Standards, Guidelines and Recommendations of the OIE relating to FMD

Normes, lignes directrices et recommandations de l’OIE en matière de fièvre aphteuse

Normas, directrices y recomendaciones de la OIE en materia de la fiebre aftosa
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**Introduction*  

In the field of animal health, the basic aim of the ‘Agreement on the Application of Sanitary and Phytosanitary Measures’ (‘SPS Agreement’) of the World Trade Organization (WTO) is to maintain the sovereign right of any government to provide the level of health precaution it deems appropriate, but to ensure that this sovereign right is not misused for protectionist purposes and does not result in unnecessary barriers to international trade. The SPS Agreement states in particular that countries should, with a view to achieving the widest possible harmonisation of the animal health measures they take to ensure the protection of human and animal life and health, establish these measures on the basis of existing international standards, guidelines and recommendations.

Furthermore, those national or sub-national animal health measures that conform to international standards, guidelines and recommendations will be deemed necessary for the protection of human and animal life and health and considered to be in accordance with the SPS Agreement.

The SPS Agreement subsequently specifies that the expression ‘standards, guidelines and recommendations... for animal health and zoonoses’ refers to the ‘standards, guidelines and recommendations developed under the auspices of the OIE’.

WTO Members are thus obliged to pay the very closest attention to the normative documents developed by the OIE when establishing the animal health conditions governing their imports and exports of animals and animal products.

These normative documents can also be referred to in the event of a dispute settlement procedure being instigated within the framework of the WTO to resolve a dispute between an exporting country and an importing country. They are also widely used by OIE Member Countries that are not members of the WTO in order to establish their trade regulations.

All these considerations are particularly relevant to foot and mouth disease (FMD), a disease that has for many years been the subject of detailed study by the International Committee and various Specialist Commissions of the OIE.

Countries have access to the following normative documents issued by the OIE relating to FMD:

- the chapter on FMD in the *International Animal Health Code* (the *Code*) and its appendix;

- the chapter on FMD in the *Manual of Standards for Diagnostic Tests and Vaccines* (the *Manual*).  

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It is important to point out that these normative documents are primarily aimed at preventing the FMD virus being introduced into a country importing live susceptible animals or products derived therefrom, whatever the FMD status of the country in question.

Within the framework of the OIE's standardisation activities relating to FMD, mention must also be made of the procedures in force for international recognition that countries or zones within countries are FMD free, even if these procedures do not constitute a standard per se, but rather a recommendation transcribed in the form of a Resolution adopted by the OIE International Committee.

Lastly, following the events related to FMD that took place in the world during the first months of 2001, the OIE and the FAO\(^1\) organised a joint International Scientific Conference on the disease. The conference was held in April 2001 at the OIE Headquarters, and resulted in the drafting of a number of important recommendations, which were endorsed by the OIE International Committee the following month.

1. **Chapter of the *International Animal Health Code* on foot and mouth disease and its appendix**

The standard relating to FMD, contained in Chapter 2.1.1. of the *Code*, is given in Part 1 of the present document. The text, which can be consulted on the OIE Web site (www.oie.int), was completely revised between 1990 and 1997, ensuring that it is fully in line with the latest scientific knowledge about the disease. It goes without saying that, as with any other standard, the contents of this chapter are not fixed once and for all but must keep pace with advances in scientific and technical knowledge.

The first part of the chapter (Articles 2.1.1.2. to 2.1.1.5.) defines the various FMD statuses that can be attributed either to countries or to zones within countries, as provided for in Chapter 1.3.4. on zoning and regionalisation. There are four such statuses:

- FMD free country where vaccination is not practised;
- FMD free country where vaccination is practised;
- FMD free zone where vaccination is not practised;
- FMD free zone where vaccination is practised.

Some of the conditions that countries must meet to obtain one of these statuses for all or part of their territory are common to all the statuses. Namely, countries should:

- have a record of regular and prompt animal disease reporting;
- have demonstrated that an effective system of surveillance is in operation (in the entire country or in the free zones, as appropriate);
- have demonstrated that all regulatory measures for the prevention and control of FMD have been implemented.

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1 FAO: Food and Agriculture Organization of the United Nations
The specific conditions that have to be met for each status are summarised in the following table:

<table>
<thead>
<tr>
<th>Status</th>
<th>Specific conditions</th>
</tr>
</thead>
</table>
| FMD free country without vaccination | No outbreak for the past 12 months  
No vaccination for at least 12 months  
No importation of vaccinated animals since the cessation of vaccination |
| FMD free country with vaccination | No outbreak for the past 2 years  
Routine vaccination with a vaccine complying with the OIE standards  
System of intensive surveillance for detection of any viral activity |
| FMD free zone without vaccination | No outbreak for the past 2 years  
No vaccination for at least 12 months  
No importation of vaccinated animals since the cessation of vaccination  
Surveillance zone or physical or geographical barriers that separate the free zone from the infected territories. |
| FMD free zone with vaccination | No outbreak for the past 2 years  
Routine vaccination with a vaccine complying with OIE standards  
System of intensive surveillance for detection of any viral activity  
Buffer zone or physical or geographical barriers that separate the free zone from the infected territories. |

If a country or zone loses its status due to an occurrence of the disease, it can regain its status under the following conditions (see Article 2.1.1.6.):

<table>
<thead>
<tr>
<th>Status</th>
<th>Conditions for regaining status</th>
</tr>
</thead>
</table>
| Free country without vaccination | 3 months after the last case where stamping out and serological surveillance are applied, or  
3 months after the slaughter of the last vaccinated animal where stamping out, serological surveillance and emergency vaccination are applied  
12 months after the last case and the last vaccination where stamping out in outbreaks, emergency vaccination not followed by the destruction of all vaccinated animals, and serological surveillance are applied |
| Free zone without vaccination | 3 months after the last case where stamping out and serological surveillance are applied, or  
3 months after the slaughter of the last vaccinated animal where stamping out, serological surveillance and emergency vaccination are applied  
2 years after the last case and 12 months after the last vaccination where stamping out in outbreaks, emergency vaccination not followed by the destruction of all vaccinated animals, and serological surveillance are applied |
| Free country or zone with vaccination | 12 months after the last case where stamping out and serological surveillance are applied  
2 years after the last case where serological surveillance is applied without stamping out |

Article 2.1.1.7. lays down the specific conditions under which animals susceptible to FMD kept in an infected zone can be transported to a buffer zone, a surveillance zone or even a free zone for immediate slaughter.
The chapter provides a precise list of the commodities from infected countries or zones that could spread the FMD virus (in Articles 2.1.1.8. and 2.1.1.28.). The list is as follows:

a) **Live animals**
- domestic and wild ruminants (including animals of the family of Camelidae) and domestic and wild pigs.

b) **Products**
- semen of ruminants and pigs;
- embryos/ova of ruminants and pigs;
- fresh meat of domestic and wild ruminants and pigs;
- meat products from domestic and wild ruminants and pigs;
- products of animal origin (from ruminants and pigs) intended for human consumption, for use in animal feeding or for agricultural or industrial use;
- products of animal origin (from ruminants and pigs) intended for pharmaceutical or surgical use;
- non-sterile biological products (from ruminants and pigs);
- straw and forage (such as hay).

The *Code* considers other commodities, including cereal grains, fruit, vegetables and tubers, as not likely to present such a risk.

The remainder of the chapter consists of articles detailing the health guarantees that should be included in international veterinary certificates, depending on the type of commodity that is to be traded and the health status of its place of origin in regard to FMD.

It is important to emphasise that the *Code* does allow for the importation of the commodities mentioned in the above list, even from countries or zones infected with the disease, subject to certain conditions for destroying the virus, as described in the chapter or in Appendix 3.6.2. These products include:

- meat from ruminants and pigs and meat products that have been processed to ensure the destruction of the FMD virus (canning, thorough cooking, drying after salting);
- milk and cream intended for human consumption that have been treated to ensure the destruction of the FMD virus (ultra-high temperature treatment [UHT], high temperature-short time pasteurisation [HTST] repeated twice depending on the pH of the milk), as well as milk powder and milk products prepared from milk subjected to one of the aforementioned processes.

Other conditions are specified for milk intended for use in animal feeding, as well as for animal products such as wool, hair, bristles, raw hides and skins from domestic and wild ruminants and pigs.
2. Chapter of the *Manual of Standards for Diagnostic Tests and Vaccines on foot and mouth disease*

The *Manual* describes internationally agreed upon laboratory methods for animal disease diagnosis and requirements for the production and control of biological products (mainly vaccines). It is revised every four years and the 2000 edition was published in early 2001. Preparation of the *Manual* is co-ordinated by the OIE Standards Commission. The *Manual* provides an essential adjunct to the *Code* as it outlines the appropriate tests that should be used for international trade in animals and animal products.

The FMD chapter in the *Manual* (Part 2) was written by internationally renowned FMD specialists, and was reviewed by other FMD experts, by the OIE Working Group on Biotechnology and by the experts at the OIE Reference Laboratories for FMD. It was then sent to Member Countries for comment and was approved by the Standards Commission and the International Committee. The chapter consists of:

- a brief description of the disease
- sample collection and submission procedures
- agent identification procedures using cell culture, enzyme-linked immunosorbent assay (ELISA) and the polymerase chain reaction
- serological tests
- requirements for vaccines.

The serological tests described in the *Manual* provide methods to screen for evidence of exposure to the FMD virus. Consequently, they play an important role in showing that a country or zone is FMD free, and in detecting subclinical infection or confirming clinical infection during an outbreak of disease. The tests can be used to meet the requirements of the *Code* with regard to qualifying animals for export and determining whether or not a country or zone is FMD free. The OIE has designated two FMD tests as prescribed tests for international trade: the ELISA and the virus neutralisation (VN) test. These two tests are used to meet the *Code* requirements. The VN is the gold standard test for confirming results obtained from other tests. Both tests are strain specific so the appropriate virus strain(s) must be included when carrying out the tests. The chapter also describes the nonstructural protein (NSP) tests, which can be used to differentiate between vaccinated and infected animals. These tests also have the advantage of being group specific so they can be used to screen for all the strains of FMD. Due to the lack of sensitivity of the NSP tests, however, the recommendation outlined in the *Manual* is that these tests be used on a herd basis and not to determine the infection status of individual animals. The Standards Commission is continually reviewing validation data on new tests. If more sensitive and specific tests become available, the Commission will publish recommendations in the OIE *Bulletin*.

The chapter provides detailed standards for FMD vaccine production, including methods to monitor sterility, purity and efficacy of vaccines. Vaccine strain selection is discussed and a detailed standard is included for insuring the potency and safety of the vaccine.
To support the Manual and to further ensure quality of diagnostic testing the OIE has designated four FMD Reference Laboratories (Part 3). These laboratories provide scientific advice or help in resolving specific problems relating to the diagnosis of FMD. One of the Reference Laboratories has prepared internationally validated reference antisera that can be used by laboratories in Member Countries to standardise testing (Part 4).

3. Recognition by the OIE of foot and mouth disease free status

The Code chapter on FMD makes provisions for countries to apply for inclusion in the category of internationally recognised FMD free countries (with or without vaccination, depending on the strategy adopted). The procedure to be followed for the OIE to recognise this classification was determined in May 1995. It is based on the following principles:

- The procedure is entirely voluntary.
- It may involve the entire country or only some of its zones.
- The country sends its proposal to the Director General of the OIE, accompanied by a comprehensive report based on the model prepared by the OIE FMD and Other Epizootics Commission.
- The Commission can support a country's proposal at this stage, if it is convinced that the application is well-founded. Otherwise, it can decide not to support the proposal, that clarification or additional information is needed, or that the visit of a group of experts is necessary. The cost of the visit and any other operations undertaken by the experts is borne by the applicant country.
- The Director General informs all OIE Member Countries of the Commission's support for a country's proposal. They have 60 days in which to obtain and evaluate any information submitted by the applicant country, and to inform the OIE in writing of any objections they may have, based on scientific or technical grounds. The Commission then examines any objections received, and decides whether or not to accept them.
- Each year, during its General Session, the International Committee adopts, by way of Resolution, the list of recognised FMD free countries and zones based on proposals formulated by the Foot and Mouth Disease and Other Epizootics Commission.
- Maintaining recognition of FMD free status is subject to continual observation of the OIE's rules and regulations and the declaration of any significant events likely to modify such status. FMD free status is immediately suspended if an outbreak of FMD is notified to the OIE.
- If, following suspension after an outbreak of FMD has occurred, a country is able to eradicate the disease under the conditions defined by the Code, the FMD free status can be recognised again by the Foot and Mouth Disease and Other Epizootics Commission, without waiting for the next General Session of the International Committee (Resolution No. XII adopted in May 1997).

The list of countries or zones recognised as free from FMD, as adopted by Resolution No. XVII during the 69th General Session of the OIE International Committee and by the decisions taken by the Foot and Mouth Disease and Other Epizootics Commission during its September 2001 meeting, is contained in Part 5 of this document.
4. OIE/FAO International Scientific Conference on Foot and Mouth Disease (April 2001)

An OIE/FAO International Scientific Conference on FMD was held at the OIE Headquarters on 17 and 18 April 2001. The decision to organise a conference was prompted by the recurrence of the disease several weeks before in a number of FMD free countries without vaccination. There was therefore a need to review the latest scientific knowledge in order to issue the appropriate recommendations for Member Countries of the OIE and the FAO and international financial agencies for development.

The members of the Foot and Mouth and Other Epizootics Commission and the International Animal Health Code Commission, the Presidents of the OIE Regional Commissions, experts from OIE Reference Laboratories for FMD, and senior staff of the FAO Animal Health Services and the OIE Central Bureau participated in the conference.

The OIE Regional Coordinators and the Head of the Regional Coordination Unit for the South-East Asia FMD Campaign, observers from Member Countries and intergovernmental organisations also attended the conference.

The following points were examined during the different sessions:

- Review of the definitions in the Code chapter on FMD (criteria for the declaration of an outbreak, freedom from disease or freedom from infection);
- Emergency FMD control methods (stamping out and restriction of animal movement procedures, criteria for the use of vaccination in livestock, criteria for the use of vaccination in special cases);
- Risk of FMD transmission due to trade of products;
- Research needed to better control and eradicate FMD;
- Development of programmes to control and eradicate FMD from countries where the disease is endemic.

At the end of the discussions that took place during the Conference, recommendations were drawn up to be presented to the OIE International Committee during the 69th General Session.

Some of the aforementioned recommendations were aimed at Member Countries whereas others were intended for international organisations.

In summary, Member Countries were invited:

- to conduct risk assessments on FMD taking into account the recent progress made in various fields (farming practices, national and international trade, illicit traffic, movement of people);
- to review their national legislation accordingly and to be better prepared for emergency intervention;
- to take appropriate measures in an emergency situation to protect rare breeds of domestic animals, wild animals of endangered species kept in zoos and animals kept in research establishments for research purposes;
- to encourage research into new diagnostic tests, improved vaccines, disease surveillance and control systems, risk analysis and economic research;

- to increase the awareness of political and professional authorities of the importance of FMD control;

- in the case of industrialised countries, to help the least developed countries to set up Veterinary Services capable of controlling epizootics.

The recommendations more specifically aimed at international organisations chiefly concerned:

- certain areas falling within the competence of the OIE (development of guidelines on the disposal of carcasses of slaughtered animals, the use of specific diagnostic tests within the framework of FMD surveillance, the conditions of application of emergency vaccination, and inclusion of new provisions in the Code chapter on FMD to cover products not currently included);

- a high level international conference to be organised by the OIE and FAO aimed at raising awareness worldwide, publicising the need for concerted international action against FMD and other animal diseases of significance for international trade, food security, food safety and public health, and proposing the components of such action.

In May 2001, the International Committee endorsed all these recommendations by adopting Resolution No. XIII (see Part 6 of the present document), and at their respective meetings in September 2001, the International Animal Health Code Commission and the Foot and Mouth Disease and Other Epizootics Commission began work on the programme thus defined.
SECTION 2.1. – LIST A DISEASES

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CHAPTER 2.1.1.

FOOT AND MOUTH DISEASE

Article 2.1.1.1.

For the purposes of this Code, the incubation period for foot and mouth disease (FMD) shall be 14 days. Standards for diagnostic tests and vaccines are described in the Manual.

Article 2.1.1.2.

FMD free country where vaccination is not practised

To be listed in FMD free countries where vaccination is not practised, a country should:

1) have a record of regular and prompt animal disease reporting;

2) send a declaration to the OIE that there has been no outbreak of FMD and no vaccination has been carried out for at least 12 months, with documented evidence that an effective system of surveillance is in operation and that all regulatory measures for the prevention and control of FMD have been implemented;

3) not have imported animals vaccinated against FMD since the cessation of vaccination.

The name of the country will be included in the list only after acceptance of submitted evidence by the OIE.

Article 2.1.1.3.

FMD free country where vaccination is practised

To be listed in FMD free countries where vaccination is practised, a country should:

1) have a record of regular and prompt animal disease reporting;

2) send a declaration to the OIE that there has been no outbreak of FMD for the past 2 years, with documented evidence that:

   a) an effective system of disease surveillance is in operation and that all regulatory measures for the prevention and control of FMD have been implemented, and
b) routine vaccination is carried out for the purpose of the prevention of FMD and that the vaccine used complies with the standards described in the Manual, and

3) have a system of intensive and frequent surveillance for detection of any viral activity.

The name of the country will be included in the list only after acceptance of submitted evidence by the OIE.

If an FMD free country where vaccination is practised wishes to change its status to FMD free country where vaccination is not practised, a waiting period of 12 months after vaccination has ceased is required.

Article 2.1.1.4.

**FMD free zone where vaccination is not practised**

An FMD free zone where vaccination is not practised can be established in an FMD free country where vaccination is practised or in a country of which parts are still infected. The free zone is separated from the rest of the country and from neighbouring infected countries by a surveillance zone, or physical or geographical barriers and animal health measures which effectively prevent the entry of the virus. A country in which an FMD free zone where vaccination is not practised is to be established should:

1) have a record of regular and prompt animal disease reporting;

2) send a declaration to the OIE that it wishes to establish an FMD free zone where vaccination is not practised, where there has been no outbreak of FMD for the past 2 years, where no vaccination has been carried out for the past 12 months, and that no vaccinated animal has been introduced into the zone since the cessation of vaccination;

3) supply documented evidence that an effective system of surveillance is in operation in the FMD free zone where vaccination is not practised as well as the surveillance zone if applicable;

4) describe in detail:

   a) the boundaries of the FMD free zone, and the surveillance zone, where vaccination is not practised,

   b) the system for preventing the entry of the virus into the FMD free zone,

and supply evidence that these are properly supervised and that all regulatory measures for the prevention and control of FMD have been implemented.

The name of the free zone will be included in the list of FMD free zones where vaccination is not practised only after acceptance of submitted evidence by the OIE.

Article 2.1.1.5.

**FMD free zone where vaccination is practised**

An FMD free zone where vaccination is practised can be established in a country with a free zone where vaccination is not practised or in a country of which parts are still infected. The free zone where vaccination is practised is separated from the rest of the country and, if relevant, from neighbouring infected countries by a buffer zone, or physical or geographical barriers and animal health measures
which effectively prevent the entry of the virus. A country in which an FMD free zone where vaccination is practised is to be established should:

1) have a record of regular and prompt animal disease reporting;

2) send a declaration to the OIE that it wishes to establish an FMD free zone where vaccination is practised, where there has been no outbreak of FMD for the past 2 years;

3) supply documented evidence that an effective system of surveillance is in operation in the FMD free zone where vaccination is practised as well as the buffer zone if applicable, that routine vaccination is carried out for the purpose of the prevention of FMD, and that the vaccine used complies with the standards described in the Manual;

4) describe in detail:
   a) the boundaries of the FMD free zone where vaccination is practised and the buffer zone if applicable,
   b) the system for preventing the entry of the virus into the FMD free zone,

and supply evidence that these are properly supervised, and that all regulatory measures for the prevention and control of FMD have been implemented;

5) have a system of intensive and frequent surveillance for detection of any viral activity in the FMD free zone where vaccination is practised.

The name of the free zone will be included in the list of FMD free zones where vaccination is practised only after acceptance of submitted evidence by the OIE.

If a country that has an FMD free zone where vaccination is practised wishes to change the status of the zone to FMD free zone where vaccination is not practised, a waiting period of 12 months after vaccination has ceased is required.

Article 2.1.1.6.

FMD infected country

An FMD infected country is a country that does not fulfil the requirements for being considered as an FMD free country.

When FMD occurs in an FMD free country or zone where vaccination is not practised, the following waiting periods are required to regain the disease free status:

a) 3 months after the last case, where stamping-out and serological surveillance are applied; or

b) 3 months after the slaughter of the last vaccinated animal where stamping-out, serological surveillance and emergency vaccination are applied.

When FMD occurs in an FMD free country or zone where vaccination is practised, the following waiting periods are required to regain the disease free status:

a) 12 months after the last case where stamping-out is applied, or
b) 2 years after the last case without stamping-out,

provided that an effective surveillance has been carried out.

Article 2.1.1.7.

FMD infected zone

An FMD infected zone is a zone where the infection is present in a country with a free zone where vaccination either is or is not practised. The infected zone should be separated from the free zone either by a surveillance zone, or a buffer zone, or by physical or geographical barriers and animal health measures which effectively prevent the escape of the virus.

Live animals from FMD susceptible species can only leave the infected zone if moved by mechanical transport to the nearest designated abattoir located in the buffer zone or the surveillance zone for immediate slaughter. In the absence of an abattoir in the buffer zone or the surveillance zone, live FMD susceptible animals can be transported to the nearest abattoir in a free zone for immediate slaughter only under the following conditions:

1) no animal in the establishment of origin has shown clinical signs of FMD for at least 30 days prior to movement;
2) the animals were kept in the establishment of origin for at least 3 months prior to movement;
3) FMD has not occurred within a 10-km radius of the establishment of origin for at least 3 months prior to movement;
4) the animals must be transported under the supervision of the Veterinary Authority in a vehicle, which was cleansed and disinfected before loading, directly from the establishment of origin to the abattoir without coming into contact with other susceptible animals;
5) such an abattoir is not export approved;
6) all products obtained from the animals must be considered infected and treated in such a way as to destroy any residual virus; in particular, meat must be processed in conformity with one of the procedures referred to in Article 3.6.2.1.;
7) vehicles and the abattoir must be subjected to thorough cleansing and disinfection immediately after use.

Animals moved into a free zone for other purposes must be taken to a quarantine station under the supervision of the Veterinary Authority. Freedom of infection of these animals must be established by appropriate tests.

Article 2.1.1.8.

Veterinary Administrations of countries shall consider whether there is a risk with regard to FMD in accepting importation or transit through their territory, from other countries, of the following commodities:

1) domestic and wild ruminants and pigs;
2) semen of ruminants and pigs;
3) embryos/ova of ruminants and pigs;
4) fresh meat of domestic and wild ruminants and pigs;
5) **meat products** of domestic and wild ruminants and pigs which have not been processed to ensure the destruction of the FMD virus in conformity with one of the procedures referred to in Article 3.6.2.1.;

6) **products of animal origin intended for human consumption, for use in animal feeding or for agricultural or industrial use**;

7) **products of animal origin intended for pharmaceutical or surgical use**;

8) **non-sterile biological products**.

For the purposes of this Chapter, ruminants include animals of the family of Camelidae.

**Article 2.1.1.9.**

When importing from FMD free countries or zones where vaccination is not practised, *Veterinary Administrations* should require:

for **FMD susceptible animals**

the presentation of an *international veterinary certificate* attesting that the animals:

1) showed no clinical sign of FMD on the day of shipment;

2) were kept in an FMD free country or zone since birth or for at least the past 3 months.

**Article 2.1.1.10.**

When importing from FMD free countries or zones where vaccination is practised, *Veterinary Administrations* should require:

for **domestic ruminants and pigs**

the presentation of an *international veterinary certificate* attesting that the animals:

1) showed no clinical sign of FMD on the day of shipment;

2) were kept in an FMD free country since birth or for at least the past 3 months; and

3) have not been vaccinated and showed a negative response to tests for antibodies against FMD virus, when destined to an FMD free country or zone where vaccination is not practised.

FMD free countries where vaccination is not practised may require additional guarantees.

**Article 2.1.1.11.**

When importing from FMD infected countries or zones, *Veterinary Administrations* should require:

for **domestic ruminants and pigs**

the presentation of an *international veterinary certificate* attesting that the animals:

1) showed no clinical sign of FMD on the day of shipment;
2) were kept in the *establishment* of origin since birth or
   a) for the past 30 days, if a *stamping-out policy* is in force in the *exporting country*, or
   b) for the past 3 months, if a *stamping-out policy* is not in force in the *exporting country*,

   and that FMD has not occurred within a 10-km radius of the *establishment* of origin for the relevant
   period as defined in points a) and b) above;

3) were isolated for the 30 days prior to quarantine in an *establishment*, were subjected to diagnostic
   tests (probang and serology) for FMD with negative results, and that FMD has not occurred within a
   10-km radius of the *establishment* during that period;

4) were kept in a *quarantine station* for the 30 days prior to shipment, were subjected to diagnostic
   tests (probang and serology) for FMD with negative results at the end of that period, and that FMD
   has not occurred within a 10-km radius of the *quarantine station* during that period;

5) were not exposed to any source of infection during their transportation from the *quarantine station*
   to the *place of shipment*.

**Article 2.1.1.12.**

When importing from FMD free countries or zones where vaccination is not practised, *Veterinary
Administrations* should require:

for fresh semen of domestic ruminants and pigs

the presentation of an *international veterinary certificate* attesting that:

1) the donor animals:

   a) showed no clinical sign of FMD on the day of collection of the semen;

   b) were kept in an FMD free country or zone where vaccination is not practised for at least
      3 months prior to collection;

2) the semen was collected, processed and stored in conformity with the provisions of either
   Appendix 3.2.1. or Appendix 3.2.3.

**Article 2.1.1.13.**

When importing from FMD free countries or zones where vaccination is not practised, *Veterinary
Administrations* should require:

for frozen semen of domestic ruminants and pigs

the presentation of an *international veterinary certificate* attesting that:

1) the donor animals:

   a) showed no clinical sign of FMD on the day of collection of the semen and for the following
      30 days;
b) were kept in an FMD free country or zone where vaccination is not practised for at least 3 months prior to collection;

2) the semen was collected, processed and stored in conformity with the provisions of either Appendix 3.2.1. or Appendix 3.2.3.

Article 2.1.1.14.

When importing from FMD free countries or zones where vaccination is practised, *Veterinary Administrations* should require:

for semen of domestic ruminants and pigs

the presentation of an *international veterinary certificate* attesting that:

1) the donor animals:

   a) showed no clinical sign of FMD on the day of collection of the semen and for the following 30 days;

   b) were kept in a country or zone free from FMD for at least 3 months prior to collection;

   c) if destined to an FMD free country or zone where vaccination is not practised:

      i) have not been vaccinated and showed a negative response to tests for antibodies against FMD virus; or

      ii) had been vaccinated at least twice, with the last vaccination not more than 12 and not less than 1 month prior to collection;

2) no other animal present in the *artificial insemination centre* has been vaccinated within the month prior to collection;

3) the semen:

   a) was collected, processed and stored in conformity with the provisions of either Appendix 3.2.1. or Appendix 3.2.3.;

   b) was stored in a country free from FMD for a period of at least one month before export, and during this period no animal on the *establishment* where the donor animals were kept showed any sign of FMD.

Article 2.1.1.15.

When importing from FMD infected countries or zones, *Veterinary Administrations* should require:

for semen of domestic ruminants and pigs

the presentation of an *international veterinary certificate* attesting that:

1) the donor animals:

   a) showed no clinical sign of FMD on the day of collection of the semen;
b) were kept in an establishment where no animal had been added in the 30 days before collection, and that FMD has not occurred within 10 km for the 30 days before and after collection;

c) have not been vaccinated and showed a negative response to tests for antibodies against FMD virus; or

d) had been vaccinated at least twice, with the last vaccination not more than 12 and not less than 1 month prior to collection;

2) no other animal present in the artificial insemination centre has been vaccinated within the month prior to collection;

3) the semen:

   a) was collected, processed and stored in conformity with the provisions of either Appendix 3.2.1. or Appendix 3.2.3.;

   b) was subjected, with negative results, to a virus isolation test if the donor animal has been vaccinated within the 12 months prior to collection;

   c) was stored for a period of at least one month between collection and export, and during this period no animal on the establishment where the donor animals were kept showed any sign of FMD.

Article 2.1.1.16.

When importing from FMD free countries or zones (where vaccination either is or is not practised), Veterinary Administrations should require:

for in vivo derived embryos of cattle

the presentation of an international veterinary certificate attesting that:

1) the donor females:

   a) showed no clinical sign of FMD at the time of collection of the embryos;

   b) were kept in an establishment located in a country or zone free from FMD at the time of collection;

2) the embryos were collected, processed and stored in conformity with the provisions of Appendix 3.3.1. or Appendix 3.3.9., as relevant.

Article 2.1.1.17.

When importing from FMD infected countries or zones, Veterinary Administrations should require:

for in vivo derived embryos of cattle

the presentation of an international veterinary certificate attesting that:

1) the donor females:

   a) showed no clinical sign of FMD at the time of collection of the embryos;
b) were kept in an establishment where no animal had been added in the 30 days before collection, and that FMD has not occurred within 10 km for the 30 days before and after collection;

2) the embryos were collected, processed and stored in conformity with the provisions of Appendix 3.3.1. or Appendix 3.3.9., as relevant.

Article 2.1.1.18.

When importing from FMD free countries or zones where vaccination is not practised, Veterinary Administrations should require:

for *in vitro* produced embryos of cattle

the presentation of an *international veterinary certificate* attesting that:

1) the donor females:
   a) showed no clinical sign of FMD at the time of collection of the embryos;
   b) were kept in a country or zone free from FMD at the time of collection;

2) fertilisation was achieved with semen meeting the conditions referred to in Articles 2.1.1.12., 2.1.1.13., 2.1.1.14. or 2.1.1.15., as relevant;

3) the embryos were collected, processed and stored in conformity with the provisions of Appendix 3.3.1. or Appendix 3.3.9., as relevant.

Article 2.1.1.19.

When importing from FMD free countries or zones where vaccination is practised, Veterinary Administrations should require:

for *in vitro* produced embryos of cattle

the presentation of an *international veterinary certificate* attesting that:

1) the donor females:
   a) showed no clinical sign of FMD at the time of collection of the embryos;
   b) were kept in a country or zone free from FMD for at least 3 months prior to collection;
   c) if destined for an FMD free country or zone where vaccination is not practised:
      i) have not been vaccinated and showed a negative response to tests for antibodies against FMD virus, or
      ii) had been vaccinated at least twice, with the last vaccination not less than 1 month and not more than 12 months prior to collection;

2) no other animal present in the establishment has been vaccinated within the month prior to collection;

3) fertilization was achieved with semen meeting the conditions referred to in Articles 2.1.1.12., 2.1.1.13., 2.1.1.14. or 2.1.1.15., as relevant;
4) the embryos were collected, processed and stored in conformity with the provisions of Appendix 3.3.1. or Appendix 3.3.9., as relevant.

Article 2.1.1.20.

When importing from FMD free countries or zones where vaccination is not practised, Veterinary Administrations should require:

for fresh meat of FMD susceptible animals

the presentation of an international veterinary certificate attesting that the entire consignment of meat comes from animals:

1) which have been kept in the country or zone since birth, or have been imported from a country or zone free from FMD;

2) which have been slaughtered in an approved abattoir and have been subjected to ante-mortem and post-mortem inspections for FMD with favourable results.

Article 2.1.1.21.

When importing from FMD free countries or zones where vaccination is practised, Veterinary Administrations should require:

for fresh meat of bovines (excluding feet, head and viscera)

the presentation of an international veterinary certificate attesting that the entire consignment of meat:

1) comes from animals which:
   a) have remained in the exporting free country or zone for at least 3 months prior to slaughter;
   b) have been slaughtered in an approved abattoir (located in the free zone, when the animals originate from such a zone) and have been subjected to ante-mortem and post-mortem inspections for FMD with favourable results;

2) comes from deboned carcasses:
   a) from which the major lymphatic glands have been removed;
   b) which, prior to deboning, have been submitted to maturation at a temperature above + 2°C for a minimum period of 24 hours following slaughter, and in which the pH value of the meat was below 6.0 when tested in the middle of both the longissimus dorsi.

If the meat is to be imported into a country or a zone of equivalent FMD status or into an infected country in which the virus types used in the vaccines are the same, the maturation and deboning processes may not be required.
Article 2.1.1.22.

When importing from FMD free countries or zones where vaccination is practised, Veterinary Administrations should require:

**for fresh meat or meat products of pigs and ruminants other than bovines**

the presentation of an *international veterinary certificate* attesting that the entire consignment of meat comes from animals:

1) which have been kept in the country or zone since birth, or have been imported from a country or zone free from FMD (where vaccination either is or is not practised);

2) which have not been vaccinated;

3) which have been slaughtered in an *approved abattoir* (located in the free zone, when the animals originate from such a zone) and have been subjected to ante-mortem and post-mortem inspections for FMD with favourable results.

Article 2.1.1.23.

When importing from FMD infected countries or zones, where an official control programme exists, involving compulsory systematic vaccination of cattle, Veterinary Administrations should require:

**for fresh meat of bovines (excluding feet, head and viscera)**

the presentation of an *international veterinary certificate* attesting that the entire consignment of meat:

1) comes from animals which:

   a) have remained in the *exporting country* for at least 3 months prior to slaughter;

   b) have remained, during this period, in a part of the country where cattle are regularly vaccinated against FMD and where official controls are in operation;

   c) have been vaccinated at least twice with the last vaccination not more than 12 months and not less than 1 month prior to slaughter;

   d) were kept for the past 30 days in an *establishment*, and that FMD has not occurred within 10 km during that period;

   e) have been transported, in a *vehicle* which was cleansed and disinfected before the cattle were loaded, directly from the *establishment* of origin to the *approved abattoir* without coming into contact with other animals which do not fulfil the required conditions for export;

   f) have been slaughtered in an *approved abattoir*:

      i) which is officially designated for export;

      ii) in which no FMD has been detected during the period between the last *disinfection* carried out before slaughter and the shipment for export has been dispatched;
g) have been subjected to ante-mortem and post-mortem inspections for FMD with favourable results within 24 hours before and after slaughter;

2) comes from deboned carcasses:
   a) from which the major lymphatic glands have been removed;
   b) which, prior to deboning, have been submitted to maturation at a temperature above +2°C for a minimum period of 24 hours following slaughter and in which the pH value was below 6.0 when tested in the middle of both the longissimus dorsi.

[Note: Article 2.1.1.23. should also apply when meat is to be imported from an infected country into another infected country, in order to prevent the introduction of new strains of FMD virus.]

Article 2.1.1.24.

When importing from FMD infected countries or zones, Veterinary Administrations should require:

for meat products of domestic ruminants and pigs

the presentation of an international veterinary certificate attesting that:

1) the entire consignment of meat comes from animals which have been slaughtered in an approved abattoir and have been subjected to ante-mortem and post-mortem inspections for FMD with favourable results;

2) the meat has been processed to ensure the destruction of the FMD virus in conformity with one of the procedures referred to in Article 3.6.2.1.;

3) the necessary precautions were taken after processing to avoid contact of the meat products with any potential source of FMD virus.

Article 2.1.1.25.

When importing from FMD free countries or zones (where vaccination either is or is not practised), Veterinary Administrations should require:

for milk and milk products intended for human consumption and for products of animal origin (from FMD susceptible animals) intended for use in animal feeding or for agricultural or industrial use

the presentation of an international veterinary certificate attesting that these products come from animals which have been kept in the country or zone since birth, or which have been imported from an FMD free country or zone (where vaccination either is or is not practised).

Article 2.1.1.26.

When importing from FMD infected countries or zones, Veterinary Administrations should require:

for milk and cream

the presentation of an international veterinary certificate attesting that:
1) these products:
   a) originate from herds or flocks which were not subjected to any restrictions due to FMD at the time of milk collection;
   b) have been processed to ensure the destruction of the FMD virus in conformity with one of the procedures referred to in Article 3.6.2.5. and in Article 3.6.2.6.;

2) the necessary precautions were taken after processing to avoid contact of the products with any potential source of FMD virus.

Article 2.1.1.27.

When importing from FMD infected countries or zones, Veterinary Administrations should require:

for milk powder and milk products

the presentation of an *international veterinary certificate* attesting that:

1) these products are derived from milk complying with the above requirements;

2) the necessary precautions were taken after processing to avoid contact of the milk powder or the milk products with any potential source of FMD virus.

Article 2.1.1.28.

When importing from FMD infected countries, Veterinary Administrations should require:

for blood and meat-meals (from domestic or wild ruminants and pigs)

the presentation of an *international veterinary certificate* attesting that the manufacturing method for these products included heating to a minimum internal temperature of 70°C for at least 30 minutes.

Article 2.1.1.29.

When importing from FMD infected countries, Veterinary Administrations should require:

for wool, hair, bristles, raw hides and skins (from domestic or wild ruminants and pigs)

the presentation of an *international veterinary certificate* attesting that:

1) these products have been processed to ensure the destruction of the FMD virus in conformity with one of the procedures referred to in Articles 3.6.2.2., 3.6.2.3. and 3.6.2.4.;

2) the necessary precautions were taken after collection or processing to avoid contact of the products with any potential source of FMD virus.

Veterinary Administrations can authorise, without restriction, the import or transit through their territory of semi-processed hides and skins (limed hides, pickled pelts, and semi-processed leather - e.g. wet blue and crust leather), provided that these products have been submitted to the usual chemical and mechanical processes in use in the tanning industry.
Article 2.1.1.30.

When importing from FMD infected countries or zones, Veterinary Administrations should require:

for straw and forage

the presentation of an international veterinary certificate attesting that these articles:

1) have been subjected:
   a) either to the action of steam in a closed chamber for at least 10 minutes and at a minimum temperature of 80°C,
   b) or to the action of formalin fumes (formaldehyde gas) produced by its commercial solution at 35-40% in a chamber kept closed for at least 8 hours and at a minimum temperature of 19°C;

OR

2) have been kept in bond for at least 3 months (under study) before being released for exportation.

Article 2.1.1.31.

When importing from FMD free countries or zones (where vaccination either is or is not practised), Veterinary Administrations should require:

for skins and trophies derived from wild animals susceptible to FMD

the presentation of an international veterinary certificate attesting that these products are derived from animals that have been kept in such a country or zone since birth, or which have been imported from a country or zone free of FMD (where vaccination either is or is not practised).

Article 2.1.1.32.

When importing from FMD infected countries or zones, Veterinary Administrations should require:

for skins and trophies derived from wild animals susceptible to FMD

the presentation of an international veterinary certificate attesting that these products have been processed to ensure the destruction of the FMD virus in conformity with the procedures referred to in Article 3.6.2.7.

[Note: International veterinary certificates for animal products coming from infected countries or zones may not be required if the products are transported in an approved manner to premises controlled and approved by the Veterinary Administration of the importing country for processing to ensure the destruction of the FMD virus in conformity with the procedures referred to in Articles 3.6.2.2., 3.6.2.3. and 3.6.2.4.]
SECTION 2.1.: LIST A DISEASES

CHAPTER 2.1.1.
FOOT AND MOUTH DISEASE

SUMMARY

Foot and mouth disease (FMD) is the most contagious disease of mammals and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals. There are seven serotypes of FMD virus, namely, O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1. Infection with one serotype does not confer immunity against another. FMD cannot be differentiated clinically from other vesicular diseases, including swine vesicular disease, vesicular stomatitis, and vesicular exanthema. Laboratory diagnosis of any suspected FMD case is therefore a matter of urgency.

Typical cases of FMD are characterised by a vesicular condition of the feet, buccal mucosa and, in females, the mammary glands. Clinical signs can vary from mild to severe and fatalities may occur, especially in young animals. In some species the infection may usually be subclinical, e.g. African buffalo (Syncerus caffer). The preferred tissue for diagnosis is epithelium from unruptured or freshly ruptured vesicles. Where this is not possible, blood and/or oesophageal-pharyngeal fluid samples taken by probang cup in ruminants or throat swabs from pigs provide an alternative source of virus. Myocardial tissue or blood can be submitted from fatal cases, but vesicles are again preferable if present.

It is vital that samples from suspected cases be transported under secure conditions and according to international regulations. They should only be dispatched to authorised laboratories.

Diagnosis of FMD is by the demonstration of FMD viral antigen or nucleic acid in samples of tissue or fluid. Detection of specific humoral antibody can also be used for diagnosis, especially in wildlife, but this requires the absence of any history of vaccination, as it is not always possible to differentiate a serological response to natural infection from that due to vaccination. Diagnosis based on serological response may also be problematical in endemic areas due to the possibility of previous infection.

Identification of the agent: The demonstration of FMD viral antigen is sufficient for a positive diagnosis.

Complement fixation (CF) has been the traditional test for diagnosis, but has been replaced in many laboratories by the enzyme-linked immunosorbent assay (ELISA), as this is more specific and sensitive and is not affected by pro- or anti-complementary factors. If the sample is inadequate or the test result inconclusive, it will be necessary to grow the virus in cell cultures or in 2-7-day old unweaned mice. The cultures should preferably be of primary bovine thyroid, but pig, lamb or calf kidney cells, or cell lines of comparable sensitivity may be used. When a cytopathic effect (CPE) appears in the cultures, the fluids can be used in CF tests or ELISAs. Similar tests can be performed on homogenised suspensions of the dissected musculo-skeletal tissues of any mice that die. In the absence of CPE or any dead mice, a further passage should be made at a 48-hour interval, with freeze-thawing of the cells, before the sample is declared to be negative.
Nucleic acid recognition tests, such as the polymerase chain reaction and in situ hybridisation, are being used increasingly as rapid and sensitive diagnostic methods. Electron microscopic examination of lesion material is sometimes useful to differentiate FMD from disease caused by pox or other viruses.

**Serological tests:** The demonstration of specific antibody titres in nonvaccinated animals, where a vesicular condition is present, is sufficient for a positive diagnosis. This is particularly useful in mild cases or where epithelial tissue cannot be collected.

Virus neutralisation (VN) tests and ELISAs are used as serotype-specific serological tests. VN tests depend on tissue cultures and are therefore more prone to variability than ELISAs; they are also slower and subject to contamination. ELISAs for antibodies have the advantage of being faster, and are not dependent on cell cultures. The ELISA can be performed with inactivated antigens, thus requiring less restrictive biocontainment facilities.

**Requirements for vaccines and diagnostic biologicals:** Inactivated virus vaccines of varying composition are available commercially. Typically, virus is used to infect a suspension or monolayer cell culture and the resulting preparation is clarified, inactivated with ethyleneimine and blended with adjuvant. Many FMD vaccines are multivalent to provide cover against the different serotypes likely to be encountered in a given field situation.

The finished vaccine must be shown to be free from residual live virus. This is usually done using a combination of in-vitro tests on the inactivated virus preparation and in-vivo tests on the finished vaccine. Challenge tests are also conducted in vaccinated cattle to establish a PD₅₀ (50% protective dose) value, although a serological test is considered to be satisfactory where the vaccine producer has established a statistically significant correlation between protection and specific antibody response.

Diagnostic and reference reagents are available from the OIE/FAO World Reference Laboratory for FMD¹, or from Regional Reference Laboratories for FMD.

**A. DIAGNOSTIC TECHNIQUES**

Foot and mouth disease (FMD) is caused by a virus of the genus Aphthovirus, family Picornaviridae. There are seven serotypes of FMD virus, namely O, A, C, SAT 1, SAT 2, SAT 3, and Asia 1, that infect cloven hoofed animals. Infection with any one serotype does not confer immunity against another. Within serotypes, many subtypes can be identified by biochemical and immunological tests.

In Africa, FMD viruses are maintained by cattle and African buffalo (*Syncerus caffer*). Available evidence indicates that although other domestic and wild species become infected, they are unable to maintain the infection for more than a few months in the absence of cattle or African buffalo. Elsewhere in the world cattle are usually the main reservoir, although in some instances the viruses involved appear to be specifically adapted to domestic pigs or sheep and goats. It is probable that these adapted viruses are able to modify their adaptation and affect other species if given the opportunity. Wildlife outside Africa have not, so far, been shown to be able to maintain FMD viruses. The evidence indicates that infection of deer in the past was derived from contact, direct or indirect, with infected domestic animals.

Of the domesticated species, cattle, pigs, sheep, goats and buffalo are susceptible to FMD (21). In addition, many species of cloven-hoofed wildlife, such as deer, antelope and wild pigs may become infected, although, apart from the African buffalo their involvement in the epidemiology of FMD in the domesticated species is not certain. Strains of FMD virus that infect cattle have been isolated from wild pigs and deer. For the diagnosis of FMD in wild species, procedures similar to those described for farm animals can be applied.

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¹ OIE/FAO World Reference Laboratory for FMD, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey GU24 ONF, United Kingdom.
Infection of susceptible animals with FMD virus leads to the appearance of vesicles on the feet, in and around the oral cavity, and on the mammary glands of females. Vesicles can also occur at other sites, such as inside the nostrils and at pressure points on the limbs – especially in pigs. The severity of clinical signs varies with the strain of virus, the exposure dose, the age and breed of animal, the host species and its degree of immunity (29). The signs can range from a mild or inapparent infection to one that is severe. Death may result in some cases. Mortality from a multifocal myocarditis is most commonly seen in young animals: myositis may also occur in other sites. Adult animals may occasionally succumb.

On premises with a history of sudden death in young cloven-hoofed livestock, close examination of adult animals may often reveal the presence of vesicular lesions if FMD is involved. The presence of vesicles in fatal cases is variable.

In animals with a history of vesicular disease, the detection of FMD virus in samples of vesicular fluid, epithelial tissue, milk, or blood is sufficient to establish a diagnosis. Diagnosis may also be established by the isolation of FMD virus from the blood, heart or other organs of fatal cases. A myocarditis may be seen macroscopically in a proportion of fatal cases.

FMD virus can replicate and be excreted from the respiratory tract of animals. Airborne excretion of virus occurs during the acute phase of infection. FMD viruses may occur in all the secretions and excretions of acutely infected animals including expired air. Transmission is generally effected by contact between infected and susceptible animals or, more rarely, exposure of susceptible animals to the excretions and secretions of acutely infected animals. Following recovery from the acute stage of infection, infectious virus disappears from all secretions and excretions with the exception, in the case of ruminants, of those of oesophageal-pharyngeal (OP) origin. Animals in which the virus persists in the OP for more than 28 days after infection are referred to as carriers. Pigs do not become carriers. Circumstantial evidence indicates that carriers are able, on rare occasions, to transmit the infection to susceptible animals with which they come in close contact: the mechanism involved is unknown. The carrier state in cattle usually does not persist for more than 6 months, although in a small proportion it may last up to 3 years. In African buffalo individual animals have been shown to harbour the virus for at least 5 years, but it is probably not a lifelong phenomenon. Within a herd of buffalo, the virus may be maintained for 24 years or longer. Domestic buffalo, sheep and goats do not usually carry FMD viruses for more than a few months.

Due to the highly contagious nature and economic importance of FMD for many countries, the laboratory diagnosis and serotype identification of the virus should be done in a virus-secure laboratory. Countries lacking access to such a specialised national or regional laboratory should send specimens to the OIE/FAO World Reference Laboratory (WRL) for FMD (26).

Diagnostic and standard reagents are available in kit form or as individual items from the OIE/FAO WRL for FMD. The use of inactivated antigens in the enzyme-linked immunosorbent assay (ELISA), as controls in the antigen-detection test or to react with test sera in the liquid-phase blocking ELISA, reduces the disease security risk involved in the use of live virus. Reagents are supplied freeze-dried and can remain stable at 4°C in this state for many years. The International Atomic Energy Agency2 has produced a manual that includes a recommended test and quality control protocols.

For laboratory diagnosis, the tissue of choice is epithelium. Ideally, at least 1 g of epithelial tissue should be collected from an unruptured or recently ruptured vesicle. To avoid injury to personnel collecting the samples, as well as for animal welfare reasons, it is recommended that animals be sedated before any samples are obtained.

Epithelial samples should be placed in a transport medium composed of equal amounts of glycerol and 0.04 M phosphate buffer pH 7.2-7.6, preferably with added antibiotics (penicillin [1000 international

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2 International Atomic Energy Agency, Wagramerstrasse 5, P.O. Box 100, A-1400 Vienna, Austria.
units (IU)], neomycin sulphate [100 IU], polymyxin B sulphate [50 IU], mycostatin [100 IU]). If 0.04 M phosphate buffer is not available, tissue culture medium or phosphate buffered saline (PBS) can be used instead, but it is important that the final pH of the glycerol/buffer mixture be in the range pH 7.2-7.6. Samples should be kept refrigerated or on ice until received by the laboratory.

Where epithelial tissue is not available from ruminant animals, for example in advanced or convalescent cases, or where infection is suspected in the absence of clinical signs, samples of OP fluid can be collected by means of a probang (sputum) cup (or in pigs by swabbing the throat3) for submission to a laboratory for virus isolation.

Before the collection of OP samples from cattle or large ruminants (e.g. buffaloes), 2 ml transport fluid (composed of 0.08 M phosphate buffer containing 0.01% bovine serum albumin, 0.002% phenol red, antibiotics (1000 units/ml penicillin, 100 units/ml mycostatin, 100 units/ml neomycin, and 50 units/ml polymyxin), and adjusted to pH 7.2) should be added to a container of around 5 ml capacity capable of withstanding freezing above solid carbon dioxide (dry ice) or liquid nitrogen.

After collection of OP fluid by probang, the contents of the cup should be poured into a wide-necked transparent bottle of around 20 ml capacity. The fluid is examined, and should contain some visible cellular material. Of this, 2 ml is then added to the 2 ml of transport fluid, ensuring that cellular material is transferred; the mixture is shaken gently and should have a final pH of around pH 7.6. Samples contaminated with ruminal contents may be unsuitable for culture. Samples seen to contain blood are not entirely satisfactory. Repeat sampling can be done after the mouth and throat of the animal have been rinsed with water or PBS.

OP samples from small ruminants are collected by putting 2 ml of transport fluid into a wide-necked bottle of about 20 ml capacity and, after collection, rinsing the probang cup in this transport fluid to discharge the OP sample. This is then transferred to a container of about 5 ml capacity for transport. The small container should be capable of withstanding freezing above solid carbon dioxide or liquid nitrogen (26).

Samples of OP fluid should be refrigerated or frozen immediately after collection. If they are to remain in transit for more than a few hours, they should be frozen by being placed either above solid carbon dioxide or liquid nitrogen. Before freezing, the containers should be carefully sealed using airtight screw caps or silicone. This is particularly important when using solid carbon dioxide, as introduction of CO₂ into the OP sample will lower its pH, inactivating any FMD virus that may be in the samples. Glass containers should not be used because there is a risk that they will explode on defrosting in the event of liquid nitrogen leaking into them. Samples should reach the laboratory in a frozen state.

Special precautions are required when sending perishable suspect FMD material both within and between countries. These regulations are mainly designed to prevent leakage and consequent contamination, but are also important in ensuring that the specimens arrive in a satisfactory state. If wet ice is put inside a package, escape of water must be prevented. The receiving laboratory must be notified in advance of the despatch.

1. **Identification of the agent**

   **a) Virus isolation**

   The epithelium sample should be taken from the PBS/glycerol, blotted dry on absorbent paper to reduce the glycerol content, which is toxic for cell cultures, and weighed. A suspension should be prepared by grinding the sample in sterile sand in a sterile pestle and mortar with a small volume of tissue culture media and antibiotics. Further media should be added until a final volume of ten times

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3 The pig should be properly restrained, ideally held on its back in a wooden cradle with its neck extended. Holding a swab in a suitable instrument, such as an artery forceps, the swab is pushed to the back of the mouth into the pharynx.
that of the epithelial sample has been added, giving a 10% suspension. This is clarified on a bench centrifuge at 2000 g for 10 minutes. Such suspensions of field samples suspected to contain FMD virus once clarified are inoculated into cell cultures or unweaned mice. Sensitive cell culture systems include primary bovine thyroid cells and primary pig, calf or lamb kidney cells. Established cell lines, such as baby hamster kidney BHK-21 and IB-RS-2 cells, may be used but are less sensitive than primary cells for detecting low amounts of infectivity (9). The cell cultures should be examined for cytopathic effect (CPE) for 48 hours. If no CPE is detected, the cells should be frozen and thawed, used to inoculate fresh cultures and examined for CPE for another 48 hours. Unweaned mice are an alternative to cell cultures and should be 2-7 days of age and of selected inbred strains. Some field viruses may require several passages before they become adapted to mice (33).

b) Immunological methods

* Enzyme-linked immunosorbent assay

At the OIE/FAO WRL for FMD (see footnote 1), the preferred procedure for the detection of FMD viral antigen and identification of viral serotype is the ELISA (20, 31). This is an indirect sandwich test in which different rows in multiwell plates are coated with rabbit antisera to each of the seven serotypes of FMD virus. These are the ‘capture’ sera. Test sample suspensions are added to each of the rows, and appropriate controls are also included. Guinea-pig antisera to each of the serotypes of FMD virus are added next, followed by rabbit anti-guinea-pig serum conjugated to an enzyme. Extensive washing is carried out between each stage to remove unbound reagents. A colour reaction on the addition of enzyme substrate, indicates a positive reaction. With strong positive reactions this will be evident to the naked eye, but results can also be read spectrophotometrically at an appropriate wavelength. In this case, an absorbance reading greater than 0.1 above background indicates a positive reaction; the serotype of FMD virus can also be identified. Values close to 0.1 should be confirmed by retesting or by amplification of the antigen by tissue culture passage and testing the supernatant once a CPE has developed. A suitable protocol is given below.

Depending on the species affected and the geographical origin of samples, it may be appropriate to simultaneously test for swine vesicular disease (SVD) virus or vesicular stomatitis (VS) virus. Ideally a complete differential diagnosis should be undertaken in all vesicular conditions.

Rabbit antiserum to the 146S antigen of each of the seven serotypes of FMD virus (plus SVD virus if required) is used as a trapping antibody at a predetermined optimal concentration in carbonate/bicarbonate buffer, pH 9.6.

Control antigens are prepared from selected strains of each of the seven types of FMD virus (plus SVD virus if appropriate) grown on monolayer cultures of BHK-21 cells (IB-RS-2 cells for SVD virus). The unpurified supernatants are used and pretitrated on ELISA plates. The final dilution chosen is that which gives an absorbance at the top of the linear region of the titration curve (optimal density approximately 2.0), so that the five-fold dilutions of the control antigens used in the test give two additional lower optimal density readings from which the titration curve can be derived. PBS containing 0.05% Tween 20 and phenol red indicator is used as a diluent (PBST).

Guinea-pig antisera prepared by inoculating guinea-pigs with 146S antigen of one of the seven serotypes of FMD virus (plus SVD virus if required) and preblocked with normal bovine serum (NBS) is used as the detecting antibody. Predetermined optimal concentrations are prepared in PBS containing 0.05% Tween 20, and 5% dried, nonfat skimmed milk (PBITM).

Rabbit (or sheep) anti-guinea-pig immunoglobulin conjugated to horseradish peroxidase and preblocked with NBS is used at a predetermined optimum concentration in PBITM.
• **Test procedure**

i) ELISA plates are coated with 50 µl/well rabbit antiviral sera in carbonate/bicarbonate buffer, pH 9.6. Rows A to H receive, respectively, antisera to serotypes O, A, C, SAT 1, SAT 2, SAT 3, Asia 1 and SVD virus (optional).

ii) Leave overnight at 4°C in a stationary position or place on an orbital shaker set at 100-120 revolutions per minute in a 37°C incubator for 1 hour.

iii) Prepare test sample suspension (with 10% original sample suspension or undiluted clarified cell culture supernatant fluid).

iv) The ELISA plates are washed five times in PBS.

v) On each plate, load wells of columns 4, 8 and 12 with 50 µl PBST. Additionally, add 50 µl of PBST to wells 2 and 3 of rows A to H on plate 1. To well 1 of row A of plate 1 add 50 µl of control antigen type O, and to well 2 of row A add 12.5 µl of control antigen type O. Mix antigen and diluent in well 2 and transfer 12.5 µl from well 2 to well 3 of row A. Mix and discard 12.5 µl from well 3 (this gives a five-fold dilution series of antigen O). Similarly repeat with antigen A, adding 50 µl of antigen type A to well 1 of row B, and 12.5 µl of antigen type A to well 2, and then mix and transfer 12.5 µl to well 3 (as done before with antigen type O), and continue for types C, SAT 1, SAT 2, SAT 3, Asia 1 and SVD (if appropriate). It is only necessary to change pipette tips on the micropipette between antigens. The remainder of the plate can be loaded with the test sample(s). Add 50 µl of sample one to wells 5, 6 and 7 of rows A to H, the second sample is placed similarly in columns 9, 10 and 11, rows A to H.

If more than two samples are to be tested at the same time, the other ELISA plates should be used as follows:

Dispense 50 µl of the PBST to the wells (rows A to H) of columns 4, 8 and 12 (buffer control columns). Note that the control antigens are not required on these plates. These test samples may be added in 50 µl volumes in rows A to H to columns 1, 2, 3; 5, 6, 7; 9, 10, 11, respectively.

vi) Cover with lids and place on an orbital shaker at 37°C for 1 hour.

vii) Wash the plates by flooding with PBS - wash three times as before and empty residual wash fluid. Blot the plates dry.

viii) Transfer 50 µl volumes of each guinea-pig serum dilution to each plate well in the appropriate order, e.g. rows A to H receive, respectively, antisera to serotypes O, A, C, SAT 1, SAT 2, SAT 3, Asia 1 and SVD virus (optional).

ix) Cover plates with lids and replace on the orbital shaker. Incubate at 37°C for 1 hour.

x) The plates are washed again three times and 50 µl of rabbit anti-guinea-pig immunoglobulin conjugated to horseradish peroxidase is added to each well. The plates are incubated at 37°C for 1 hour on a rotary shaker.

xi) The plates are washed again three times and 50 µl of orthophenylene diamine containing 0.05% H₂O₂ (30% w/v) is added to each well.

xii) The reaction is stopped after 15 minutes by the addition of 50 µl of 1.25 M sulphuric acid. The plates are read at 492 nm on a spectrophotometer linked to a computer.
Complement fixation test

The ELISA is preferable to the complement fixation (CF) test because it is more sensitive and specific, and it is not affected by pro- or anti-complementary factors. If ELISA reagents are not available, however, the CF test may be performed as follows:

Antisera to each of the seven types of FMD virus are diluted in veronal buffer diluent (VBD) in 1.5-fold dilution steps from an initial 1/16 dilution to leave 25 µl of successive antiserum dilutions in U-shaped wells across a microtitre plate or appropriate volumes in test tubes. To these are added 50 µl of 3 units of complement, followed by 25 µl of test sample suspension(s). The test system is incubated at 37°C for 1 hour prior to the addition of 25 µl of 1.4% standardised sheep red blood cells (SRBC) in VBD sensitised with 5 units of rabbit anti-SRBC. The reagents are incubated at 37°C for a further 30 minutes and the plates are subsequently centrifuged and read. Appropriate controls for the test suspension(s), antisera, cells and complement are included. CF titres are expressed as the reciprocal of the serum dilution producing 50% haemolysis. A CF titre ≥36 is considered to be a positive reaction. Titre values of 24 should be confirmed by retesting an antigen that has been amplified through tissue culture passage.

c) Nucleic acid recognition methods

The polymerase chain reaction (PCR) can be used to amplify the genome fragments of FMD virus in diagnostic material (2, 7). Specific primers have been designed to distinguish between each of the seven serotypes. In situ hybridisation techniques have been developed for investigating the presence of FMD virus RNA in tissue samples (39). These techniques are only in use in specialised laboratories.

The molecular epidemiology of FMD is based on the comparison of genetic differences between virus isolates. Dendrograms showing the genomic relationship between vaccine and field strains for all seven serotypes have been published based on sequences derived from the 1D gene. Reverse-transcription polymerase chain reaction (RT-PCR) amplification of FMD virus RNA, followed by nucleotide sequencing, is the current preferred option for generating the sequence data to perform these comparisons. The WRL and other laboratories have developed techniques for performing these studies, and a database of over 2000 sequences is currently held.

The recommended method is to:

i) Extract FMD virus RNA directly from epithelial suspensions, or from a low cell culture passage.

ii) Perform an RT-PCR of the complete VP1 gene (or if only part of the VP1 gene, then the 3’ end of the gene is more useful).

iii) Determine the nucleotide sequence of the PCR product (or at least 170 nucleotides [preferably 420 for the SAT types] at the 3’ end of the gene).

A protocol, complete with primer sequences is available from the WRL on request or can be downloaded from the following World Wide Web URL:

http://www.iah.bbsrc.ac.uk/virus/picornaviridae/aphthovirus/fmdv.htm

Research on monoclonal antibodies (MAbs) that identify individual antigenic sites on the surface of the FMD virus may have future potential. Panels of neutralising antibodies are being developed for each serotype and characterised by nucleotide sequencing of escape mutant virus. The neutralising site against which each MAb acts can be deduced by identifying the sequence change in the mutant virus that allowed it to escape neutralisation.
2. Serological tests

FMD virus infection can be diagnosed by the detection of a specific antibody response. The tests generally used are virus neutralisation (VN) and ELISA (23, 24, 36). These are also the prescribed tests for trade. The VN test is serotype specific, requires cell culture facilities and takes 2-3 days to provide results. The ELISA is also serotype specific, sensitive and quantitative, and has the advantage that it is quicker to perform, is less variable, and is not dependent on tissue culture systems. Low titre false-positive reactions can be expected in a small proportion of the sera in either test. An approach combining screening by ELISA and confirming the positives by the VN test minimises the occurrence of false-positive results.

The detection of antibody to the nonstructural (NS) proteins of FMD virus has been used to identify past or present infection with any of the seven serotypes of the virus, whether or not the animal has also been vaccinated. Conventionally this has been carried out by measuring antibody to the virus infection-associated antigen (VIAA; the viral RNA polymerase protein 3D) using agar gel immunodiffusion (AGID) (28). Although relatively insensitive, the test is inexpensive, easy to perform and has been used extensively in South America to detect viral activity on a population basis during FMD eradication campaigns. The VIAA test has now largely been superseded by assays that measure antibody to FMD virus NS proteins produced by recombinant techniques in a variety of *in-vitro* expression systems. Antibody to the polyproteins 3AB or 3ABC are the single most reliable indicator of infection (10, 27, 34). In animals seropositive for antibody to 3AB or 3ABC, antibody to one or more of the other NS proteins including the L, 2C, 3A or 3D protein is further confirmation of infection (8, 27, 34). The test can be used on a herd basis to detect FMD virus infection in vaccinated and unvaccinated populations. Care must be taken when interpreting results from single animals as some repeatedly vaccinated animals produce antibody to 3ABC. It has not yet been established whether or not all animals that have been both vaccinated and infected seroconvert to this protein. A negative result for antibody to NS proteins cannot therefore be taken as definitive proof that an individual animal has not been exposed to FMD virus. This must be taken into account if NS protein antibody tests are used for assessment of risks for animals involved in international trade.

**a) Virus neutralisation (a prescribed test for international trade)**

The quantitative VN microtest for FMD antibody is performed with IB-RS-2, BHK-21, lamb or pig kidney cells in flat-bottomed tissue-culture grade microtitre plates.

Stock virus is grown in cell monolayers and stored at −20°C after the addition of 50% glycerol. (Virus has been found to be stable under these conditions for at least 1 year.) The sera are inactivated at 56°C for 30 minutes before testing. The control standard serum is 21-day convalescent serum (usually pig). A suitable medium is Eagle’s complete medium/LYH (Hank’s balanced salt solution with yeast lactalbumin hydrolysate) with antibiotics.

The test is an equal volume test in 50 µl amounts.

**Test procedure**

i) Starting from a 1/4 dilution, sera are diluted in a twofold dilution series across the plate, using at least two rows of wells per serum, preferably four rows, and a volume of 50 µl.

ii) Previously titrated virus is added; each 50 µl unit volume of virus suspension should contain about 100 TCID<sub>50</sub> (50% tissue culture infective dose) within an accepted range (e.g. 35-350 TCID<sub>50</sub>).

iii) Controls include a standard antiserum of known titre, a negative serum, a cell control, a medium control, and a virus titration used to calculate the actual virus titre used in the test.

iv) Incubate at 37°C for 1 hour with the plates covered.
v) A cell suspension at $10^6$ cells/ml is made up in medium containing 10% bovine serum (specific antibody negative) for cell growth. A volume of 50 µl of cell suspension is added to each well.

vi) Plates are sealed with pressure-sensitive tape and incubated at 37°C for 2-3 days. Alternatively, the plates may be covered with loosely fitting lids and incubated in an atmosphere of 3-5% carbon dioxide at 37°C for 2-3 days.

vii) Microscope readings may be feasible after 48 hours, the plates are finally fixed and stained routinely on the third day. Fixation is effected with 10% formalin/saline for 30 minutes. For staining, the plates are immersed in 0.05% methylene blue in 10% formalin for 30 minutes. An alternative fixative/stain solution is naphthalene blue black solution (0.4% [w/v] naphthalene blue black, 8% [w/v] citric acid in saline) (22). The plates are rinsed in tap water.

viii) Positive wells (where the virus has been neutralised and the cells remain intact) are seen to contain blue-stained cells sheets; the negative wells (where virus has not been neutralised) are empty. Titres are expressed as the final dilution of serum present in the serum/virus mixture at the 50% end-point, i.e. a well where there is an incomplete cell sheet (Kärber). The test is considered to be valid when the amount of virus used per well is in the range log10 1.5-2.5 TCID50, and the positive standard serum is within twofold of its expected titre.

ix) Interpretation of tests can vary between laboratories in regard to end-points taken. Laboratories should establish their own criteria by reference to standard reagents that can be obtained from the OIE/FAO WRL for FMD (see footnote 1). At the WRL, a titre of 1/45 or more of the final serum dilution in the serum/virus mixture is regarded as positive. Titres of 1/16 to 1/32 are considered to be doubtful, and further serum samples are requested for testing. Animals are considered to be positive if the second sample has a titre of 1/16 or greater. A titre of 1/8 or less is considered to be negative.

b) Liquid-phase blocking enzyme-linked immunosorbent assay (a prescribed test for international trade)

Rabbit antiserum to the 146S antigen of one of the seven types of FMD virus is used as the trapping antibody at a predetermined\(^4\) optimal concentration in carbonate/bicarbonate buffer, pH 9.6.

Antigens are prepared from selected strains of FMD virus grown on monolayers of BHK-21 cells. The unpurified supernatants are used and pretitrated according to the VN protocol but without serum. The final dilution chosen is that which, after addition of an equal volume of diluent (see below), gives an absorbance on the upper part of the linear region of the titration curve (optical density approximately 1.5). PBS containing 0.05% Tween 20 and phenol red indicator is used as a diluent (PBST).

Guinea-pig antisera prepared by inoculating guinea-pigs with 146S antigen of one of the seven serotypes and preblocked with NBS is used as the detecting antibody. Predetermined optimal concentrations are prepared in PBS containing 0.05% Tween 20, and 5% dried, nonfat skimmed milk (PBSTM).

Rabbit (or sheep) anti-guinea-pig immunoglobulin conjugated to horseradish peroxidase and preblocked with NBS is used at a predetermined optimum concentration in PBSTM.

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\(^4\) A chequerboard titration of the rabbit-trapping antiserum, the guinea-pig antiserum and the anti-guinea-pig antiserum is performed. Before using the antigen-trapping ELISA or the liquid-phase blocking ELISA, each of these reagents is titrated one against another, keeping the third reagent at a fixed concentration. In this way the optimal dilutions (for positive colour and low background colour) can be determined. These ‘predetermined’ dilutions are then used for all future tests using these particular batches of reagents.
Test sera are diluted in PBST.

- **Test procedure**
  
i) ELISA plates are coated with 50 µl/well rabbit antiviral sera and left overnight in a humid chamber at room temperature.

ii) The ELISA plates are washed five times with PBS.

iii) In U-bottomed multiwell plates (carrier plates) 50 µl of a duplicate, twofold series of each test serum are prepared, starting at 1/4. To each well, 50 µl of a constant dose of homologous viral antigen is added and the mixtures are left overnight at 4°C, or incubated at 37°C for 1 hour. The addition of the antigen increases the starting serum dilution to 1/8.

iv) Then 50 µl of serum/antigen mixtures are transferred from the carrier plates to the rabbit-serum coated ELISA plates and incubated at 37°C for 1 hour on a rotary shaker.

v) After washing, 50 µl of guinea-pig antiserum homologous to the viral antigen used in the previous step (iv) is added to each well. The plates are then incubated at 37°C for 1 hour on a rotary shaker.

vi) The plates are washed and 50 µl of rabbit anti-guinea-pig immunoglobulin conjugated to horseradish peroxidase is added to each well. The plates are incubated at 37°C for 1 hour on a rotary shaker.

vii) The plates are washed again and 50 µl of orthophenylene diamine containing 0.05% H$_2$O$_2$ (30%, w/v) is added to each well.

viii) The reaction is stopped after 15 minutes by the addition of 50 µl of 1.25 M sulphuric acid. The plates are read at 492 nm on a spectrophotometer linked to a microcomputer.

ix) **Controls:** A minimum of four wells each of strong positive, weak positive and negative bovine reference sera at a final dilution of 1/32 should be included on each plate together with an equivalent number of reaction (antigen) control wells containing antigen in diluent alone without serum. For end-point titration tests, duplicate twofold dilution series of positive and negative homologous bovine reference sera should be included on at least one plate of every run.

x) **Interpretation of the results:** Antibody titres are expressed as the 50% end-point titre, i.e. the dilution at which 50% of the wells show greater than 50% inhibition of the median OD of the reaction (antigen) control wells (Kärber). Titres greater than 1/40 are considered to be positive. Titres close to 1/40 should be retested using the VN test.

c) **Nonstructural protein antibody tests**

Antibody to VIAA is conventionally detected by AGID. The test is based on immunoprecipitation lines formed in the agar between the FMD antigen (concentrated cell-culture fluids rich in FMD virus RNA-dependent RNA polymerase or 3D) located in a centre well, and hexagonally arranged adjacent wells containing standard positive sera or unknown test sera. Precipitation lines forming between the test sera and the control antigen well that show identity with the lines of precipitation formed by the reference sera, confirm the specificity of the reactions (28).

Antibody to expressed, recombinant FMD virus NS proteins can be measured by ELISA or immunoblotting. No single test format has yet been conclusively demonstrated to be optimal. An MAb trapping (MAT) ELISA for detecting antibody to 3ABC (10) and blocking ELISAs for detecting antibody to 3AB or 3ABC (34) have been shown to be sensitive, specific and reliable in a number of laboratories. The simultaneous detection of antibody to several NS proteins in a single test by ELISA (27, 34) or by enzyme-linked immuno-electrotransfer blot (EITB), a type of Western blot,
(8) is useful for confirmation of animals positive for antibody to 3AB or 3ABC. There are currently no internationally recognised standards for antibody to FMD virus NS proteins, but an application for these tests is described in detail below.

- **Indirect enzyme-linked immunosorbent assay**

- **Test procedure**

  i) Microplates are coated overnight at 4°C with 1 µg/ml of the fusion antigen 3ABC in carbonate/bicarbonate buffer, pH 9.6 (100 µl per well). Antigen 3ABC was expressed and purified as indicated for the EITB tests (30).

  ii) The plates are washed six times with PBS, pH 7.2, supplemented with 0.05% Tween 20.

  iii) Test sera (100 µl per well) are added in a 1/20 dilution in blocking buffer consisting of PBS, 0.05% Tween 20, 5% nonfat dry milk, 10% equine sera and 0.1% *Escherichia coli* lysate. Each plate includes a set of reference standards as defined for the EITB assay.

  iv) The plates are incubated for 30 minutes at 37°C and washed six times in PBS-Tween.

  v) Horseradish-peroxidase-conjugated rabbit anti-species IgG is diluted optimally in the blocking buffer, added at 100 µl per well and the plate incubated for 30 minutes at 37°C.

  vi) After six washings, each well is filled with 100 µl of 3.3’, 5.5’-tetramethylbenzidine plus 0.004% (w/v) H₂O₂ in phosphate/citrate buffer, pH 5.5.

  vii) The reaction is stopped after 15 minutes of incubation at room temperature by adding 100 µl of 0.5 M H₂SO₄. Absorbance is read at 450 nm and at 620 nm for background correction.

- **Interpreting the results**

  For the test system to be valid the following performance criteria are applied: the absorbance of negative controls should be <0.10 after correction for absorbance of blank wells. The cut-off serum, obtained as described in the EITB test, should give absorbance values of 0.15-0.40. Results are expressed as an index derived by dividing the absorbance value of the serum tested by that of the cut-off control. The ratio of the weak positive/cut-off controls should be 2.5 with a coefficient of variation <20%. Test sera with ratios >0.8 are considered to be suspect or positive and are retested by EITB. Coated plates and secondary standards are available from the PANAFTOSA on request.

- **Enzyme-linked immunoelectrotransfer blot assay**

  The FMD situation in South America is complex and includes a variety of ecosystems and animal populations of varying immune status immunised with vaccines of different formulation. Tests for serological diagnosis therefore must accommodate many variables. To date, the EITB assay has been widely applied in South America for serosurveillance and risk assessment associated with animal movement. Currently, the procedure is to perform an initial screening test using an indirect ELISA for antibody to 3ABC, and to follow that by a confirmatory EITB assay if samples give positive or suspect results. This combination of tests is particularly recommended when serosurveillance involves a large number of samples. Further information is available from the OIE Reference Laboratory in Brazil (see Table given in Part 4 of this *Manual*).

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PART 2: FMD Chapter 2.1.1. of the OIE *Manual*
• Preparation of test strips containing the recombinant antigens

The five bioengineered FMD virus NS proteins 3A, 3B, 2C, 3D and 3ABC are expressed in *E. coli* C600 by thermo-induction. The 3D polypeptide is expressed in its complete form (30), whereas the rest of the proteins are obtained as fusions to the N-terminal part of the MS-2 polymerase gene (35).

The expressed polymerase is purified over phosphocellulose, followed by poly(U) Sepharose columns. The fused proteins 3A, 3B, 2C and 3ABC are purified by sequential extraction of the bacterial extracts with increasing concentrations of urea. The 7M fraction containing the fusion proteins is further purified on a preparative 10% SDS/PAGE (sodium dodecyl sulphate/polyacrylamide gel electrophoresis). The fusion protein band is excised from the gel and electroeluted (30).

A mixture containing 20 ng/ml of each one of the purified recombinant polypeptides is separated on 12.5% SDS/PAGE and electrophoretically transferred to nitrocellulose (30).

• Test procedure

i) The required amount of test strips should be assessed, taking into account that for each nitrocellulose sheet, which defines one transferred gel, a positive, a weakly positive, a cut-off and a negative control serum should be assayed. In general, 24 nitrocellulose strips, each 3 mm wide, should result from a gel.

ii) A volume of 0.8 ml of saturation buffer (50 mM Tris-HCl, pH 7.5; 150 mM NaCl; 0.2% Tween 20; 5% nonfat dry milk; and 0.05% bacterial *E. coli* lysate) is added to each well. The antigen-coated strips are blocked by placing the trays on a rocker and agitating for 30 minutes at room temperature (20-22°C).

iii) A dilution of 1/200 of test sera and of each of the controls is added to the appropriate trough. The strips must be completely submerged and facing upwards, and maintained in that position during the whole process.

iv) Strips are incubated for 60 minutes on a rocker at room temperature.

v) Liquid is removed from the trays, and each test strip is washed three times with washing solution (50 mM Tris-HCl, pH 7.5; 150 mM NaCl; and 0.2% Tween 20) by agitation for 5 minutes.

vi) The alkaline-phosphatase-conjugated rabbit anti-bovine solution is added to each test well, and the strips are incubated with shaking for 60 minutes at room temperature.

vii) The liquid is removed from the trays and each test strip is washed three times with washing solution as above.

viii) Substrate solution (0.015% bromochloroindolylphosphate/0.03% nitroblue tetrazolium) is prepared in substrate buffer (100 mM NaCl; 5 mM MgCl₂; and 100 mM Tris-HCl, pH 9.3), and is added to each test well.

ix) Strips are incubated by placing the test tray on the orbital mixer and agitating until the cut-off control shows five distinct, discernible bands. Strips are washed with running deionised water and air-dried.
• **Reading the results**

A sample is nonreactive if all the bands are below the reactivity of the cut-off control, or a maximum of two bands are above the reactivity of the cut-off control. A sample is reactive if all four antigens (3ABC, 3A, 3B and 3D) Ä2C have a reactivity equal to or higher than the cut-off control. A sample has indeterminate reactivity if the above-mentioned criteria for reactive or nonreactive samples are not met. Densitometric reading is possible, but visual reading is recommended not only because it is less expensive, but also because borderline reactions rarely occur. The cut-off control serum is derived from a pool of sera and/or dilution of positive sera, representing background EITB reactivities observed individually for the different antigens in nonvaccinated animals from FMD-free regions. These controls are secondary standards, validated against a primary standard corresponding to a serum from an animal at 714 days after experimental infection from which no virus had been recovered from OP fluid for the past 364 days (8) This primary standard is diluted 1/2 when the test is used as an input for evaluation of risk analysis during selection of animals for import/export testing.

Sensitised strips and secondary standards are available on request from the PANAFTOSA (see footnote 5).

**B. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS**

The control of FMD is usually a national responsibility and, in many countries, the vaccine may be used only when authorised.

Routine vaccination against FMD is used in many countries where the disease is endemic. In contrast, a number of disease-free countries have never vaccinated their livestock but have preferred the use of strict movement controls and slaughter of infected and contact animals when outbreaks have occurred. Nevertheless, many disease-free countries maintain the option to vaccinate and have their own strategic reserves of highly concentrated inactivated virus preparations. Such antigen reserves offer the potential of supplying formulated vaccine in an ‘emergency’ at short notice (16).

FMD vaccines are chemically inactivated cell-culture-derived preparations of the virus that have been blended with a suitable adjuvant. In the case of vaccines destined for use in swine, oil adjuvants are preferred.

Because of the presence of multiple serotypes of the virus, many FMD vaccines are multivalent and it is common practice to prepare vaccines from two or more different virus strains. In areas where the disease is maintained by free-living buffalo, it is necessary to include more than one virus per serotype to ensure broad antigenic coverage against prevailing viruses.

1. **Seed management**

   a) **Characteristics of the seed**

   Selection of seed viruses should ideally be based on their ease of growth in cell culture, virus yield, stability and broad antigenic spectrum (32). The production strains should be characterised and distributed by the official control laboratories; they should be selected in accordance with the epidemiological importance of each variant.

   b) **Method of culture**

   Many manufacturers of FMD vaccines derive their vaccine strains from local field isolates and, for those grown in cell culture, adapt them for growth in suspension or monolayer cells by serial passage. The number of passages in cell culture should be kept to a minimum as there is evidence of antigenic ‘drift’ of FMD virus during this procedure.
c) Validation as a vaccine

Seed viruses must be antigenically characterised and proven to be free from contaminating microorganisms, to establish homology to the original candidate isolates, purity and effectiveness against the circulating strains for which they were developed. This often encompasses a number of methods, but to establish applicability to field strains a virus neutralisation is often used. Seed viruses may be stored at –20°C if glycerinated or at a lower temperature (e.g. –70°C) if not glycerinated. Working seed viruses may be expanded in one or a few more passages from the mother seed stock and used to infect the final cell culture at an approximate rate of 1 PFU (plaque-forming unit) per 100 cells.

2. Method of manufacture

FMD virus is usually produced in either large-scale monolayer or suspension cell systems under aseptic conditions. It is essential that all pipework and vessels be thoroughly sterilised ensuring that no areas in the system harbour microorganisms. In addition to general considerations of sterility, it is important to note that the virus is vulnerable to attack by proteolytic enzymes, such as those produced by microorganisms (12). Control of pH and temperature are also critical because of the acid and temperature lability of the virus (11). Optimum temperature for cell, virus growth and inactivation, normally around 37°C, should be precisely controlled. During other stages of manufacture, the temperature should be reduced to 4-6°C. Virus should be maintained at approximately pH 7.6 and should never be below pH 7.0.

A suitable strain of the virus is used to infect a suspension or monolayer of a transformed cell line, such as BHK. Such cell cultures should be free from contaminating microorganisms. It is common practice to keep stocks of BHK cells over liquid nitrogen and revive as necessary. On revival, they are expanded in nutrient medium to a volume and cell density appropriate to seeding the main culture. As an approximation, the main culture is seeded to give an initial density of 0.2-0.5 \( \times 10^6 \) cells/ml, which is allowed to multiply to 2-3 \( \times 10^6 \) cells/ml before being infected with virus.

When the virus has reached its maximum titre, which is variously determined by infectivity, CF or other tests, the culture is clarified and filtered, often with centrifugation. The virus is subsequently inactivated by addition of ethyleneimine (EI), usually in the form of binary ethyleneimine (BEI). This is usually prepared by dissolving, to a concentration of 0.1 M, 2-bromoethylamine hydrobromide in 0.2 N sodium hydroxide solution, and incubating at 37°C for 1 hour (4). The BEI formed is then added to a virus suspension held at 20-37°C, to give a final concentration of 0.001 M. Inactivation is usually continued for 24 hours, followed by a second dose of EI for a further 24 hours. After inactivation any residual BEI in the harvest can be neutralised by adding sodium thiosulphate solution to a final concentration of 2%. To decrease the likelihood of live virus failing to contact the EI at the second application, it is essential to transfer the vessel contents immediately to a second sterile vessel where inactivation is allowed to go to completion at 48 hours.

The inactivated virus may be concentrated by ultrafiltration, polyethylene glycol precipitation or polyethylene oxide adsorption (1, 38). These concentrated antigens can be kept at -70°C or lower temperatures for many years, if necessary, and made into vaccine when required by dilution in a suitable buffer and addition of adjuvants (14).

Conventional FMD vaccines are usually formulated in one of two ways. The vaccine most commonly used for cattle is prepared by adsorbing the virus on to aluminium hydroxide gel, one of the adjuvant constituents of the final vaccine blend. Other components of the final blend include antifoam, phenol red dye (if permitted by the country requiring vaccine), lactalbumin hydrolysate, tryptose phosphate broth, antibiotics, amino acids, vitamins and buffer salts. A second adjuvant, saponin, derived from the South American tree *Quillaja saponaria mollina*, is also incorporated, as well as merthiolate/chloroform as a preservative.
An alternative formulation uses mineral oils, such as Marcol and Drakeol, as adjuvants. These preparations offer a number of advantages over the standard aluminium hydroxide/saponin vaccine, not least of which is their efficacy in pigs. They are widely used for vaccinating cattle in South America because of the longer duration of immunity obtained. The mineral oil is usually premixed with an emulsifying agent, such as mannide monooleate, before the addition of an equal volume of the aqueous phase of the vaccine, and emulsified by use of a colloid mill or continuous mechanical or flow ultrasonic emulsifier. More complex double emulsions (water/oil/water) may be produced by emulsifying once more in an aqueous phase containing a small amount of Tween 80 (25).

Significant advances made in recent years have seen the introduction of alternative ‘ready-to-use’ oil adjuvants. Oils containing esters of octadecenoic acid and 2,5 anhydro-d-mannitol, for example, readily form double or mixed emulsions (water/oil/water), that are both stable and of low viscosity, without the requirement of sophisticated emulsification equipment (5, 16).

3. In-process control

In general, virus titres reach optimum levels within about 24 hours of the cell culture being infected. The time chosen to harvest the culture may be based on a number of assays; for instance cell death. Virus concentration may be assessed by plaque assay, sucrose density gradient (13) or serological techniques. It is preferable to use a method for measuring antigenic mass, such as sucrose density gradient analysis, as well as one that measures infectivity, as the two properties do not necessarily coincide and the different methods may complement one another.

During inactivation of the virus, timed samples should be taken at regular intervals for the purpose of monitoring the rate and linearity of the inactivation process. Virus titres in the samples are determined by inoculation of cell cultures proven to be highly susceptible to FMD virus, e.g. BHK or bovine thyroid cells. Such cultures permit the testing of statistically meaningful samples under reproducible conditions. The log_{10} infectivities of the timed samples are plotted against time, and the inactivation procedure is not considered to be satisfactory unless at least the latter part of the slope of the line is linear and extrapolation indicates that there would be less than one infectious particle per 10^4 litres of liquid preparation at the end of the inactivation period.

4. Batch control

a) Sterility

The bulk inactivated antigen, the adjuvants, the dilution buffers and the final formulated product should all undergo sterility testing. This may be carried out directly with components of the vaccine or the final product, but the preferred method is to collect any contaminating microorganisms by membrane filtration of the material to be examined and to detect them by incubation of the membranes with culture media. The latter procedure allows the removal of preservatives, etc., which may inhibit the detection of microorganisms. Guidelines on techniques and culture media, which allow the detection of a wide range of organisms, are described in the European Pharmacopoeia 1997 (ref. 19; also refer to Chapter 1.4.).

b) Safety

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 3 days, after which the spent medium is transferred to fresh monolayers and the original monolayers replenished with fresh medium. Using this method, traces of live virus can be amplified by the passage procedure and detected on the basis of CPE observed. Two to 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.

c) Potency

Potency is only examined on the final formulated product (see Section B.5.b.).

d) Duration of immunity

In order to establish a satisfactory level of immunity it is usual to give a primary course of two inoculations, 2-4 weeks apart, followed by revaccination every 4-12 months. The frequency of revaccination will depend on the epidemiological situation and the type and quality of vaccine used. Where access to the animals is difficult, it is preferable to use oil adjuvanted vaccine at 4 months and 1 year of age, followed by annual revaccination.

For calves born of vaccinated dams, the first vaccination should be delayed as long as possible to allow decline of maternal antibody, but not beyond 4 months, as at that time a high proportion can be expected to respond effectively to vaccination. For calves born of nonvaccinated dams, the first vaccination may be at 1 week of age (3).

e) Stability

The shelf life of conventional FMD vaccines is usually 1-2 years at 4°C, but they are temperature labile and should neither be frozen nor stored above 4°C.

f) Preservatives

The most commonly used preservatives are chloroform and merthiolate. The latter is used at a final concentration of 1/30,000 (w/v).

g) Precautions (hazards)

Current FMD vaccines are innocuous and present no toxic hazard to the user. Care must be taken to avoid self-injection with oil-emulsion vaccines.

5. Tests on the final product

a) Safety

It is necessary to test FMD vaccines to ensure that the final product is noninfectious and is not unduly toxic. Some laboratories determine noninfectivity by eluting the virus from the vaccine, but this is not universally applicable to all formulations. For example, saponin influences greatly the elution of FMD from aluminium hydroxide/saponin vaccines (15). If the elution procedure is appropriate to a particular formulation, then it may be validated by seeding parallel samples of vaccine with trace amounts of live virus (6).

Toxicity and noninfectivity may be assessed simultaneously in an in-vivo test in cattle (18). Each of three healthy seronegative cattle are inoculated intradermally on the dorsal surface of the tongue with 0.1 ml of vaccine at 20 sites (four rows with five inoculation sites each). The animals are observed for no fewer than 4 days, after which three full bovine doses of vaccine are administered by the manufacturer’s recommended route to each animal. The animals are observed for a further 6 days. Should any of the animals develop signs of FMD, the vaccine will fail the safety test. Equally, any undue toxicity attributable to the vaccine should be assessed and may prevent its acceptance. Ideally, vaccines prepared for species other than cattle should be safety tested in the species for which they are intended, administering a double dose of vaccine according to the manufacturer’s recommended
route and dose volume. The animals should be examined daily for a minimum of 7 days for evidence of toxicity or signs of FMD.

Provided that laboratory rodents have been shown to be a satisfactory alternative to the target species for toxicity testing of FMD vaccines, these may be used to assess toxicity (17). The test involves the subcutaneous inoculation of two guinea-pigs and five mice with 2 ml each and 0.5 ml each of vaccine, respectively. The animals are observed for 7 days and the test is considered to be satisfactory if none of the animals dies or shows significant local or systemic reaction.

b) Potency

Cattle of at least 6 months of age, obtained from areas free from FMD, that have not previously been vaccinated against FMD and are free from antibodies neutralising the different types of FMD virus should be used. Three groups of no fewer than five cattle 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 injecting 0.5 ml, and a 1/10 dose would be obtained by injecting 0.2 ml. These animals and a control group of two nonvaccinated animals are challenged 3 weeks after vaccination with a suspension of bovine virus that is fully virulent and appropriate to the virus types in the vaccine under test by inoculating 10,000 ID$_{50}$ intradermally into two sites on the upper surface of the tongue (0.1 ml per site). Animals are observed for 8-10 days. Unprotected animals show lesions at sites other than the tongue. Control animals must develop lesions on at least three feet. From the number of animals protected in each group, the PD$_{50}$ (50% protective dose) content of the vaccine is calculated. The vaccine should contain at least 3 PD$_{50}$ per dose for cattle, when employed for routine prophylactic use, or 6 PD$_{50}$ per dose when used for emergency (‘ring’) vaccination. In some cases, vaccine of high potency will prevent the development of local tongue lesions at the site of challenge. In South American countries a variation of the potency test is performed, the PGP test (percentage of protection against generalised foot infection).

Potency tests in other target species, such as sheep, goats or buffalo are not common, as a successful test in cattle is considered sufficient to endorse its use in other species. Under circumstances where a vaccine is produced for use primarily in one particular species, it may be more appropriate to potency test the vaccine in that same species. However, in respect to the limited data for African buffalo or Asiatic buffalo (Bubalus bubalis) and sheep, and the often inapparent nature of the disease in these species, potency results from a cattle test should be a good indicator of the vaccines applicability in these other species.

A similar protocol to the cattle test can be adopted for potency testing FMD vaccines in pigs. Using three groups of five pigs, one group is vaccinated with the full pig dose recommended by the manufacturer, one group receives a 1/4 dose, and a third group receives a 1/16 dose of vaccine. Traditionally, the response to oil vaccine is allowed longer to develop, and not until day 28 after vaccination are the three groups, plus two unvaccinated control pigs challenged. Challenge is by intradermal injection into the heel bulbs of one foot with 10,000 TCID$_{50}$ (0.2 ml) of a virulent challenge virus homologous to a strain used in the vaccine. The animals are observed daily for 10 days after challenge for clinical signs of FMD, but animals are removed as soon as they develop generalised FMD to avoid excessive challenge to those remaining. Both control animals should develop clinical signs on more than one foot. Probit tables are used to determine the potency of the vaccine under test. The vaccine should contain at least 3 PD$_{50}$ per dose for pigs.

Other tests, including measurement following vaccination of virus neutralising antibodies in cell culture, or ELISA antibodies, or serum-protecting antibodies in suckling mice, 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 (37). For example, the expected percentage of protection is used to analyse the sera of a group of at least 16 vaccinated cattle and to express the probability of an animal being protected by measuring neutralising, ELISA or protecting antibodies. In a single group of animals given a half dose of vaccine, the expected percentage protection should be equal to or greater than 80%.

The presence of more than one serotype in a vaccine does not interfere with the induction of antibodies against another serotype or the correlation of antibody titre with protection.

REFERENCES


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## List of the OIE Reference Laboratories for FMD

<table>
<thead>
<tr>
<th>Experts</th>
<th>Laboratories</th>
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<tbody>
<tr>
<td><strong>Dr A. Donaldson</strong></td>
<td>Institute for Animal Health</td>
</tr>
<tr>
<td></td>
<td>Pirbright Laboratory</td>
</tr>
<tr>
<td></td>
<td>Ash Road, Pirbright</td>
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<td>Woking</td>
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<td></td>
<td>Surrey GU24 ONF</td>
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<td></td>
<td>UNITED KINGDOM</td>
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<tr>
<td></td>
<td>Tel.: (44.1483) 23.24.41</td>
</tr>
<tr>
<td></td>
<td>Fax: (44.1483) 23.24.48</td>
</tr>
<tr>
<td><strong>Dr M. Proteau</strong></td>
<td>Botswana Vaccine Institute</td>
</tr>
<tr>
<td></td>
<td>Department of Animal Health and Production</td>
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<tr>
<td></td>
<td>Broadhurst Industrial Site</td>
</tr>
<tr>
<td></td>
<td>Lejara Road</td>
</tr>
<tr>
<td></td>
<td>Private Bag 0031</td>
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<td></td>
<td>Gaborone</td>
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<td></td>
<td>BOTSWANA</td>
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<tr>
<td></td>
<td>Tel.: (267) 31.27.11,</td>
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<tr>
<td></td>
<td>Fax: (267) 35.67.98,</td>
</tr>
<tr>
<td></td>
<td>Email: <a href="mailto:tmbvi@mega.bw">tmbvi@mega.bw</a></td>
</tr>
<tr>
<td><strong>Dr M.S. Söndahl</strong></td>
<td>Centro Panamericano de Fiebre Aftosa - HPV/OPS</td>
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<tr>
<td></td>
<td>Caixa Postal 589</td>
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<tr>
<td></td>
<td>20001-970 Rio de Janeiro</td>
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<tr>
<td></td>
<td>BRAZIL</td>
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<tr>
<td></td>
<td>Tel.: (55.21) 671.31.28</td>
</tr>
<tr>
<td></td>
<td>Fax: (55.21) 671.23.87</td>
</tr>
<tr>
<td><strong>Dr V.M. Zakharov</strong></td>
<td>All-Russian Research Institute for Animal Health</td>
</tr>
<tr>
<td></td>
<td>600900 Vladimir</td>
</tr>
<tr>
<td></td>
<td>Yur’evets</td>
</tr>
<tr>
<td></td>
<td>RUSSIA</td>
</tr>
<tr>
<td></td>
<td>Tel.: (7.0922) 26.06.14 ou 26.15.25</td>
</tr>
<tr>
<td></td>
<td>Fax: (7.0922) 24.36.75 ou 26.17.55</td>
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</table>
The following standard sera were developed by the Institute for Animal Health, Pirbright Laboratory (UK) as the OIE FMD Reference Standard Sera for ELISA and VNT.

<table>
<thead>
<tr>
<th>FMD Reference Sera</th>
<th>Virus inoculum</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>–</td>
<td>This serum has been tested for antibodies against all 7 FMD virus serotypes with negative results by ELISA and VNT.</td>
</tr>
</tbody>
</table>
| A 22 Irak          | A22 Irak (IRQ 24/64) | This original serum has been diluted in negative serum to produce 3 standards:  
- high positive (1:10 dilution)  
- weak positive (1:50 dilution)  
- cut off (1:100)  
These give the following titres when finally tested:  
**Viral neutralisation test**  
high positive 102:1  
weak positive 43:1  
cut off 18:1 |
| C Noville          | C Noville      | This original serum has been diluted in negative serum to produce 3 standards:  
- high positive (1:12 dilution)  
- weak positive (1:25 dilution)  
- cut-off (1:50)  
These give the following titres when finally tested:  
**Viral neutralisation test**  
high positive 107:1  
weak positive 54:1  
cut off 27:1 |
| O Manisa           | O Manisa (TUR 1/78) | This original serum has been diluted in negative serum to produce 3 standards:  
- high positive (1:4 dilution)  
– weak positive (1:12 dilution)  
- cut-off (1:48)  
These give the following titres when finally tested:  
**Viral neutralisation test**  
high positive 90:1  
weak positive 38:1  
cut off 14:1 |
The term “International Standard Serum” is synonymous with primary standard. It represents the standard serum by which all others (secondary standards) are compared and calibrated. Secondary standards may represent national standards or working standards that are in routine use at the diagnostic laboratory level. The secondary standard and not the International Standard are to be used on a daily basis to standardize testing. The International Standard Sera listed above may be obtained, in small quantities, at the corresponding address. Some laboratories may make charge for this service.
Recognition of the foot and mouth disease status of Member Countries

**Restoration of free status without vaccination**

The OIE Foot and Mouth Disease and Other Epizootics Commission evaluated documentation concerning the eradication of foot and mouth disease, submitted by the Delegates of France, Ireland, the Republic of Korea and the Netherlands, and, in accordance with Resolution No. XVII ("Restoration of recognition of the foot and mouth disease status of Member Countries") adopted by the OIE International Committee during its 65th General Session (May 1997), recognised on 19 September 2001 that these countries have regained their previously recognised FMD-free status without vaccination.

**RESOLUTION No. XVII**

**Recognition of the Foot and Mouth Disease Status of Member Countries**

(Simplified version of resolution adopted by the OIE International Committee on 31 May 2001)

CONSIDERING THAT

1. During the 63rd General Session, the International Committee adopted Resolutions XI and XII, 'Establishment of a list of foot and mouth disease (FMD) free countries where vaccination is not practised', and 'Procedure for the recognition of the foot and mouth disease status of Member Countries';

2. During the 64th General Session, the International Committee adopted Resolution XII, which asks that the Director General publish in the Bulletin a list of the countries or zones within national territories that fulfil the criteria of one of the FMD free categories described in Chapter 2.1.1. of the International Animal Health Code (the Code),

3. The Foot and Mouth Disease and Other Epizootics Commission has continued to apply the procedure approved by the International Committee, and has supported the recognition of the FMD free status of additional countries and zones within national territories for annual adoption of the list by the International Committee,

4. During the 65th General Session, the International Committee adopted Resolution XII, which stated that the Delegates of Member Countries where countries or zones within their national territories are recognised as FMD free annually confirm by letter each November both their status and that the criteria by which their status was recognised remain the same,

5. During the 65th General Session, the International Committee adopted Resolution XVII delegating to the Foot and Mouth Disease and Other Epizootics Commission the authority to recognise, without further International Committee consultation, that a Member Country or zone within its territory has regained its previously recognised FMD free status following outbreaks that are eradicated in accordance with the relevant provisions of Chapter 2.1.1. of the Code,
6. Information published by the OIE is derived from declarations made by the official Veterinary Services of Member Countries. The OIE is not responsible for inaccurate publication of country disease status based on inaccurate information or changes in epidemiological status or other significant events that were not promptly reported to the Central Bureau subsequent to the time of declaration of freedom.

THE COMMITTEE

RESOLVES

That the Director General publish in the Bulletin the following list of Member Countries recognised as FMD free countries where vaccination is not practised, according to the provisions of Chapter 2.1.1 of the Code:

<table>
<thead>
<tr>
<th>Albania</th>
<th>Germany</th>
<th>Mexico</th>
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<tr>
<td>Australia</td>
<td>Guatemala</td>
<td>New Caledonia</td>
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<td>Austria</td>
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<td>Denmark</td>
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<td>El Salvador</td>
<td>Luxembourg</td>
<td>Switzerland</td>
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<tr>
<td>Estonia</td>
<td>Madagascar</td>
<td>Ukraine</td>
</tr>
<tr>
<td>Finland</td>
<td>Malta</td>
<td>United States of America</td>
</tr>
<tr>
<td>Former Yug. Rep. of Macedonia</td>
<td>Mauritius</td>
<td>Vanuatu</td>
</tr>
</tbody>
</table>

And

That the Director General publish in the Bulletin the following Member Countries as having an FMD free zone where vaccination is not practised, according to the provisions of Chapter 2.1.1. of the Code:

- Botswana: zone designated by the Delegate of Botswana in documents addressed to the Director General on 26 August 1996 and 24 September 1997;
- Colombia: Northwest region of Choco Department;
- Republic of Korea: Island of Cheju;
- Namibia: zone designated (see map) by the Delegate of Namibia in a document addressed to the Director General on 6 February 1997;
- Philippines: Mindanao.

1 For information on the status of non-contiguous territories of Member Countries recognised as FMD free, please address enquiries to that country’s Delegate or to the Director General
AND

That the Director General publish in the Bulletin the following Member Countries as having FMD free zones where vaccination is practised, according to the provisions of Chapter 2.1.1. of the Code:

- Brazil: States of Bahia, Espírito Santo, Goiás, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Rio de Janeiro, São Paulo, Sergipe, Tocantins and the Federal District;
- Colombia: zone designated (see map) by the Delegate of Colombia in documents addressed to the Director General on 7 December 2000.

AND

That the Director General publish in the Bulletin the following Member Country as being an FMD free country where vaccination is practised, according to the provisions of Chapter 2.1.1. of the Code:

- Paraguay.
An OIE/FAO International Scientific Conference on foot and mouth disease (FMD) was held at the Office International des Epizooties (OIE) headquarters from 17 to 18 April 2001.

The Members of the OIE FMD and Other Epizootics Commission and the International Animal Health Code Commission, the Presidents of the OIE Regional Commissions, the Experts of the OIE FMD Reference Laboratories, as well as Senior Staff of the FAO Animal Health Services and the OIE Central Bureau participated at the Conference.

The OIE Regional Coordinators and the Head of the Regional Coordination Unit for the South-East Asia FMD Programme, Observers from Member Countries and intergovernmental organisations also attended the Conference.

Dr Tony Garland, Consultant FAO-EMPRES1, was appointed Rapporteur.

List of Participants is given Appendix I.

Dr Romano Marabelli, President of the OIE and Dr Bernard Vallat, Director General of the OIE welcomed the participants and thanked Delegations and Representatives of Member Countries for attending the Conference. The opening addresses of Dr Marabelli and Dr Vallat were followed by further words of welcome from Dr Yves Cheneau, Chief of the FAO Animal Health Service. Both Dr Vallat and Dr Cheneau wished the Conference every success.

Dr James Pearson, Head, OIE Scientific and Technical Department, presented the Conference Objective and the Conference Agenda. The Agenda was approved by the participants. Each session was presided over by a Chairperson and a Rapporteur, who lead the discussion on each of the listed Agenda items. The recommendations developed during the meeting were discussed, modified as needed, and approved by the Conference participants. The final recommendations of the Conference are listed below.

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1 EMPRES: Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases
**Conference objective:**

To consider current foot and mouth disease issues and draft science-based recommendations and resolutions, addressed to the Member Countries of the OIE and FAO, to be presented to the International Committee of the OIE at its May 2001 General Session and to the Governing Bodies of the FAO.

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**RECOMMENDATIONS of the OIE/FAO International Scientific Conference on Foot and Mouth Disease**

**RECOMMENDATION NO. 1**

Review the following definitions in foot and mouth disease Chapter of the OIE International Animal Health Code:  

a) The criteria for the declaration of an outbreak  
b) Freedom from disease or freedom from infection

**CONSIDERING THAT**

In the OIE International Animal Health Code (the Code), Chapter 1.1.1. General definitions, an ‘infected zone’ is defined as an area ‘in which a disease included in this Code has been diagnosed’ and the definition of an ‘outbreak’ is restricted to an ‘Outbreak of Disease’. Neither of these definitions covers subclinical infection.  

The inclusion of subclinical infection in these definitions is important for disease control and trade.  

In Code Chapter 2.1.1. Foot and mouth disease (FMD), only two categories for freedom from FMD are recognised, namely: ‘FMD free where vaccination is practised’ and ‘FMD free where vaccination is not practised’  

Vaccinated animals can become subclinically infected or develop a carrier state  

The OIE FMD and Other Epizootics Commission has proposed changes to Chapter 2.1.1. of the Code to address FMD infection.  

**THIS CONFERENCE RECOMMENDS THAT**

1. The relevant chapters of the Code be amended to include a definition of FMD infection, encompassing the category of subclinical infection.  
2. The OIE FMD and Other Epizootics Commission and the OIE International Animal Health Code Commission finalise the proposed changes to Chapter 2.1.1. of the Code to redefine ‘freedom from FMD’ as ‘freedom from FMD infection’.  
3. The FMD and Other Epizootics Commission of the OIE define the surveillance standards required to verify the status of ‘Freedom from FMD Infection’.
CONSIDERING THAT

The speed and extent of spread of outbreaks of the Pan-Asian strain of type O FMD in certain European countries during 2001 has been unprecedented

The spread of FMD virus within newly infected countries during the European outbreaks of 2001 occurred mainly through the movement of subclinically infected animals, and principally of sheep, and via contact with contaminated vehicles used for the transport of these animals

The exact initial mode of FMD contamination has not always been identified, but vehicles, markets, and other places where animals are collected have played a major role in the spread of the disease

There is increasing evidence from North Africa, the Middle East and Europe to suggest that subclinically infected sheep are a factor of major significance in the transmission and spread of FMD, both within and between countries and over long distances

The safe and timely disposal of large numbers of carcasses constitutes a serious impediment to the implementation of a successful stamping-out policy

There is currently insufficient evidence of alternative options to rendering, burning and burial for large-scale disposal of carcasses

National veterinary authorities should negotiate in advance with the environmental authorities as to the availability of options for carcass disposal for use in different areas of the country

International trade and movements of live animals and certain animal products are the main route of the international diffusion of FMD virus and other diseases

Early suspicion, recognition and laboratory diagnosis of FMD is essential for efficient control

Countries that are FMD free where vaccination is practised need a sensitive and specific test that can identify infection in vaccinated animals

There is a requirement for the establishment of the validity and suitability of serological tests for the detection and quantification of antibodies to the non-structural proteins of FMD virus and also for guidance on their use in surveillance to assist in the definition of the FMD status of a country/zone following implementation of a stamping-out policy

The imposition of restrictions on the movement of species that are not susceptible to FMD, and particularly of Equidae, beyond the existing provisions of Chapter 2.1.1. of the OIE Code has posed problems for several Member Countries

THIS CONFERENCE RECOMMENDS THAT

1. Member countries recognise the emergence of new and/or increasing risks to animal health including: changes to the livestock industry; increased movement of people, product and livestock; illicit traffic; and other factors predisposing to disease. Accordingly, Member Countries should undertake risk analyses and ensure that the risks are communicated to the relevant authorities and other sectors.
2. Countries engaged in international trade recognise the increased risk and impact of the occurrence of FMD and review their legislation, contingency planning, emergency preparedness, prevention measures and the availability of resources to ensure their adequacy.

3. Member Countries define the roles of their Veterinary Services and recognise that a national crisis planning exercise may be necessary, involving other agencies, for the effective management of a major FMD emergency.

4. The Director General of the OIE set up an ad hoc group of specialists to examine the problem of the safe and timely disposal of carcasses arising from the enforcement of a stamping-out policy, and to recommend international guidelines for this purpose.

5. Member countries free of FMD establish procedures for the immediate restriction of markets, gatherings of animals and transportation of susceptible species throughout the country, when the veterinary authorities first confirm the presence of FMD.

6. Member Countries establish equipment, facilities and procedures for the effective disinfection of all relevant modes of transport used for the movement of animals and animal products, including as appropriate both the exterior surfaces and the interior transport compartment(s), both before and after their use for these purposes.

7. The OIE Standards Commission evaluate the validity of tests for the detection and quantification of antibodies to the non-structural proteins of FMD virus and their suitability for monitoring and surveillance programmes and prepare guidelines for their application.

8. The OIE FMD and Other Epizootics Commission evaluate the risk of transmission of FMD by the movement of Equidae and develop appropriate guidelines for consideration by the OIE International Animal Health Code Commission.
RECOMMENDATION NO. 2

Emergency foot and mouth disease control methods:

b) Criteria for the use of vaccination in livestock

CONSIDERING THAT

FMD vaccines should comply with internationally recognised pharmacopoeia standards

Emergency FMD vaccination is not fully addressed in the OIE Code

National circumstances may alter the objectives and the available options for the control of FMD

THIS CONFERENCE RECOMMENDS THAT

1. The use of FMD vaccines that comply with the criteria defined in the OIE Manual of Standards for Diagnostic Tests and Vaccines be strongly encouraged, and the use of live FMD vaccines be strongly discouraged.

2. The definition, conditions of application and the consequences of emergency vaccination against FMD be considered and finalised for inclusion in the OIE Guidelines.
RECOMMENDATION NO. 2

Emergency foot and mouth disease control methods:
c) Protection against FMD, including by the use of vaccination in special cases
(e.g. zoos, wildlife parks, rare breeds, rare genetic material, endangered species,
animals in special research programmes)

CONSIDERING THAT

Certain rare and/or valuable animals and genetic resources are susceptible to foot and mouth disease

Member Countries have an obligation to conserve endangered species and in particular those covered by The Convention on International Trade in Endangered Species

In the case of a foot and mouth disease outbreak in a country/zone previously free of FMD, every measure should be taken to prevent the exposure of such animals to the virus

When the veterinary authorities consider that the risk of FMD infection appears imminent, vaccination is one measure that might be considered for the protection of such animals

In general, available FMD vaccines protect animals against the development of clinical disease, but do not protect them against the acquisition of infection

There is little scientific information available on the effects of FMD vaccination in wildlife species

Chapter 2.1.1., Article 2.1.1.6. of the OIE Code requires that, in order to regain the status of an ‘FMD free country/zone where vaccination is not practised’, either such vaccinated animals would have to be destroyed or a waiting period of at least 12 months would have to elapse

THIS CONFERENCE RECOMMENDS THAT

1. Member Countries ensure their national contingency plans against FMD include specific provision for the protection of such animals and materials.

2. The OIE consider the applicability of implementing compartmentalisation for the control of FMD and other transboundary diseases.

3. Chapter 2.1.1., Article 2.1.1.6. of the Code be modified, where appropriate, to allow for emergency vaccination of certain rare or valuable animals to be permitted without prejudice to the FMD free without vaccination status of the country/zone, provided that such vaccinated animals are individually identified and are maintained in a location that has physical barriers and where zoosanitary procedures are applied that are adequate to prevent contact with any susceptible animals that may be situated beyond the confines of the location, and that measures are in place that would prevent the spread of infection by fomites. This location could be considered as an ‘FMD free zone where vaccination is practised’, where all the attendant Code restrictions will apply to the vaccinated animals, their progeny, embryos, ova and semen and other products derived from these animals.

4. In respect of zoological collections, stakeholders should consider the funding of research into the efficacy of vaccines in non-domesticated ruminants and other species and the application of diagnostic tests in such species.
RECOMMENDATION NO. 3

Risk of FMD transmission due to trade in commercial products and the illegal carriage of animal products by international travellers

CONSIDERING THAT

The recent introduction of the Pan-Asian strain of type O FMD virus into new territories and countries, as into South Africa and Europe, is likely to have been through the feeding of contaminated swill or contact with contaminated fomites.

Certain commodities of animal origin (e.g. sausages, sausage casings and intestines) are traded internationally and some are carried by travellers without adequate precautions against their potential spread of the FMD virus.

Some Member Countries have recently imposed restrictions on some products that are more stringent than those currently prescribed in Chapter 2.1.1 of the OIE Code.

THIS CONFERENCE RECOMMENDS THAT

1. Member Countries re-evaluate their legislation and enforcement practices to minimise the risk of the introduction of FMD and other infections through swill feeding.

2. Member Countries strengthen measures to control the illegal carriage of animal products by international travellers.

3. The OIE International Animal Health Code Commission in association with the OIE FMD and other Epizootic Commission develop standards for the importation of commodities that are not currently listed in the Code Chapter 2.1.1., Articles 2.1.1.8. and 2.1.1.28., such as sausage casings and other offal.

4. Member Countries apply the standards of the OIE Code, unless there is scientific justification for more or less stringent restrictions. If such scientific evidence does exist, it should be brought promptly to the attention of the Director General of OIE, who will ask the specialist Commission to assess the information provided.
RECOMMENDATION NO. 4

Research needed to better prevent, control and eradicate foot and mouth disease

CONSIDERING THAT

Changes in farming and trading practices have exacerbated the distribution and severity of recent FMD outbreaks in previously FMD free countries

The role of subclinically infected animals and contaminated commodities is prominent in the dissemination of FMD

Molecular biology and immunological techniques have proved invaluable in the characterisation of FMD virus strains and the tracing of FMD infections

Current test systems for the surveillance and detection of FMD infection in individual animals and commodities pose some serious deficiencies

Currently available vaccines and vaccination systems have limitations

The slaughter of animals and disposal of carcasses pose logistical, welfare, environmental and social problems

There is little specific data on the certain risks of transmission of FMD infection by animal and animal products; particularly those derived from vaccinated animals

THIS CONFERENCE RECOMMENDS THAT

Research should be encouraged in the following domains:

1. **Early detection systems** including robust, pen-side diagnostic tests for use on live animals, and commodities, such as IgA, IgM detection, preclinical and subclinical diagnosis based on antigen/viral relevant genomic or immunological methods.

2. **Surveillance systems**

   É Development of practical and statistically based sampling programmes.

   É Development and validation of serological tests that are applicable to a wide variety of livestock and wildlife susceptible to FMD virus. Of particular and immediate need are the serological tests to differentiate infection from vaccinal immunity, to establish that herds are free of FMD infection, and to develop assay systems that can be reliable for individual animal certification. Critical to accomplishing this task is the acquisition of standard reference sera.

   É Establishment of antigen profiles against well described polyclonal and monoclonal antibodies to better appreciate and anticipate vaccine protection and relevant viruses around the world. (To achieve the creation of antigen banks, Member Countries are urged to submit virus samples to a reference laboratory on a regular basis.)
3. **Vaccines** that, ideally, encompass some or all of the following improved characteristics over currently available vaccines:

- Readily affordable; easily applied; induce a broad spectrum of antigenic coverage (cross serotype and strain), engender rapid onset and long duration of immunity; engender protection of the oropharynx against the acquisition of persistent infection.
- Induce vaccinal antibody response(s) that can readily be distinguished from that (those) due to infection.
- Avoid requirements for refrigerated conservation.
- Can be combined with a proven antiviral drug, without modifying efficacy, and protect against viral infection.

In addition the following activities are encouraged

- Marker-type vaccine development and companion diagnostic tests.
- Development of vaccines that do not engender antibodies to the non-structural proteins of FMD virus in vaccinated animals.
- Development of vaccine efficacy studies in species other than cattle.

4. **Epidemiology** with special emphasis on:

- Risk analysis, impact and cost-benefit analysis.
- The role of subclinically infected animals (including wildlife, small ruminants, and camellidae), The role of Equidae in transmission of FMD infection to susceptible hosts.
- Infection tracking and predictive modelling.
- Re-evaluation of wind-borne spread of FMD virus.
- Development of performance indicators to assist countries with a particular epidemiological status of FMD to advance to a more favourable status.

5. **Risk analysis** on animal products and products from convalescent/vaccinated animals using epidemiological and experimental data, including predictive modelling studies.

6. **Economic research** and surveys on trends and market forces for animals and animal products as well as on the financial and other resource needs for the effective functioning of national Veterinary Services in dealing with disease emergencies and the management of national programmes for the control of major epizootics.

7. **Other methods to improve control**

- Disinfectants that are effective in the presence of organic matter, are noncorrosive, and do not harm the environment.
- Investigation of the survival of FMD virus in smoke plumes, when infected carcasses are burned.
- Methods of carcass disposal.
CONSIDERING THAT

The World Food Summit (November 1996), in recognising the importance of epidemic diseases of livestock (including foot and mouth disease) to both international trade and world food security, committed the governments of countries and the civil society to seek to ensure effective prevention and progressive control of such diseases.

Infectious diseases, especially those of an epidemic nature or of public health concern, are assuming increasing economic importance in the changing farming and trading systems of both industrialised and developing countries.

Emerging or evolving infectious diseases have the potential to quickly move from local to international significance.

In recent years epidemic diseases have caused heavy losses both in industrialised and developing countries and that there have been dramatic incidents of transboundary and international spread of disease.

The events involving the spread of the type O Pan-Asian strain of FMD virus to many countries and most recently to South Africa, the United Kingdom, Ireland, France and the Netherlands, and of type A virus in South America, have shown just how vulnerable countries are to epidemic livestock diseases, even countries with sophisticated quarantine controls.

Effective disease control requires efficient disease detection systems, competent diagnostic laboratories and close co-operation and joint management of official Veterinary Services with private veterinarians, farmers, industry, consumers and other organisations, especially medical authorities and national emergency services, as well as regional and international co-operation.

When national programmes are jointly developed, managed and financed between the public and the private sectors, notable successes have been achieved, for example in the Global Rinderpest Eradication Programme and the Hemispheric Plan for the Eradication of FMD in the Americas.

THIS CONFERENCE RECOMMENDS THAT

1. The control of FMD and other animal diseases of significance to international trade, food security, food safety and public health be considered as a service for the good of the international public.

2. National Veterinary Services undertake to increase the awareness and the involvement and education of political authorities, veterinarians, farmers, other involved organisations, industry and consumers in the early detection, reporting and control of infectious diseases, as well as in the joint management and financing of national eradication programmes.

3. The provision of financial support, technical assistance and other support by countries with highly developed livestock industries to less developed countries to improve emphasis on their national Veterinary Services and to better control and eradicate their important animal diseases will provide very substantial mutual benefits to both parties.

4. Special attention should be given to international co-ordinated programmes for rinderpest, foot and mouth disease and classical and African swine fever. This initiative would also provide templates for the surveillance and tactical control of other important diseases.
5. As a first step, the OIE and FAO should convene a high level international meeting to improve awareness and establish agreement that there international concerted actions are needed against foot and mouth disease and other animal diseases that are of significance for trade, food security, food safety and public health.
Appendix I

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Publications

Orders can be made directly through the OIE or through one of the OIE book distributors.
The following publications are available and free of charge on the OIE Web site (www.oie.int):

- Diagnostic Manual for Aquatic Animal Diseases, 3rd Ed., 2000
- Disease Information
- World Animal Health in 2000

International standards

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