XI
TESTS FOR REGISTRATION AND CHANGING REGISTRATION

Article 21. To grant registration to the product and for changes in registration that require efficacy tests, an official PPG test (in addition to those laid down for finished product in Article 10) will be demanded, or any other serological test defined by MAPA, to evaluate the duration of immunity, with bleeding of primovaccinated animals at 56 and 168 DPV.
Sole Paragraph - Any batch of vaccine obtaining an EPP below the value established for approval, in any of the tested periods, will be deemed to have failed.
Article 22. The request for registration must also contain stability data obtained for a minimum of 180 days from the pilot batch.
Sole Paragraph - In the case set forth in the head provision, the initial expiration deadline to be granted will be eighteen months.

CHAPTER XII
THE STORAGE, SALE AND USE OF THE VACCINES

Article 23. The vaccines registered must present an indication for use in bovines and buffaloes.
Article 24. The dose volume must be 2.0 mL (two milliliters) administered intramuscularly or subcutaneously.
Article 25. The vaccines must always be kept at a temperature between 2ºC and 8ºC.
Sole paragraph - if storage is detected at a temperature other than that laid down in this article, sale of the product will be banned, and the vials rendered useless, and no type of technical evaluation shall be performed.
Article 26. The expiration date of FMD vaccines is at most 24 (twenty-four) months from the date of manufacture.

CHAPTER XIII
GENERAL PROVISIONS

Article 27. FMD vaccines must contain a pH indicator substance in their formulation.
Sole Paragraph - if the product present signs of changes in pH, the corresponding batch shall be deemed unfit for use.
Article 28. The vials used in bottling the FMD vaccine must enable the color of the contents to be observed.
Article 29. The volume of each vial may not be less than that declared on the product license.
Sole Paragraph - The vials must present a minimum surplus of 2% (two per cent) over the total labeled volume.
Article 30. Criteria for passing or failing in a B sample test are the same as those for the test itself.
Sole Paragraph - The result of the B sample test shall be deemed conclusive within the administrative sphere.
Article 31. A batch of vaccine may only be taken for official testing after completion by the manufacturer of at least a residual virus and a sterility test and presentation of their results in specific protocols approved by MAPA.
Article 32. No batch of vaccine produced and presented for official control for sale in Brazil may have fewer than two million doses: variation of up to -10% (minus ten per cent) is accepted.

Article 33. A B sample may be granted provided that it is requested of the Inspection Service of the Federal Agriculture Superintendent's Office in the state having jurisdiction, by the interested party, within ten days counting from the date of receipt of the official result.
Article 34. The date of bottling will be taken into consideration for counting the deadline for expiration of the product.
Paragraph 1. Vaccines may not be manufactured using monovalents whose inactivation date was over 6 (six) months prior to the production date of the batch.
Paragraph 2. Paragraph One does not apply to antigens stored for exclusive use in antigen banks and preserved at -180ºC (minus one hundred and eighty degrees centigrade).
Article 35. A B sample test will not be allowed for a batch of vaccine that has failed active residual virus tests, sterility tests or tolerance tests.
Article 36. All batches of vaccine that have failed in official tests and for which a B test was not requested within ten days, or that failed in the B test allowed for in the present regulation, must be rendered useless immediately.
Sole Paragraph - The procedure for rendering useless shall be the responsibility of the product-owning company.
Article 37. - Official tests for registration or product alteration purposes, and B sample tests, will be performed in accordance with the availability in the calendar for official tests.
Article 38. Results of tests to assess the quality of a batch of FMD vaccine must be provided in protocols accompanying the official sampling of the product.
Sole Paragraph - should there be a need for concluding internal tests after harvesting, they will be finalized and their results made available to MAPA before the completion of the official tests.
Article 39. The test bench, manufacturing records, and quality control protocols are documents that guarantee product quality.
Article 40. The granting of registration to vaccines containing Genetically Modified Organisms or GMO derivatives in their formulations will depend on advance positioning of CTNBio (Brazil's National Biosecurity Technical Commission), as laid down in Article 16 of Law no. 11,105, enacted 24 March, 2005.
Article 41. Cases not covered herein and queries raised in the application of this Regulation will be clarified by the Department of Inspection of Livestock Inputs of the Secretariat of Animal and Plant Health of the Ministry of Agriculture, Livestock and Food Supply: DFIP/SDA/MAPA.
Article 42. Supplementary acts required for applying this Regulation will be drafted and published by the Secretariat of Animal and Plant Health (SDA/MAPA).
Article 43. This Normative Instruction shall come into force as of the date of its publication.
Article 44. Normative Instruction 50 of 23 September, 2008 is hereby revoked.

EUMAR ROBERTO NOVACKI