

**Appendix 10**

**MINIMUM STANDARDS FOR LABORATORIES WORKING WITH FMDV IN VITRO/IN VIVO**

*Standard adopted by the 38<sup>th</sup> General Session of the European Commission for the Control of Foot-and-Mouth Disease (EuFMD), 30<sup>th</sup> April 2009*

**Foreword**

In 1985 the European Commission for the Control of Foot-and-Mouth Disease (EuFMD) at the Food and Agriculture Organization (FAO) of the United Nations adopted a document entitled "*Minimum Standards for Laboratories working with FMDV in vitro and in vivo*", hereinafter "the FMD-lab standards". This document described a set of precautions to be taken by Foot-and-Mouth Disease (FMD) laboratories to avoid an escape of virus. It was prepared at a time when the majority of countries on continental Europe employed systematic annual prophylactic vaccination of their cattle. Council Directive 90/423/EEC amending Directive 85/511/EEC on Community control measures for FMD made the above FMD-lab standards a condition for the approval and operation of laboratories handling live FMD virus (FMDV).

Although the above document dealt with all important aspects of FMD containment, it had been found necessary to review it with special reference to the need for more specific technical and general requirements as a consequence of the change in Europe to a policy of non-vaccination. The security standards as specified in the 1993 revision had to be considered as minimum requirements for FMD laboratories located in FMD-free countries with or without systematic prophylactic vaccination. Article 65 of Council Directive 2003/85/EC on Community measures for the control of FMD and repealing Directive 85/511/EEC makes the FMD-lab standards, as amended in 1993, a condition for the approval and operation of laboratories handling live FMDV. Even in countries where FMD is present it is important to avoid the escape of FMDV from laboratories so the standards in this document are recommended as the minimum for FMD laboratories regardless of the prevailing disease situation. However, countries endemically infected with FMD should use, *mutatis mutandis*, at least the provisions described in "Minimum standards of biorisk management for laboratories undertaking diagnostic investigations of low-risk samples during an outbreak of FMD", as a guideline to minimize the risk of escape of FMDV, of possibly a different serotype or topotype, from laboratories not meeting the FMD-lab standards.

Following the 2007 FMD outbreak in an EU Member State that was related to virus escape from a laboratory, EuFMD undertook to review, and where necessary to adapt, the aforementioned FMD-lab standards. The present edition of the "*Minimum Standards for Laboratories working with foot-and-mouth disease virus in vitro and in vivo*" were adopted at the 38<sup>th</sup> General Session of EuFMD on 29 April 2009 and supersedes the edition adopted by EuFMD in 1985 and revised in 1993.

This document relates to facilities that handle or intend to handle materials containing foot-and-mouth disease virus (FMDV) in a form that could give rise to animal infection ("live FMDV"). The updating of the FMD-lab standards reflects the changes in the terminology of biorisk management practises, the change in the performance characteristics and standards relating to air filtration, and also addresses concerns relating to security of facilities.

The terminology used and the biorisk management principles incorporated into this document were adapted from the latest available draft of the CEN/CWA "Laboratory Biorisk Management Standard", Edition April 2008.

**Introduction**

Foot-and-Mouth Disease (FMD) is one of the most contagious diseases known, and manipulating the virus in the laboratory without adequate precautions is a hazard. It has been shown that as few as 10 TCID can be infective to cattle by the airborne route. However, this is under

experimental conditions and the low infective dose may relate to the relatively large size of aerosol droplets, which can be efficiently contained by HEPA filtration of air exhaust from facilities handling infective FMD virus (FMDV). As a consequence of the low infective dose, laboratories handling FMDV must work under high containment conditions, **in which the principle objective of the containment measures is to prevent release of virus that would give rise to animal infection outside of the laboratory (veterinary containment)**. The principles on which the containment measures are based are as follows:

- FMD virus is an animal health but not a human health hazard;
- containment measures for FMDV laboratories will differ in certain respects from those required of high containment facilities handling pathogens which present a significant human health hazard;
- effective implementation and maintenance of the containment measures will reduce the risk of an accidental release of virus to a level that can be considered acceptable in a risk management balancing those risks against the expected benefits of the services provided by such laboratory.

The containment measures were prepared on the basis of the documented evidence on the physico-chemical properties of FMDV, its inactivation kinetics, and the form and quantity of FMDV required to infect susceptible species.

Key factors in establishing and implementing a successful containment system include:

1. Physical and operational barriers to the release of FMDV that involve three containment layers and multiple fail-safe mechanisms as follows:
  - 1.1. Primary containment layer:
    - Contain the live FMDV at source within closed containers or a class I, II or III safety cabinet, or
    - In the case of infected animals, contain the live FMDV by physical containment in specially constructed rooms with treatment of all waste and the HEPA filtration of air.
  - 1.2. Secondary containment layer:
    - Containing FMDV of infected materials and staff working with such materials within a closed and highly controlled physical environment, and
    - Subject solids, fluids and air to a treatment by validated procedures that will remove or inactivate FMDV;
  - 1.3. Tertiary containment layer:
    - Prevent contact between the live FMDV and susceptible livestock outside containment by appropriate measures, such as restrictions placed on access of staff to such livestock.
2. Commitment by senior management:
  - To provide the resources required to attain and maintain the containment measures, including the physical and human environment;
  - To recognise the top priority of the management of the risks associated with facilities handling live FMDV;
  - To establish and maintain a management system and a working culture in the facility that facilitates continual improvement in preventing possible release of virus, the effectiveness of containment processes and root cause analysis of possible release incidents so as to prevent their recurrence;
  - To recognise and promote continual improvement.

## General requirements

*FMD risk management system:* Each facility should establish, implement and maintain a FMD risk management system, appropriate to the level of risk associated with each of the mechanisms and routes by which FMDV could escape or be released.

*Policy:* The management of the facility should have in place a policy that clearly states the FMD risk management objectives and the commitment to improving the FMD risk management performance.

*Risk assessment:* To operate a FMD risk management system, a risk assessment system should be in place in order to:

- Identify and address the risks (likelihood and extent of impact) of release or escape of FMDV by each facility (plant);
- Define the circumstances which would trigger a new or revised assessment, for example plans to construct new or modify existing facilities, changes to the programme, changes to volume of activities, following incidents or as a result of elevated levels of biosecurity threats to the facility.

*Hazard identification:* The Hazard identification system should identify the situations, and other hazards, associated with the work of the facility that may impact on the risk of FMDV release, including emergencies (such as electrical failure, fire, flood, medical emergencies etc). The requirements in this standard do not necessarily identify all hazards that may occur, but are written to reduce the risk associated with the hazards in facilities handling live FMDV.

The main sources of FMDV are:

- Diagnostic specimens;
- Infected tissue cultures;
- Infected laboratory animals, e.g. baby mice and guinea pigs;
- Laboratory based physical and chemical processing of large quantities of virus, and
- Infected pigs, cattle, sheep, goats and other susceptible large animals.

The principal routes by which the FMDV may escape or be carried out from laboratories include:

- Personnel;
- Air;
- Liquid effluent;
- Solid waste;
- Equipment, and
- Samples and reagents.

Although RNA derived from FMDV may still be infectious under very specific conditions, for practical purposes samples can be considered "inactivated" after an approved treatment with an appropriate lysis buffer and a disinfection of the sample tube by an approved method. However, as a precaution, such samples should not be handled without appropriate risk management measures, which must, in particular ensure that such samples are at no stage of processing added to cell cultures or injected into animals, except in laboratories meeting the "Minimum Standards for Laboratories working with Foot-and-Mouth Disease virus *in vitro* and *in vivo*", hereinafter "the FMD-lab standards".

*Risk control:* Under the direct responsibility of the management of each facility (plant), the hazards which could lead to a risk of FMD escape should be identified, quantified, prioritised and control options identified. The requirements indicated in this Standard should be considered a minimum, and do not release the management of each facility from the responsibility to undertake a formal risk assessment process.

Special attention should be given to:

- Replacement and reduction in use of live virus where possible;
- Security and recording of access to the facility;
- Security check of personnel handling live FMD virus;
- The responsible behaviour of personnel within and when they leave the laboratory, including the use of changing and showering facilities;
- The application of rules for primary containment;
- The maintenance of the physical containment including the air handling systems to ensure a negative air pressure where virus is manipulated and the effective particulate filtration of exhaust air;
- The decontamination of effluent;
- The disposal of carcasses in a safe manner;
- The decontamination of equipment and materials before removal from the restricted area.

*Use of alternative procedures:* The use of alternative procedures for inactivation of FMD virus to those specified in this Standard is permissible provided that the information from the validation

of the process has been examined and found equal or superior in performance to those currently specified. Decisions on equivalence of the proposed procedures can be made by national competent authorities. However, national authorities have to inform the EuFMD Standing Technical Committee of such decisions and their scientific basis, which will be reviewed and findings published in the "Report of the Sessions of the EuFMD Standing Technical Committee."

*Residual Risk:* The residual risk is the risk of a consequential release of FMDV, after application of the control measures. The Biorisk Officer (BRO), management and ultimately the national regulatory body should consider the overall biorisk management system together with the hazard identification and risk control procedures, and identify if there are residual risks requiring either more effective controls to be put into place, or work to be suspended.

### **Authorization of laboratories in respect to FMD:**

In respect to work with FMDV, laboratories may be authorized by the competent authorities to carry out one or more of the following types of work:

- (1) Infection of experimental and/or large animals with FMDV;
- (2) Activities which produce high amounts of infectious FMDV, e.g. large scale virus production at a capacity that involves more than 10 litres of cell culture;
- (3) Activities involving the handling, and in particular, the propagation of infectious FMDV, but are limited to 10 litres of cell culture, and during which the FMDV is enclosed in containers which can be effectively autoclaved or disinfected;
- (4) To test diagnostic samples for antibody to FMDV, by methods that do not involve live FMDV manipulation;
- (5) To test diagnostic samples for FMDV genome by methods that do not involve live FMDV manipulation (e.g. RT-PCR);
- (6) To apply on the genome of FMDV methods of molecular biology that do not involve live FMDV manipulation.

Laboratories carrying out the type of work mentioned under points 1, 2 and 3 must comply with the "Minimum Containment Standards for FMD Laboratories".

In accordance with EU legislation, and in most cases national legislation, the manipulation of live FMDV requires a mandatory authorisation by the competent authority.

The FMDV -associated risk of laboratories not manipulating live FMDV but carrying out the type of work mentioned under points 4, 5 and 6 is usually much lower.

However, authorisation of such facilities also requires a proper risk assessment.

Laboratories that receive samples from areas not free from FMD from susceptible animals should comply with the "Minimum standards of biorisk management for laboratories undertaking diagnostic investigations of low-risk samples during an outbreak of FMD" given in Annex II.

### **SPECIFIC REQUIREMENTS**

The requirements below are intended to assist self-assessment, bio-risk audit and inspection of facilities.

#### ***I. Management***

##### **Specific management requirements:**

1. *Bio-risk policy, delegation of responsibilities and communication:* The management of a facility is ultimately responsible for biorisks (biosafety and biosecurity) of its premises. The management should therefore define and document roles, responsibilities and authorities related to biosafety and biosecurity management in a formal policy statement and communicate this to all staff members;

2. *Formal process of Risk assessment / threat assessment:* The management should ensure that a formal process is in place to conduct, review and update a risk assessment. The need for a structured security threat assessment should be considered for each facility;
3. *System for continual improvement:* The management should put a system in place to guarantee that biosafety and biosecurity procedures and elements are thoroughly reviewed and audited on a regular basis. Records should be maintained of findings of audits, including actions taken to comply with the containment policy;
4. *Standard operating procedure (SOP):* A system should be in place to maintain a complete set of SOPs for all operational processes that are considered critical to the containment of FMDV;
5. *Biorisk Officer(BRO):* It is the duty of the management to properly monitor the biosafety and biosecurity by appointing a BRO (Biosafety / Biosecurity Officer), arranging for a deputy or replacement, and creating the necessary framework conditions in the facility. To ensure that biosafety and biosecurity is given full consideration in its activities the management should carefully define the status, duties and responsibilities of a BRO:
  - (a) The BRO should report directly to the top management representative (Director-General, site Director or similar) and should have authority to stop the work in the facilities in the event that it is considered necessary to do so;
  - (b) The status of the BRO should ensure his/her independence and the absence of any potential conflict of interest;
  - (c) Adequate financial and personnel resources should be allocated to the BRO to carry out his or her duties;
  - (d) The BRO should have the possibility of a direct link to the competent authorities responsible for the enforcement of biosafety /biosecurity regulations within the country or geographical/administrative area;
  - (e) The BRO should have appropriate training in virology, containment techniques and procedures to fulfil his/her duties. It is to be expected that he/she would also have a broad based knowledge of the FMDV with particular respect to its physico-chemical properties, mode of transmission and other topics of relevance to his/her role;
  - (f) The BRO should review regularly both technical reports concerning the various containment facilities as well as data relating to their day to day operation and monitoring. On the basis of such information, the officer should inform senior management of any concerns he/ she may have and as they arrive as well as prepare an annual report on all relevant containment elements of the facilities.
6. *Record keeping – accessibility to live FMDV:* Access to live FMDV should be limited to key personnel authorised and adequately instructed by the management. Detailed records of handling live FMDV (eg virus strains and dates used) should be kept and stored at least 5 years. Inventory lists including information on the location where a virus strain is stored should be maintained and periodically inspected and crosschecked (*previous sentence: Inventory information including the location, the virus strain should be maintained and periodically inspected and crosschecked*). (Laboratory books or other daily records of procedures by staff working with FMDV should be in place to enable retrospective analysis of activities for at least 12 months);
7. *Accident/incident reporting system:* Each facility should have an accident / incident reporting system in place, with a procedure for rating of the risk of the event and a decision making process for recording, reporting and remedial actions. An example of a risk rating system and associated decision tool is given in Section I of ANNEX I;
8. *Accident/Incident review system:* there should be a system in place to ensure each incident/accident is reviewed to ensure that the lessons learned have been identified, the type of failing in control measures is recognised, and adequate and proportionate remedial measures set in place. A statistic concerning accidents / incidents should be made available to the management at least annually;
9. *Systems to review biorisk changes:* changes to the design, operation and maintenance of a facility including biosafety/biosecurity procedures and risk assessment should be reviewed, verified, approved and documented through a formal change control process before implementation. Trigger points for review or drafting of new risk assessments should be identified;

10. *Emergency management plans* (contingency plans): types of emergency should be identified, including fire, flooding, loss of essential services, security breaches and major events affecting integrity of buildings, and standard management procedures for each event developed, documented and made permanently available to staff.
11. *Access to site*: management should implement and document a system for controlling access to areas of the site where the activities of the area pose a potential hazard. There should be physical security measures to restrict access;

Management should define the different zones on the site, taking into consideration the hierarchy of risk of activities in each zone. A suggested typology is:

<i>RED</i>	<i>[=restricted area = where FMDV is manipulated and/or which contain infected animals]</i>
<i>ORANGE</i>	<i>[= support services and access to the restricted area]</i>
<i>GREEN</i>	<i>[general access and administration].</i>

*RED, ORANGE and GREEN* zones are situated within the *controlled area* = area within the outer security barrier or fence of the facility.

The minimum requirements are to clearly define and document the zones under control of the BRO, including definition of the outer perimeter of the site, lower risk areas for personnel and plant access, the location and barriers of the laboratories in which FMDV is handled, and the location and access points to waste treatment (including ventilation systems).

## **II. Training**

12. The organisation should ensure that personnel are competent for their designated roles and receive appropriate training on a regular basis. In particular, training requirements and procedures for biosafety and biosecurity related training of personnel should be identified (training programme) and established (training manual) and training records should be maintained;
13. Training content and training tools should be defined taking into account the different target audiences and the individual learning differences within a facility. Training efficacy assessment should be considered wherever possible and appropriate. Training should be reviewed on a regular basis;

The BRO should be in charge of providing information and advice on biosafety and biosecurity to laboratory staff, cleaning personnel, visitors, contractors as well as to other persons working either in locations in which FMD is handled or adjacent facilities such as service areas. Personnel should be made aware of the responsibilities, the specific containment features and the risks associated with such activities;

14. Training should be provided on the specific properties of FMD, the primary and secondary containment features and the biosafety / biosecurity procedures pertinent to each facility;
15. All staff members must be appropriately informed and regularly trained in emergency evacuation procedures with special attention being given to security requirements in cases of fire;

## **III. Laboratory Biosecurity**

*Note:* Additional considerations and notes are given in Sections II and III of ANNEX I.

The objective of Laboratory biosecurity is to protect biological materials containing FMD virus against deliberate removal from the facility.

16. It is part of the duty of care of every facility handling FMDV to ensure that it minimizes the risk of virus misappropriation by intruders and people with access rights to the facility, through measures taken following a *formal threat assessment process*.

In a threat assessment the critical assets of a facility should be identified and the facilities' vulnerability to threats should be assessed. Any decision not to undertake such an assessment requires documentation and justification. Based on the threat assessment, structural (eg. building design, IT etc.), physical (cameras, fences, access etc.) and organisational (security policy, accessibility etc.) measures should be taken.

17. To comply with point 16, the minimum requirements are:
  - (a) *Security system* that is appropriate to detect and alert security to the presence of intruders, with a security plan in place for rapid response to intrusion;
  - (b) *Entry Recording system*: Access to the facility should be recorded to provide an audit trail of who was in the facility at any given time.
18. *Threat reduction/control measures*: Due to the unpredictability of the actual threat, controls are required to reduce the risk to an acceptable level. These controls should consider structural, physical and organisational measures and must address at least the following scenarios:
  - Intruder attempting to remove FMDV from the facility by forced or fraudulent entry;
  - Staff member removing FMDV from the facility;
  - Shipment of virus containing materials.

#### **IV. Personnel**

19. Control of entry into and exit from the restricted zones ("RED zones") must take place only through changing and showering facilities. This means a complete change from private or controlled area working clothes to dedicated restricted area working clothes on entry and the reverse process on exit but with a shower before leaving the restricted area;
20. A code of FMDV containment practice, including instructions for entry into and exit from control zones/restricted areas, must be available for all employees and visitors on site;
21. The FMDV containment rules and other relevant documents provided by the management must have been read and signed by each employee at the beginning of their employment. At this time, it should also be made clear to new staff that any violation of such and similar regulations may result in disciplinary actions by the management and the terms of employment should indicate this;
22. *Control of access to controlled zones/critical areas*: A level of security checks is recommended for all individuals with access to FMDV laboratories or critical plant/service areas of these laboratories. The performance of such checks will depend on the legislation of the country and procedures should have been developed in consultation with the police and relevant government agencies of the country;  
  
Access to FMDV containing materials in the laboratory should be restricted to trained and dedicated staff on the basis of legitimate needs. The number of individuals with access to virus storage areas should be kept as small as reasonably possible.
23. *Visitors*: There must be rules in place governing the access to controlled zones by visitors, covering at least the record keeping and the possible use of background checks. The security system should verify the identity of visitors through use of unique identifiers including passport or ID card details. The reasons for each visit and the responsible person must be recorded;
24. Visitors have to be instructed in the specific containment procedures (eg. decontamination) of each facility before entering the controlled / restricted zones. There must be a system in place that guarantees that these procedures are properly followed;
25. *Oversight (mentoring)*: A system for oversight of new personnel should be established, such that all new staff has someone assigned for oversight who has sufficient understanding of the biosafety rules;
26. A human resources support system should be in place, with appropriate protocols to support staff that may be under pressure that affects their participation in the biosafety practices of the facility;
27. *Quarantine*: each facility must define and apply quarantine periods for persons authorised to work in each category of controlled zone/restricted area, to reduce the risk that personnel will cause a release of FMD virus as a result of virus carriage on their body. A range of quarantine periods depending on the level of exposure to virus. Depending on the risk assessment application of quarantine rules may be applied to other areas of a facility as well;

28. Persons, including visitors, authorised to enter the FMDV restricted area must agree not to keep any animals which are susceptible to FMD, nor reside on premises where such animals are kept and to abide by minimum standards of quarantine, i.e. no contact with animals susceptible to Foot-and-Mouth Disease for at least three days;
29. Personal protective equipment; regular supply of appropriate laboratory clothing for use within the restricted area ["Red zone"];

#### V. Facility Design

30. General construction of buildings and their surfaces, including ducting of the air conditioning system:
  - Maintain inward flow of air through doorways and other openings at all times;
  - Properly maintained condition with a high standard of airtightness;
  - Insect, rodent and bird proof.
31. Windows:
  - Sealed, toughened and preferably double glazed, and able to withstand operating pressures and all but major impact.
  - Equivalent standard in animal rooms and at a height where animals are not able to break.
32. Doors:
  - Warning signs at entrances:

**ACCESS FOR AUTHORISED PERSONNEL ONLY**  
**BIOLOGICAL HAZARD**
  - Access restricted by locked doors where locks are operated from the outside. The advantages of a key-less lock system centrally controlled by the biosafety department should be explored that prevents unauthorised cutting of falsified spare keys and allows the biosafety department to reset access rights as necessary;
  - Airlocks provided with airtight doors which are interlocked to prevent opening of both doors simultaneously, in particular following a gaseous decontamination cycle;
  - Doors should be fitted with windows to allow staff outside of a room to see actions inside and provide assistance if necessary.
33. Walls, floors, ceilings:
  - In many respects, the surfaces and material appropriate to Pharmaceutical facilities respecting GMP standards are also relevant to laboratories handling FMD virus. Notably, surfaces should be impervious, smooth, crevice free and easily cleaned and disinfected. Cavities within the fabric of the facility should be avoided (eg cavity walls) unless all penetrations of the walls, floors and ceilings are thoroughly sealed with suitable materials such as silicone mastic. Crevices and joints between surfaces should also be sealed with similar materials. Continuity of seal should be maintained between floors and walls. A continuous cove floor finish up the wall is recommended in particular for areas where major spillages will occur, e.g. animal and post mortem rooms;
  - Sealed (airtight) entry of service lines.
34. *Communication*: All areas equipped with telephones and, in some areas, cameras, to ensure additional security outside of normal operations and allow staff to report issues including accidents and incidents without leaving work area;
35. *Emergency back-up power*: The laboratory facility should be equipped with a back-up source of electricity (an emergency generator) which starts with a delay of no more than a few minutes in the event of power failure. Alternatively, it is acceptable if the commercial power supplier is able to guarantee a supply from an alternative source within a few minutes of the main power failure. The delay period that is permissible will depend on the airtightness of the key buildings in the facility where virus in aerosol form may be present. In the design of a restricted area facility, special attention should be paid to the critical electrical supply circuits such as air handling systems, cold stores, safety cabinets, and other equipment and installations relating to the security and safety of the facility. There should be no possibility of the emergency supply being diverted from critical circuits by less important demand from non-critical equipment. Thus, the

critical supply circuits would include air handling systems, cold stores, safety cabinets and other equipment and installations relating to security and safety of the facility;

#### **VI. Handling of FMD virus**

36. *Recording receipt of virus containing materials:* A system should be in place for recording receipt of specimens or samples known or reasonably be suspected (to contain FMDV. The accompanying type and strain identification, or such information generated by the laboratory, respectively, should be recorded;
37. Except in cases when this is not technically feasible (e.g. during large animal experiments and post-mortem examinations), materials known or expected to contain FMD virus must either be kept within closed vessels or in devices that in combination with suitable operating procedures will function as primary containment. Such devices should be equipped with suitable filters, for example HEPA filters for which the requirements are defined in the Glossary, or equivalent off-gas or vent filters (primary containment). A suitable disinfectant should be kept close to the work areas such that a spillage can be rapidly dealt with;
38. In areas where only small quantities of virus are handled (10 litres or less of cell culture), liquids and suspensions containing FMDV should be inactivated by a validated procedure, for example, dilution in disinfectants, before disposal into the liquid waste system of the facility;
39. When large quantities of virus are processed (e.g. for vaccine production), it is necessary to transfer virus with a contained system of vessels, pipes and other equipment. To permit fluid transfers, air needs to enter and exit equipment and infectivity must be efficiently removed by a suitably validated procedure. Usually, this is done by filtration and a number of manufacturers supply filters capable of removing FMD virus with very high levels of efficiency. Procedures are also required for decontamination of vessels, pipes and other equipment after the process has finished and before the process is either repeated or items are opened or stripped down for cleaning or maintenance. Usually this will require a chemical decontamination stage followed by steam sterilization;
40. Inoculation of animals, maintenance of infected animals and post-mortem examinations must take place within the restricted area in rooms (normally dedicated animal or post-mortem rooms, respectively) that in combination with suitable operating procedures function as a primary containment. [see glossary] Personnel must wear appropriate and comprehensive protective clothing to minimise exposure of body surfaces to virus splashes and aerosols when handling virus suspensions and when inoculating or handling infected animals. On exit from an animal and post-mortem rooms, protective clothes and footwear must be left inside these rooms or in ante-rooms to these rooms. Showering and complete change of clothes is required before the operator can move to an area not operating under a negative pressure/air filtration system;
41. Movement of materials known or expected to contain FMD virus out of one zone (eg laboratory), to another zone (e.g. animal rooms) on the same site must be governed and made by a set of procedures that prevent possible loss or spillage of virus in a non-restricted area of the facility. As a minimum requirement, such materials are transported between the zones within a leak and break proof container. Staff making such transfers should be fully authorised to do so and be familiar with the emergency response procedures in the event of accident or incident;
42. Laboratory facilities and equipment must be cleaned and appropriately disinfected at regular intervals. In particular, benches and other flat surfaces exposed to virus should be wiped down with a suitable disinfectant as soon as open work has finished;

## VII. Air Handling – Live Virus Facilities

Note: Additional considerations and notes are given in Section VII of ANNEX I.

### Ventilation systems

43. *Negative pressure ventilation system:* All facilities used for the handling of FMDV must operate under a negative pressure ventilation system with HEPA filtration of exhaust air and systems to prevent air escape on the inlet supply;

In areas where only small quantities of virus are handled (10 litres or less of cell culture), the minimum negative pressure should be 35 Pa<sup>3</sup> but due consideration needs to be given to ensure a gradient from the periphery of the restricted area to the area where virus is handled. From a practical perspective, it is difficult to achieve gradient steps of less than 10 Pa and this will tend to dictate the choice of pressure in the most negative part of the restricted area. For areas where larger quantities of virus are handled such as large scale virus production rooms and large animal rooms, the minimum negative pressure should be 50 Pa. A system should be in place to prevent a positive pressure occurring within the building due failures or faults within the restricted area ventilation system;

44. *Exhaust air filtration system:*

Laboratories: Double HEPA filtration of exhaust air. Use of a single HEPA filter may be acceptable, provided that it is demonstrated that open work with live virus is at all times restricted to within biological safety cabinets (BSC) which have HEPA filtration of exhaust air, thereby maintaining an effective double HEPA filtration following open work;

Animal rooms: Double HEPA filtration of exhaust air is obligatory;

Production laboratories: Double HEPA filtration of exhaust air is obligatory.

45. *Inlet air supply:* A system must be in place to prevent escape of air via the inlet in case of ventilation shut-down. This may be achieved by a single HEPA filter or automatic dampers in the air inlet system.
46. The air pressures within the different rooms of a restricted area should be continuously monitored by manometers and a system must be in place so that staff working in these areas are informed if significant loss of air pressure occurs and the actions to be taken. Manometers should be labelled to indicate the working pressure and the minimum and maximum limits within which open virus work is permitted. Under any of these alarm conditions, the primary action is to cease all open virus work and secure the workplace by sealing virus containers and disinfection of surfaces and protective clothing. The opening of doors leading to the contained area or to rooms containing infected animals or carcasses should be avoided as far as possible until the pressure difference has been restored;
47. All critical filters (HEPA) should be incorporated into a preventative maintenance programme. In particular, the efficiency of HEPA filters should be checked at least once per year, and in line with requirements of EN 14644;
48. When HEPA filters are installed or replaced, an in-situ efficiency test must be carried out by trained personnel with validated equipment. Replacement of HEPA filters must be performed in accordance with an authorised procedure. Strict precautions must be taken to prevent the spread of virus with used filters or contaminated air. Replacement of filters from outside the restricted area must take place after decontamination "in situ" or in "safe change" air-handling units. Filter specifications and test results supplied by the manufacturer should be incorporated into the maintenance records but cannot replace in-situ testing because filters may have been damaged during transportation or may not have been fitted into the gaskets properly during installation;
49. Filters must be changed when the pressure difference exceeds certain limits in accordance with the instructions given by the manufacturer, or sooner if the filter fails one of the prescribed efficiency tests. Additionally, it may be necessary to change some filters more frequently if they are subject to high humidity or high particle challenge;

<sup>3</sup> 1 Pascal (Pa) = 1 N/m<sup>2</sup> = 1 J/m<sup>3</sup> = 1 kg/(m·s<sup>2</sup>) = 0.102 mm water column.

50. Animal rooms – prefilters should be designed in a way that they can be changed without shut-down of the ventilation system;
51. HEPA filters in safety cabinets should also be checked at least once per year. Movement of safety cabinets should be accompanied by re-validation of the filter integrity due to possible flexing and movement on the filter cartridge or filter housing;
52. Off-gas or vent filters require testing on installation and at least once per year;

## VIII. Waste management

### Effluent

53. Effluent from restricted area laboratories and from facilities holding FMD infected or potentially infected animals must be treated in a manner which ensures that there is no residual infectivity in the effluent using a suitable validated procedure. Both heat and chemical treatment may be used to process the effluent provided all of the material in the effluent is exposed to the specific treatment;
54. The treatment must be validated for the highest virus load and the most difficult matrix that can reasonably be expected. The possibility that virus particles may be protected from inactivation by proteins or lipids, and/or by aggregation or precipitation, must be taken into account in the validation process;
55. The entire effluent treatment system must comply with high containment conditions. In every case it must be ensured that no leakage from the primary containment system into the environment can occur;
56. There must be sufficient storage capacity (tanks) for the storage of untreated effluent;
57. The equipment must have automatic monitoring systems to ensure proper function. These systems must ensure that the required conditions for inactivation of FMDV have been reached before the effluent is discharged. The systems should be continuously monitored and all critical data recorded. The system should be designed in a way that in case of any failure, the likelihood of a release of potentially infectious material is minimised;
58. Treatment options:

*Heat treatment:* FMD virus is quite sensitive to heat at 100°C for 1 hour or an equivalent heat effect has been shown to be sufficient to inactivate FMDV in effluent to the extent that no residual infectivity can be detected. The treatment process should be monitored by multiple, automatic and continuous time and temperature measurements, combined with automatic measurement of flow rates or volumes. Any treatment system must ensure homogeneity of the effluent during the inactivation process. All data relevant to the inactivation process and the release of effluent must be recorded. Critical data measuring and logging equipment must be validated by qualified personnel at least annually;

*Chemical treatment:* FMD virus is quite sensitive to acid and alkaline pH conditions. NaOH or Na<sub>2</sub>CO<sub>3</sub> or other alkaline treatment at pH 12 for at least 10 hours has been shown to be sufficient to inactivate FMDV in effluent and are particularly effective because of their action on concentrated biological effluents. As with heat, thorough mixing of the materials must be ensured. The treatment process should be monitored by multiple, automatic and continuous time and pH measurements. After treatment, the materials must be neutralized and the pH checked before the effluent is released. All data relevant to the inactivation process and the release of effluent must be recorded. Critical data measuring and logging equipment must be validated by qualified personnel at least annually.

### Solid waste (animal carcasses, feedstuffs, laboratory waste etc.)

59. The principle requirement is on-site inactivation of FMDV in waste using a validated method;
60. These methods include:
  - Sterilisation by steam using an autoclave (at least 115°C for 30 minutes or equivalent heat effect). It is essential that the different autoclave load types (eg

plastic waste, paper waste, waste liquids) are each validated for the maximum load size with thermocouples at different locations within the load including the centre of the load. Typically, autoclave periods are 30 min or more. Autoclaves should be double-ended so that treated waste does not need to re-enter the restricted area. Autoclaves should be revalidated at least annually by experienced personnel. Depending on the national requirements, it may be necessary to dispose of the autoclaved waste by incineration on or off the site;

Rendering of carcasses, in compliance with the requirements of Chapter III of Annex V to Regulation (EC) No 1774/2002;

- Incineration on site. The incinerators must comply with current safety standards and be fitted with afterburners.

61. *Emergency procedures:* A similar level of safety must be demonstrated for procedures used when normal waste treatment procedures can not be followed, e.g. because of a breakdown of equipment. Emergency procedures must be documented in the laboratory emergency plans, and include procedures for storage until treatment and final disposal;

### **IX. Equipment and Materials**

#### **Laboratory fittings**

62. *Benches* shall be smooth, impervious and resistant for any chemicals used in the facility. Junction between horizontal and vertical surfaces should be covered;
63. *Centrifuges, sonicators, homogenizers and other equipment* must be designed so as to contain aerosols or be used within safety cabinets where any aerosols generated will not escape to the atmosphere of the restricted laboratory;

#### **Removal of equipment and other material**

64. Before removal from restricted areas, equipment must be decontaminated according to the size and use of the equipment:
  - Either by steam sterilization within an autoclave, at 115°C for 30 minutes, or an equivalent heat effect, or
  - After surface disinfection, fumigation with formaldehyde (10 g/m<sup>3</sup> at 70 % RH) for at least 10 minutes or (3 g/m<sup>3</sup> for 24 hours or equivalent with other aldehydes, e.g. glutaraldehyde, or ethylene oxide (0.8 g/litre at 50°C for 1.5 hours ). Equipment, for example contractors' tool boxes, laptops, etc. which is fumigated out of a restricted area should be cleaned and be opened as much as reasonably possible to allow penetration of the gaseous fumigant; or
  - Thorough washing in an appropriate chemical disinfectant<sup>4</sup> such as:
    - 4 % Sodium Carbonate or 10% washing soda (Na<sub>2</sub>CO<sub>3</sub> Dehydrate);
    - 0.5 % caustic soda (NaOH);
    - 0.2 % citric acid;
    - 4 % formaldehyde or equivalent with other aldehydes, e.g. glutaraldehyde;or
  - An equivalent disinfection protocol officially approved for the purpose.
65. Decontamination of clothing before removal from the restricted area for laundry must include a wet heat treatment step (autoclaving at a temperature of at least 115°C for 30 min, or equivalent heat effect). A laundry process without autoclavation is permitted if performed on-site in a double-ended pass-through laundry device. Such a laundry process must include a validated alternative inactivation step;
66. Documents should be sent out of the restricted zone preferably in electronic format (fax, scans, electronic documents, e-mails etc.). In case papers have to be taken out of the restricted zone, they must be treated by a validated procedure e.g. autoclaving, irradiation or ethylene oxide treatment. In cases when only low levels of contamination can reasonably be expected and following risk assessment, paper can be sealed and kept at > 20 °C for two years before being taken out of the restricted zone;

#### **Removal of biological material from the restricted area**

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<sup>4</sup>Note: The efficiency of these chemical disinfectants is considerably improved by the addition of a non-ionic detergent.

67. Before sending non-FMD biological material to another laboratory which lacks the required level of containment, the necessary precautions must be taken to ensure that the material does not contain FMDV;

Thus if the source of the biological material is a restricted laboratory area, it is essential that it is subject to an innocuity test to demonstrate freedom from FMDV or a validated treatment that destroys FMDV infectivity;

The recipient laboratory must be informed about the potential risk of material coming from a laboratory manipulating FMDV. The recipient laboratory must further sign a statement that it is prepared to receive the material and that it will take the necessary precautions;

68. For the shipment of FMDV containing materials to other laboratories an innocuity test is not required if the material is sent to a high containment laboratory licensed to handle live FMDV;

The laboratory which provides FMDV to another laboratory has a duty of care to ensure that the recipient laboratory is authorised to handle FMDV. Before shipment, it has to ask for a statement from the recipient laboratory that it is requesting the virus only for legitimate purposes and will not redistribute the virus to other laboratories without written consent. The sending of materials containing FMDV is subject to international requirements governing transportation;

**X. *Decommissioning containment compartments for maintenance or renovation purposes.***

*Note:* Additional considerations and notes are given in Section X of Annex I.

69. Maintenance or renovation work that may compromise the integrity of the containment barrier thus possibly allowing the escape of air or liquids must be preceded by an assessment of the risk and a safety plan;
70. Decontamination of rooms/compartments, to reduce the risks to an acceptable level, are required before these can be decommissioned permanently or temporarily, for example during renovation;

*Standard Treatment procedures* include fumigation with formaldehyde after making the room effectively air-tight.

71. Waste building materials generated by demolition and redevelopment and other potentially contaminated materials must be treated in a way that any residual infectivity is inactivated. If autoclaving is not feasible, it should be sprayed or fumigated to disinfect surfaces, and then stored on site for 6 months before removal.

## Glossary

Terms are in line with the proposed “Laboratory Biorisk Management Standard” (CEN draft document for public comment, 2007-07-25)

**Biorisk (adapted from OHSAS 18001:2007):** combination of the likelihood of the occurrence of an adverse event involving exposure to biological agents and toxins and the consequence (in terms of accidental infection, toxicity or allergy or unauthorised access, loss, theft, misuse, diversion or release of biological agents or VBMs) of such an exposure.

**Biorisk officer (BRO) or biorisk advisor (Biosafety / Biosecurity Officer):** a staff member of an institution who has expertise in the biohazards encountered in the organisation and is competent to advise top management and staff on biorisk management issues.

**Biosafety (adapted from: WHO/CDS/EPR/2006.6):** Laboratory biosafety describes the containment principles, technologies and practices that are implemented to prevent the unintentional exposure to biological agents and toxins, or their accidental release.

**Biosecurity (adapted from: WHO/CDS/EPR/2006.6):** Laboratory biosecurity describes the protection, control and accountability for valuable biological materials within laboratories, in order to prevent their loss, theft, misuse, diversion of, unauthorised access, or intentional release.

**Restricted Area:** area of the facility where FMDV is manipulated and/or which contain infected animals, bounded by physical barriers to prevent air and fluid escape except through air filtration and waste treatment systems.

**Controlled area:** area within the outer security barrier or fence of the facility, containing the restricted area, the services for the restricted area, and zones for access and administration.

**Open virus work, or open work:** describes the handling of materials containing FMDV (usually liquids) in which exposure to room air occurs, for example during the pipetting of liquids into containers, and the subsequent exposure of the liquid handling object (pipettes etc) to air.

**Primary containment:** measures that contain the live virus at source, within closed containers or within a class I, II or III safety cabinet, or for animals, by physical containment in specially constructed rooms with treatment of all waste including the HEPA filtration of air.

**HEPA filter:** High Efficiency Particulate Air filter: the classification of HEPA filters is on the basis of efficiency of removal of the most penetrating particle size, and set by international standards (EN1822). In the context of this minimum standard, all HEPA filters must at least meet H13 requirements. However in order to increase the margin of safety, H14 filters are recommended. HEPA filter performance requirements are defined by EN1822; to classify as H13, the filter must remove > 99.95% of particles of the most penetrating particle size (0.3 µm). A leak is defined as penetration > 5 times the required integral efficiency, i.e. 5 times 0.05% = 0.25%. To classify as H14, the filter must remove > 99.995% of particles of the most penetrating particle size (0.3 µm). A leak is defined as penetration > 5 times the required integral efficiency, i.e. 5 times 0.005% = 0.025%

## ANNEX I

### ADDITIONAL CONSIDERATIONS AND EXAMPLES

#### **Section I:** *Establishing an FMD incident risk rating system*

Each facility should establish a risk rating system and an associated set of incident management procedures, including reporting and responsibilities in the event that a high risk incident occurs.

Risk is the product of consequence and likelihood. The consequence of an FMD escape into susceptible livestock (resulting in an outbreak) is huge.

In establishing a risk rating system, the following factors should be considered:

- Where does the incident occur? (for example in an animal room);
- What type of event? (for example a visitor leaving without showering);
- How much potential virus exposure or loss? (for example number of persons, time or volume);
- To where was the virus release? (for example outside of the high containment area, to ruminants, to areas within the perimeter of the facility).

Each facility should establish their own risk rating system, taking into consideration e.g. the history of incidents, estimations of likelihood, objective data, and computer simulations. The risk rating system and reporting requirements should be agreed at the level of the top management of the facility, and reviewed on a regular basis.

Once established, the risk rating system can be used in training of staff on their reporting requirements, setting out the types of event or that should be reported to the line manager and/or biorisk officer.

## Example of a risk rating system

Where	What	How much*	To where
5	Animal room containing FMD infected pigs.	5	5
4	Animal room containing FMD infected animals (not pigs).	4	4
3	Lab undertaking FMD virus work Or During the first half of the FMDV disinfection process of formaldehyde or steam autoclaves or EthyleneOxide sterilizers.	3	3
2	Lab not handling FMD virus but within common building/containment to labs handling FMDV Or During the second half of the FMDV disinfection process of formaldehyde or steam autoclaves or Ethylene Oxide sterilizer.	2	2
1	In engineering maintenance areas – HEPA filter replacement, etc	1	1

\* temperature, humidity, expired time will also have influence on this issue;  
Relative risk = where x what x how much x to where;

**Example**

A person who was working in the laboratory where live FMD is handled was observed to pass to the area outside of high containment, without taking a shower, but did not leave the perimeter of the facility.

Risk rating:  $3 \times 5 \times 2 \times 2 = 60$

<b>relative risk</b>	<b>&lt;20 is 'Acceptable'</b>	<b>21 – 60 is 'Low'</b>	<b>61 – 250 is 'Substantial'</b>	<b>&gt;250 is 'Catastrophic'</b>
decisions	Report Biorisk Officer.	Report Biorisk Officer. Report Biorisk Committee. Report General Manager.	Report Biorisk Officer. Report Biorisk Committee. Report General Manager. Call together Crisis Team. Decision about the necessity to inform authorities.	Report Biorisk Officer. Report Biorisk Committee. Report General Manager. Call together Crisis Team. Report to Regulatory authority/Chief Vet. Officer

## **Section II: Improvement of biorisk management through analysis of incidents**

Management should take a high interest in learning from reported incidents. Each may be considered a form of failure or non-conformity to the expected performance of the risk control measures, and occur as a result of failure in the engineering controls and/or personnel related control measures.

The cause of each event may be categorised as:

Related to engineering:

- Hardware (as facilities and equipment);
- Design (as irrational lay-out and ergonomics);
- Maintenance (as planning and availability);
- Procedures (as standard operations and relevance);
- Defences (as protective equipment and signals).

Related to personnel management:

- Error-enforcing conditions (as occupational health and attitude);
- Housekeeping (as tidiness and discipline);
- Incompatible goals (as costs and safety);
- Communication ( as interpretation and point of time);
- Organization (as responsibilities and authority);
- Training (as knowledge and experience).

## **Section III: Threat assessment, reduction and control**

In deciding upon undertaking a threat assessment, the following should be considered:

- (a) The threat of criminal use of FMDV for any malicious purpose has to be carefully assessed to determine the additional risk that arises from operating FMDV facilities. FMDV laboratories have exclusively peaceful objectives concerned with development and implementation of control measures. They are critical for the technical cooperation with veterinary services around the world in order to minimize the economic impact of FMD on livestock and economies. The threat of criminal use of FMDV is subject to major change as the political agenda of terrorist group changes;
- (b) The threat and consequences of a terrorist attack will vary by country. Because of the transboundary nature of FMD, there is also the possibility that a deliberate release may occur in another, possibly neighbouring, country. For this reason, effective control measures need to be consistently applied throughout all EU member states that operate FMD laboratories;

As the motivation for a deliberate release may change unpredictably over a very short period, effective control measures need to be sustained at all times and be sufficiently flexible to allow an enhanced response if required;

Facilities permitted to handle FMDV are obliged to prevent illegal access and removal of the virus. As a consequence, such access to laboratory-held virus must be substantially more difficult than acquiring the virus in the field.

*Threat reduction/control measures:* due to the unpredictability of the actual threat, controls are required to reduce the risk to an acceptable level. These controls should consider structural, physical and organisational measures and must address the following:

- (c) Intruder attempting to remove FMDV from the facility by forced or fraudulent entry;  
Appropriate controls include 1) physical security measures restricting access to authorised staff and contingency plans in the event of intrusion, 2) secure storage of virus containing materials including maintenance of inventories of stocks.
- (d) Staff member removing FMDV from the facility;  
Appropriate controls include 1) vetting of persons before authorisation of access, and escorts for persons allowed temporary access when security clearance is not available; 2) restricted access to FMDV virus material in the lab to trusted staff on the basis of a legitimate need, 3) access to the facility is logged [and records maintained for at least

two years] to provide an audit trail of who was in the facility at any given time. 4) Design of the laboratory or facility such that the number of staff needing to enter the secure areas is limited. Eg some engineering aspects of the design of the facility can be arranged so that certain services can be maintained from outside of the security envelope.

(e) Shipment of virus containing materials.

Appropriate controls include standard procedures before authorisation, including receipt of adequate information from the intended recipient of its authority to handle FMDV, and written agreement that the recipient laboratory will not redistribute the virus to other laboratories without applying the same risk assessment and will adhere to relevant national or international legislation relating to shipment and supply of dangerous animal pathogens.

**Section VII: Air-handling**

1. Depending on the small animal species, route and nature of infection and method of animal containment and handling, quite high titres of virus in relatively uncontrolled conditions might be produced. Consideration should be given to the appropriate negative air pressure requirements, with 35 *pascal* negative pressure as the minimum;
2. Provisions must be in place to ensure that in the restricted area no overpressure is generated. One approach is to interlock the inlet and extract fans so that the most that can occur is that the air supply and extract fails and the negative envelope decays solely depending on the airtightness of the building. An emergency back-up extract fan is recommended so that the negative envelope can be restored in the event of the main extract fan failing and this also should be interlocked to the supply fan to avoid very high negative pressures which may cause damage to the fabric of the building. As an alternative, the air extraction plant can be divided into several parallel sections so that the negative pressure can be maintained if one section fails or is shut down;
3. It is advisable to have and maintain other filters within the air handling system, notably, prefilters upstream of the HEPA filters. These other filters will conserve the life of the HEPA filters and reduce the need to change at the annual maintenance interval. In properly maintained systems, it is relatively rare to change the terminal extract filter due to the efficiency of particulate removal by all of the filters upstream. However, high levels of humidity will shorten the life expectancy of filters and large amounts of dust generated by nearby building works or other activities will soon blind filters even with efficient prefilters up-stream;
4. Off-gas or vent filters: This type of filter is often steam sterilised and filter efficiency testing involves different approaches such as the water intrusion test. At the smaller scale, disposal cartridge filters may be appropriate as vent filters to allow gas exchange while preventing virus escape from the container to the laboratory environment;
5. Although not widely used, sterilisation of extract air may be done by heating the air as it passes through an in-line furnace;
6. To save energy, air extracted from a restricted area may be partially recirculated into the same restricted area provided it passed through a HEPA filter before it re-enters the laboratory. However, the advisability of recirculation and the proportion of air recirculated will need to be considered against the quality of the air leaving and re-entering the work place and the activities within the workplace;
7. In the event that HEPA filters become blocked prematurely (ie prior to annual testing), this does not normally represent a problem in terms of the integrity of the affected filter(s), but it probable that the increased resistance to airflow and consequent problems of balancing the pressures in the different rooms of the restricted area will necessitate changing the affected filters.

**Section X:** *Decontamination of compartments*

The compartment must be made airtight to make fumigating possible, if necessary by means of temporary panels.

Formaldehyde procedure:

- Check the compartment and accompanying drawings for connections with containment facilities that must be closed. Close down utilities as gas, water, electricity, sewerage, steam and if possible ventilation;
- Empty the compartment, for example by moving objects to other containment facilities. Remove porous material. Discard material via validated procedures like autoclaves and formaldehyde airlocks. Open non removable installation parts to make them accessible to vapour;
- Clean the compartment and disinfect critical points which are possibly contaminated;
- Prepare the fumigating equipment and shut the compartment airtight;
- Disinfect (air)ducts and HEPA filters for example separately by injecting formalin:
  - ✓ Use a fumigating method in conformance with a validated procedure used for formaldehyde airlocks;
  - ✓ Use bioindicators, (preferably a rapid bioindicator system) to prove the efficacy of the fumigating process;
  - ✓ Set restrictions for access such as clothing, quarantine for people and demolition material, in order to be able to make corrections in case of accidents.
- Inspect the maintenance and renovation activities to be performed in the compartment.

## ANNEX II

### MINIMUM STANDARDS OF BIORISK MANAGEMENT FOR LABORATORIES UNDERTAKING DIAGNOSTIC INVESTIGATIONS OF LOW-RISK SAMPLES DURING AN OUTBREAK OF FMD

#### Introduction

The following Minimum standards for laboratories undertaking diagnostic investigations during an FMD emergency or during FMD surveillance after an outbreak refer to the laboratories mentioned in point 13 of Annex XV to Council Directive 2003/85/EC. These standards only apply to the use of laboratory tests which do not contain or require live FMD virus for the testing of

- (a) Blood samples from holdings without clinical signs;
- (b) Any samples from any holding that have been treated in a way that ensures the inactivation of FMDV infectivity.

Serology by commercially produced FMDV-ELISA kits can be performed in many laboratories, e.g. regional veterinary laboratories, which can process samples with a high throughput. In case of an outbreak, this allows to increase the throughput of diagnostic samples significantly, which will often be a crucial factor for successful disease control and timely recovery of the previously free status.

Blood sampling is often combined with surveillance and staff taking samples may also examine the mouth of possibly infected animals, which may increase the risk of a surface contamination of packing material. This risk, as well as the risk of leakage during transport has to be mitigated by appropriate provisions. The risk of FMD occurring as a result of sero-diagnostic activities within laboratories is associated with escape of virus following receipt of blood samples from viraemic animals. While the likelihood of virus being present in samples originating from holdings without clinical signs during an FMD epidemic generally is moderate to low, it is almost impossible to exclude the presence of FMDV due to the dynamic nature of an epidemic. However, the maximum virus titres in blood of viraemic animals are much lower than in vesicular material.

Real-time PCR has been introduced in many laboratories, e.g. regional veterinary laboratories, which can process samples with a high throughput. Testing samples that have been treated at the site under suspicion in a way that infectivity is inactivated for FMDV genome in regional laboratories may facilitate a significantly increased sample throughput and a shortened time between sampling and reception of results.

## Requirements for testing samples during an FMD outbreak (for any disease or purpose)

### Classification of samples and overview on required laboratory

Sample FMD Risk Level	Origin of samples	Sample Risk	Laboratory Requirements
A	Holding with signs indicative of FMD	High risk, material has to be considered to contain infectious virus	"Infectious FMDV Lab"
B	Holding without clinical signs indicative of FMD	Low risk, to be mitigated by appropriate bio-risk management measures	"Serology/inactivated sample FMDV Lab"
	Holding with or without signs indicative of FMD, samples treated in a way that FMDV infectivity is inactivated		
C	FMD free area	No obvious risk	No specific requirements

### Packaging of samples classified as FMD sample risk level B

Samples must be put into watertight primary containers (e.g. plastic tubes) and the primary containers must be packed in watertight secondary packaging, which should be a strong crushproof and leak-proof container, with absorbent material that can absorb the entire contents of all the primary containers. The packaging process must include a disinfection of the secondary packaging. The packaging should comply with the European agreement concerning the international carriage of dangerous goods by road (ADR). Samples should be labelled as biological substance, category B (UN3373).

### Treatment of samples to inactive FMDV infectivity

Samples from holdings with signs indicative of FMD must be treated in a way that FMDV infectivity is inactivated before shipment by a procedure authorised by the competent national authority in order to be permitted to be investigated at a laboratory not meeting the minimum containment standard for FMD laboratories.

*Remark:* although RNA derived from FMDV, under very specific conditions may still be infectious, for practical purposes samples can be considered "inactivated" after an approved treatment with an appropriate lysis buffer and a disinfection of the sample tube by an approved method. However, as a precaution, such samples should not be handled without appropriate risk management measures. In particular, these risk management measures have to ensure that such samples do not, at any stage of processing, be added to cell cultures or be injected into animals except in laboratories meeting the "MINIMUM STANDARDS FOR LABORATORIES WORKING WITH FOOT-AND-MOUTH DISEASE VIRUS *in vitro* AND *in vivo*".

### Minimum Requirements for laboratory biorisk management

#### Personnel

1. A biorisk officer (BRO) and deputy (DBRO) must be designated, and one or both present on-site at all periods in which samples are being received, and contactable at all periods when diagnostic activities are ongoing;
2. The BRO/DBRO must have sufficient experience and technical training to enable assessment of FMD risk and risk management procedures;
3. There must be a designated restricted area or areas with controls in place to limit human access;

4. Personnel must be authorised to enter the restricted area by the BRO/DBRO;
5. Authorised personnel working in the restricted area must be trained in biorisk management and evidence of the training recorded. Where facilities for the inactivation of waste from the restricted area are located outside of this area, also staff working with such waste must be trained in biorisk management and evidence of the training recorded;
6. Authorised personnel must:
  - Change clothing before entering and after leaving the restricted area and shower-out before leaving the laboratory premises;
  - For at least 3 days after leaving the restricted area not have any contact to animals of susceptible species, nor enter buildings or enclosed fields where animals of susceptible species are kept, and not handle items used in the care of susceptible species.

The agreement of the authorised personnel to these conditions must be recorded and a reminder notice of these conditions placed in a visible location at the exit point of the restricted area;

7. Entry and exit of personnel to the restricted area should be recorded;
8. Entry and exit points to the restricted area will be kept to the minimum– preferably a single point of entry/exit;
9. A step-over line, or other clearly demarcated boundary, shall indicate the exit point;
10. In case the shower facilities are not placed at the border of the restricted area, outer protective garments, including shoes or shoes coverings, shall be removed before exit from the restricted area. All clothing worn in the restricted area must be stored in a secure way, e.g. in designated lockers, until treatment;
11. An incident recording system, SOPs for risk identification and notification procedures and target response time, must be in place to ensure early notification of the authorities responsible for FMD surveillance in the event that:
  - Samples have been received which are considered FMD sample risk level A;
  - Samples have been received in unsatisfactory state of packaging.

### **Buildings**

12. Access doors to the restricted area should display a warning sign that access is restricted to authorised personnel only;
13. Changing facilities and lockers are required to enable staff to deposit unessential items outside the restricted area;
14. Entering of the laboratory premise by farmers or staff working on farms should be avoided. If possible, it should be attempted to separate vehicles bringing samples from vehicles entering the premise for other purposes;
15. Shower facilities must be available onsite, preferably at the border of the restricted area;
16. Sample reception area:
  - (a) The restricted area must contain a specified area for reception of packages.
  - (b) This area must:
    - (i) Be easily disinfected in the event that leakage of samples occurs into packing materials or following opening of the packages;
    - (ii) Be equipped to enable packages considered to potentially contain samples of FMD sample risk level A to be re-packaged into appropriate transport containers for dispatch to laboratories licensed for handling FMD virus,
    - (iii) Have suitable facilities for waste disposal and have hand-washing facilities at exit points.
17. Sample preparation area:

- (a) The restricted area must contain a specified area for serum separation and/or RNA extraction;
  - (b) This area must have suitable facilities for surface disinfection and waste disposal and have hand-washing facilities at exit points.
18. Testing area:
- (a) The restricted area must contain a specified area for testing.
  - (b) This area must have suitable facilities for surface disinfection and waste disposal and have hand-washing facilities at exit points.
19. Sample storage area:
- (a) The restricted area must contain a specified area for the storage of samples;
  - (b) This area must have suitable facilities for surface disinfection;
20. Communications and reporting office space.
- The laboratory must have an adequate provision of office space, computing and communications facilities (e.g. electronic communications, facsimile) to reduce the need to a minimum for staff, papers and physical records to exit the restricted area.
21. Rest rooms:
- The restricted area should have sufficient rest rooms and lavatory facilities in relation to the staff number expected at peak periods of activity, sufficient to reduce the need to a minimum for staff to exit the restricted area.
22. Location of autoclave:
- Facilities for wet heat treatment must be present on the site, preferably with sufficient capacity for throughput at the maximum operating capacity of the laboratory.

## Waste

23. Liquid waste:
- (a) Heat or chemical treatment of all waste water is the preferred treatment, in compliance with the prescribed standards specified for FMD laboratories;
  - (b) Alternatively, or additionally, the laboratory may demonstrate that it has put in place a system for inactivation of virus if present in liquid waste that has contacted risk materials. If treatment of all liquid waste from the restricted area (including waste water from the showers) is not possible, at least the ELISA buffers and washing fluids must be collected and treated.
24. Solid waste:
- (a) For biological, solid waste, and all solid, disposable materials that have been in contact with specimens, treatment by wet-heat in an autoclave within or at an entrance point to the restricted area is the preferred option.
  - (b) If such a treatment of all solid waste is not possible, it may be packed into suitable watertight containers and, after spraying of the containers with disinfectant, removed for treatment at a different site.
26. Removal of equipment, materials and clothing from the restricted area:
- (a) Removal of any material and equipment from the restricted area shall be subject to authorisation by the BRO;
  - (b) The reason for removal, date and destination will be recorded;
  - (c) The BRO will ensure that materials and equipment which has been in contact with risk materials (specimens) will not be removed from the restricted area without a validated treatment to inactivate FMDV.
27. Declassification of the restricted area:
- (a) A decontamination plan must be agreed with the competent authorities, before restrictions can be lifted;
  - (b) If heat treatment or scanning of all paper from the restricted area is not possible, it should be packed into suitable containers, which should be disinfected and kept under lock for at least two years. If the containers have to be opened before, this has to be done in a restricted area meeting the standards described above.