Appendix III

New Section for OIE Diagnostic Manual on Vaccine Matching Tests

Vaccine matching tests

1. Introduction

Vaccination against one serotype of FMDV does not cross-protect against other serotypes and may also fail to protect fully or at all against other subtypes of the same serotype. The most direct and reliable method to measure cross-protection is to vaccinate relevant target species and then to challenge them by exposure to the virus isolate against which protection is required. This will take account of both potency and cross-reactivity. However, such an approach is slow and expensive, and the animal numbers needed for statistically significant results raise welfare concerns.

A variety of in vitro serological methods can be used to quantify antigenic differences between the FMDV capsid proteins and thereby estimate the likely cross-protection between a vaccine strain and a field isolate. Genetic characterisation and antigenic profiling can also reveal the emergence of new strains for which vaccine matching may be required and conversely indicate that an isolate is similar to one for which vaccine matching information is already available.

Appropriate vaccine strain selection is a critical element in the control of foot-and-mouth disease virus (FMD) and is necessary for the application of vaccination programmes in FMD-affected regions as well as for the establishment and maintenance of vaccine antigen reserves to be used in the event of new FMD incursions.

Vaccine potency also contributes to the range of antigenic cover provided by a vaccine. A highly potent vaccine that stimulates a strong immune response may give greater protection against a heterologous virus than an equally cross-reactive vaccine that stimulates a weaker immune response. Furthermore, booster doses of vaccine can increase potency and the subsequent breadth of antigenic cover provided by a given vaccine, although the onset of full protection may be delayed.

2. Selection of field viruses for vaccine matching studies

Serological matching of field isolates to vaccine strains requires that isolates have been serotyped and adapted to grow in cell cultures. The serotype is usually determined by ELISA or CFT using type-specific serological reagents, although methods based on monoclonal antibodies (mAbs) or genetic typing may also be used. BHK or IB-RS-2 cell cultures are usually used for in vitro virus replication. For vaccine matching, preferably, at least two isolates should be evaluated from any outbreak and inconsistent results should be followed up to determine whether this is due to genuine antigenic differences or is an artefact of testing.

Viruses can be selected based on epidemiological information, for instance isolation at different stages of an epidemic, from different geographic locations or from different hosts. Field evidence for a lack of vaccine induced protection is an important criterion for vaccine matching.

Antigenic profiling by CFT or by ELISA or sequence analysis of the VP1 gene are suitable approaches for selecting representative virus isolates for vaccine matching. Antigenic profiling is performed by CFT using panels of hyperimmune guinea-pig sera raised against epidemiologically

1 To be inserted “en bloc” into the Manual as a new section between current sections B and C.
Proposed section on vaccine matching for incorporation into the OIE Diagnostic Manual

relevant field isolates (Bergmann et al., 1988) or by ELISA using panels of well-characterised monoclonal antibodies (Alonso et al., 1993).

3. Selection of vaccine strains to be matched

The serotype of the virus, the region of origin and any information on the characteristics of the virus may give indications of the vaccines most likely to provide an antigenic match. The availability of reagents for matching to particular vaccines may limit the choice of what is possible. Vaccine matching has two purposes; firstly to chose the most effective vaccine for use in a particular circumstance and secondly to monitor, on an ongoing basis, the suitability of vaccines maintained in vaccine antigen reserves.

4. Choice of vaccine matching test

4.1. The serological relationship between a field isolate and a vaccine virus ('r' value) can be determined by ELISA, VNT or CFT (Alonso, 1986; Pereira, 1977; Sutmoller et al., 1984; Ferris and Donaldson, 1992; Kitching et al., 1988; 1989; check). One way testing is recommended ($r_1$) with a vaccine antiserum, rather than two way testing ($r_2$) which also requires an antiserum against the field isolate to be matched. **Repeatability.** In vitro neutralisation may be more relevant to in vivo protection than other measures of virus-antibody interaction, although some studies have shown that non-neutralising antibodies can also be protective (McCullough et al., 1992). Advantages of ELISA are that the test is rapid and utilises smaller volumes of post-vaccination sera which are often available in limited quantities. A disadvantage of the ELISA method is that it is harder to standardise the virus antigen concentration used in the test. One approach is to screen viruses by ELISA and use neutralisation tests to confirm or refute unexpected results. Another is to screen by CFT followed by expected percentage of protection (EPP). For either VNT or ELISA, post-vaccination sera are derived from at least five cattle 21-30 days after immunisation. The titre of antibody to the vaccine strain is established for each serum which are used individually or pooled, excluding low responders. The CFT method utilises hyperimmune guinea-pig sera.

4.2. A more thorough evaluation is provided by the EPP method (Alonso et al., 1987) which measures the reactivity of a panel of post-vaccination antisera using either VNT or ELISA and relates the serological titres to the probability of protection, established through correlation tables associating antibody titres with protection against the relevant vaccine strain. These correlation tables derive from previously performed challenge tests using this vaccine. However, the requirement for a panel of antisera and accompanying potency test data for the vaccine in question currently cannot be met for a wide range of vaccine strains.

5. Vaccine matching by ELISA

5.1. This test utilises an antiserum raised against a vaccine strain. The blocking ELISA titres of this reference serum against antigens prepared from the homologous vaccine strain and a field isolate are compared to determine how antigenically 'similar' the field virus is to the vaccine virus.

5.2. The test procedure is similar to that of the liquid phase blocking ELISA (see section 2c). Additional biological reagents are: 21-30 day post-vaccination bovine vaccine sera (inactivated at 56ºC for 45-60 min); the homologous vaccine strain; the test virus, a field isolate of the same serotype as the vaccine strain.

5.3. Grow the field isolate and the vaccine strain in BHK or IB-RS-2 cells. The number of cell culture passages should be kept to a minimum (normally less than four) to avoid
selection of antigenic variants unrepresentative of those in the original material. A sufficient quantity of virus should be present if cell cultures are destroyed by cytopathic viral effect within 24 hours of inoculation.

5.4. Harvest and titrate the vaccine and field viruses using a panel of trapping rabbit antisera and detector guinea pig antisera raised against the same or closely related vaccine strains. If necessary, the virus antigens may be inactivated prior to use by binary ethyleneimine (BEI).

5.5. Select the optimum trapper/detector combination and the working dilution of the field virus. This should not be less than 1:6. If there is no suitable trapper/detector combination then a back-titration of the antigen stock must be performed to confirm that sufficient virus was present. Once confirmed, this indicates that none of the available vaccines are suitable.

5.6. Titrate 21-30 day post-vaccination serum of a chosen vaccine strain against the field isolate and the homologous vaccine strain. A control titration of reference serum and virus is included on every test plate and this titre should not fluctuate more than two-fold either side of a mean value.

5.7. To determine the serum titre, calculate the average optical density (OD) of 24 antigen control wells without blocking serum. This represents the maximum OD value for the test, i.e., the 100% control value. Divide this by 2 to determine the 50% inhibition value. Score wells with blocking serum positive if the OD is less than or equal to 50% and negative if the OD value is greater than this. The end-point is defined as the dilution at which half of the wells show 50% inhibition or more (i.e., identify the dilution at which one out of the two duplicate wells has an OD less than 50% of the antigen control). If the end-point falls between two dilutions, it is taken as the mid-point between these dilutions, as estimated by the Spearmann-Karber method.

5.8. Derive an \( r_1 \) value, the relationship between a field and a vaccine strain, as:
\[
r_1 = \frac{\text{titre of reference serum against field virus}}{\text{titre of reference serum against vaccine virus}}
\]

At least two consistent results are needed for acceptance.

5.9. Interpretation

For \( r_1 \) values derived by ELISA the following guidelines are used for interpretation: (Kitching et al., 1988 - check)

- 0.4-1.0 Close relationship between field isolate and vaccine strain. A potent vaccine containing the vaccine strain is likely to confer protection.

- 0.2-0.39 The field isolate is antigenically related to the vaccine strain. The vaccine strain might be suitable for use if no closer match can be found provided that a potent vaccine is used and animals are preferably immunised more than once.

- <0.2 The field isolate is only distantly related to the vaccine strain and the vaccine strain is unlikely to protect against challenge with the field isolate.

6. Vaccine matching by two-dimensional neutralisation test
6.1. This test also utilises an antiserum raised against a vaccine strain. The titres of this serum against 100 TCID50 of the homologous vaccine strain and the same dose of a field isolate are compared to determine how antigenically 'similar' the field virus is to the vaccine strain.

6.2. The procedure is similar to that of the microtitre plate virus neutralisation test (see Section 2a). Additional biological reagents are: 21-30 day post-vaccination bovine vaccine sera (inactivated at 56°C for 45-60 min); the homologous vaccine strain; the test virus, a field isolate of the same serotype as the vaccine strain

6.3. Field isolates are passaged on cell cultures until adapted to give 100% CPE in 24 hours. Passages should be kept to a minimum. When adapted, determine the virus log10 titre by end-point titration.

6.4. For each test and vaccine virus a chequerboard titration is performed of virus against vaccine serum along with a back-titration of virus alone. Cells are added and incubated at 37°C for 48-72 h after which time CPE is assessed.

6.5. Antibody titres of the vaccine serum against the vaccine strain and field isolate for each virus dose used are calculated using the Spearmann-Karber method. The titre of the vaccine serum against 100 TCID50 of each virus can then be estimated by regression. The relationship between the field isolate and the vaccine strain is then expressed as an 'r' value as described for the strain characterisation ELISA.

6.6. Interpretation. In the case of neutralisation, r1 values greater than 0.3 indicate that the field isolate is sufficiently similar to the vaccine strain that use of the vaccine is likely to confer protection against challenge with the field isolate (ref). Conversely, values less than 0.3 suggest that the field isolate is so different from the vaccine strain that the vaccine is unlikely to protect. In these cases, either the field isolate should be examined against alternative vaccine strains or, rarely, it will be necessary to adapt a suitable field isolate to become a new vaccine strain.

6.7. Tests should always be repeated more than once. The confidence with which 'r' values can be taken to indicate differences between strains is related to the number of times that the examination is repeated. In practice, a minimum of at least three repetitions is advised.

7. Vaccine matching by CFT

7.1. The relationship between a field isolate and a vaccine strain can also be determined by complement fixation using guinea-pig hyperimmune serum raised against the relevant vaccine strain (Alonso, 1986).

7.2. The CFT 50% titres of this reference serum against antigens prepared from the homologous vaccine strain and a field isolate are compared to determine how antigenically 'similar' the field virus is to the homologous vaccine virus.

7.3. Field isolates are passaged on cell cultures until adapted to give 100% CPE in 24 hours. Passages should be kept to a minimum. When adapted, determine the virus titre that fixes 2.5 50% complement fixing units (CFU50).

7.4. A relationship is established by titration of hyperimmune sera through a 2-fold dilution series against 4 CFU50 of the homologous and heterologous antigens in veronal buffer
diluent (VBD) or borate saline solution (BBS) placed in separate tubes. Four haemolysis units of complement are then added to each reaction.

7.5. The test system is incubated at 37°C for 30 minutes prior to the addition of 2% of standardised sheep red blood cells (SRBC) in VBD or BSS sensitised with rabbit anti SRBC. Reagents are incubated at 37°C for a further 30 minutes and the tubes are subsequently centrifuged and read.

7.6. The CF 50 titres are calculated by the Spearmann-Karber method and an 'r' value is derived from the relationship between the reactivity of the field isolate and the vaccine strain, as:

\[
r_1 = \frac{\text{Reciprocal titre of hyperimmune serum against field virus}}{\text{Reciprocal titre of hyperimmune serum against vaccine virus}}
\]

7.7. Interpretation. In the case of CFT, \(r_1\) values greater than 0.25 indicate that the field isolate is sufficiently similar to the vaccine strain that use of the vaccine is likely to confer protection against challenge with the field strain.

8. Expected Probability of Protection

8.1. The Expected Probability of Protection (EPP) estimates the likelihood that cattle would be protected against a challenge of 10,000 infective doses after a single or boosted vaccination.

8.2. Individual sera are required from sixteen or thirty 18-24 month-old cattle at thirty days post vaccination and thirty days post revaccination, using a full dose of the vaccine strain to be matched.

8.3. This panel of sera is tested for antibody titres to the homologous FMD vaccine strain and the field isolate to be matched using VNT or LPB-ELISA (see Sections 2a and 2c).

8.4. If necessary, the antigens used in the ELISA may be inactivated prior to use by binary ethylenimine (BEI).

8.5. The EPP is determined from the serological titre obtained, for each individual serum, by reference to predetermined tables of correlation between serological titres and clinical protection The mean EPP is then calculated from the EPP for each individual serum.

8.6. The clinical protection data is derived from previously performed experiments carried out on hundreds of cattle that had been immunized using the vaccine strain in question and challenged with a homologous virus (similar to the PGP potency tests described in Section X of the Manual, Potency tests on final product). Each animal is scored as protected or not and tables of correlation based on logistic regression models are established between antibody titre and clinical protection.

8.7. An EPP < 0.75 (when sera from a group of sixteen vaccinated animals are used) and < 0.7 (when sera from a group of thirty vaccinated animals are used) is an indication that the vaccines will give a low protection against the field strain (Bol. Centro Panamericano Fiebre Aftosa 49-50: 27-30, 1984).

9. References
Proposed section on vaccine matching for incorporation into the OIE Diagnostic Manual


Alonso et al., 1993. Viral Immunology 6: 219


