PROPOSAL:
MINIMUM REQUIREMENTS FOR THE MANAGEMENT OF BIOLOGICAL RISK AND BIOSAFETY IN LABORATORIES HANDLING THE FOOT-AND-MOUTH DISEASE VIRUS AND/OR ITS DERIVATIVES

(following COSALFA 43 Resolution No 2, reviewed by the Expert Team - July 07 2016)

OBJECTIVE

The present document describes the minimum requirements for the Management of Biological Risk and Biosafety in Laboratories Handling the Foot-and-Mouth disease Virus and/or its Derivatives, as requested by Resolution N° 1 of COSALFA 41. This document will be considered a work input in order that the Regional Commission for the Management of Biological Risk and Biosafety[1] can elaborate a final document describing the necessary requirements for the management of biological risk and biosafety for the control of risks associated with activities involving the foot-and-mouth disease virus and animal facilities in COSALFA member countries.

INTRODUCTION

The foot-and-mouth disease virus (FMDV) represents a risk for animal health although no scientific evidence exists of an impact on human health. Containment measures for pathogens affecting animal health are slightly different from those required for pathogens that pose a risk to human health. When containment measures are implemented for pathogens that pose a risk only for animal health, it is expected that they can reduce the possibility of virus escape to the environment to "manageable" levels.

Considering the low infective dose required for virus transmission through the air, laboratories handling the foot-and-mouth disease virus should work under high containment conditions. The main goal of these containment conditions is to prevent escape of virus into the environment and subsequent infection of susceptible animals.

Basically, implementing a biological containment system requires two factors **a)** establishment of physical and operational barriers and **b)** institutional commitment.

a) Physical and operational barriers
1. Primary containment level
   a. FMDV specimens (tissue/cells) are stored in closed containers and handled within biological safety cabinets.
   b. FMDV infected animals should be kept in specially constructed rooms equipped with effluent treatment and HEPA-filtered air.

[1] Described in the document: EVALUATION MECHANISM OF BIOSAFETY CONDITIONS IN LABORATORIES HANDLING THE FOOT-AND-MOUTH DISEASE VIRUS AND/OR ITS DERIVATIVES

2. Secondary containment level

   a. Personnel handling FMDV works in a closed and strictly controlled physical environment.
   b. Solid and liquid waste, as well as air, is subjected to validated treatment for viral inactivation/removal of viral particles.

3. Tertiary containment level
   a. It consists in the implementation of measures tending to prevent contact between FMDV and vulnerable animals outside of containment (restricting the contact of staff working in the foot-and-mouth disease unit with susceptible animals; permanent staff training).

b) Institutional commitment:
   a) Providing the financial resources to sustain containment measures
   b) Giving priority to the management of risks associated to the biocontainment unit
   c) Keeping a continuous process of staff training to provide insight on the relevance of implementing measures to prevent virus escape and the immediate notification of emergencies in the workplace environment.

<u>Sources of foot-and-mouth disease virus in laboratories and potential escape routes</u>:

The main sources of FMDV in laboratories are:

- Specimens for diagnosis
- Infected cell culture
- Infected small laboratory animals (mice, rabbits, guinea pigs)
- Large-scale biologics manufacturing
- Infected large animals (pig, cattle, sheep, etc.).

Main viral escape routes out of the laboratory are:

- Laboratory staff
- Air
- Effluents
- Waste material
- Laboratory equipment
- Biological specimens and reagents

The laboratory manager is responsible for the identification, quantification and monitoring options of threats that may pose a risk of FMDV escape.

The main activities developed with the FMDV can be summarized as follows:

1. Activities of isolation and characterization of foot-and-mouth disease virus through

viral replication in cell cultures, in a total volume of up to 10 liters/week. Replication is performed in bottles that can be easily disinfected and autoclaved. This laboratory is aimed at: diagnosis and research.

2. Activities for the production of foot-and-mouth disease virus suspensions in volumes above 10 liters/week. This laboratory is aimed at: biologics manufacturing.
3. Experimental animal infection with foot-and-mouth disease virus. This laboratory is aimed at: vaccine control and research.
4. Analysis of foot-and-mouth disease antibodies using the virus neutralization technique. The purpose of this laboratory is diagnosis, vaccine control and research.
5. Analysis of foot-and-mouth disease antibodies using immunoenzyme techniques. This laboratory is aimed at diagnosis, vaccine control and research.
6. Application of molecular techniques in viral genome without the use of infectious foot-and-mouth disease virus. This laboratory is aimed at research.

Note: it was suggested to consider mentioning (non-vesicular) laboratories that receive specimens for the diagnosis of other diseases, but coming from animals in foot-and-mouth disease endemic areas.

Laboratories performing the activities mentioned in 1, 2, 3, and 4 pose a higher biological risk than those performing only the activities mentioned in 5 and 6. Regardless of the biological risk level, all laboratories are subjected to evaluation of biosafety conditions by the Regional Commission for the Management of Biological Risk and Biosafety.

MINIMUM REQUIREMENTS FOR ACTIVITIES WITH THE FOOT-AND-MOUTH DISEASE VIRUS

Laboratories and animal facilities in which the foot-and-mouth disease virus is handled should comply with the general requirements recommended by the World Organization for Animal Health – OIE – for OIE Containment Group 4 (OIE, 2015) and specific requirements that will be detailed and defined by the Regional Commission for the Management of Biological Risk and Biosafety, briefly summarized in this document.

General requirements recommended by the OIE (Terrestrial Manual 2015)

A) Laboratory/ animal facility setting and structure

1. It is advisable that the laboratory/animal facility be located in an isolated area.
2. Not next to fire hazard
3. Workplace separated from other activities.
4. Restricted personnel access.
5. Protected against entry of rodents and insects
6. Liquid effluents must be decontaminated and monitored

7. Liquid effluents from sterilizers must be sterilized and monitored

8. Isolated by airlock. Continuous airflow.
9. The laboratory/animal facility must be under negative pressure and pressure differentials must be monitored.
10. Input air should be filtered using HEPA or equivalent as tight damper; exhaust air must have single HEPA filtration for laboratories and double HEPA filtration for animal facilities (single for incoming, double for exhausted airflow)
11. HEPA filters should be regularly verified (usually once a year)
12. Mechanical air supply system with fail/safe system and equipped with an alarm in case any problems occur.
13. Laboratory/animal facility sealable to permit fumigation.
14. Incinerator, pressure steam sterilizer or renderer for disposal of carcasses and waste. Available *in situ*
15. The laboratory/animal facility should be easy to clean, with surfaces impervious to water and resistant to chemicals. There shall be a wash-hand basin and emergency shower, including an eye bath, in each laboratory suite as appropriate for the chemicals and other risks present. Procedures shall be established for frequent cleaning and disinfection during and at the end of the work period.

B) <u>Additional laboratory/animal facility requirements</u>

16. Class I or II biological safety cabinet available.
17. Class III biological safety cabinet available
18. Regular verification of HEPA filters (usually once a year).
19. Direct access to autoclave/pressure steam sterilizer (access with double doors)
20. Specified pathogens stored in laboratory.
21. Double-ended dunk tank required.
22. Clothes or protective equipment should not be taken outside the laboratory.
23. It is necessary to take a shower and change clothes before exiting laboratory.
24. Safety officer responsible for containment
25. Staff receives special training and demonstrates competence in the required skills.

C) <u>Laboratory discipline</u>

26. Warning notices for containment areas to indicate the risks present and the name and telephone number of responsible person(s).
27. Informative notices about emergency protocols should be posted within the laboratory to advice personnel of steps to be followed in case of pathogen spill or the need to evacuate the laboratory in the event of fire or other emergency.
28. The laboratory must be lockable.

29. Personnel should have entry authorization.
30. Appropriate protective clothing, including gowns, and laboratory clothing with sleeves, closed footwear, disposable gloves, masks, protective eye shields, nose-mouth masks shall be worn in the laboratory and removed when leaving it.
31. Laboratory door should be closed when work is in progress and ventilation should be provided by extracting air from the room (when biosafety cabinets are used, ventilation systems should be balanced).
32. Food shall not be stored or consumed in laboratories.
33. Smoking or application of cosmetics shall not take place in the laboratory.
34. Pipetting shall not be done by mouth.
35. Aerosol production should be minimized.
36. No infectious material shall be discarded down laboratory sinks or any other drain.
37. Laboratory glassware and other materials used shall be safely stored before disinfection. Materials for disposal shall be transported in leak/spillage proof resistant containers. Waste material should be autoclaved, incinerated, or otherwise its safety should be guaranteed before disposal. Reusable material shall be decontaminated by appropriate procedures.
38. Any accident or incident shall be recorded and reported to the safety officer.
39. On entering, all street clothing should be removed and clean work clothes worn.
40. On exiting, all laboratory clothes should be removed and individuals shall wash and go to a clean area (outside the biocontainment area).
41. Individuals shall shower prior to going to a clean area.

D) <u>Handling of specimens</u>

42. Request advice on packaging prior to submission.
43. Incoming packages shall be opened by trained staff in reception areas with appropriate containment.
44. The transfer of pathogens from one authorized laboratory to another requires a license
45. Standard operating procedures for all laboratory areas should be available.

Guidelines on specific requirements for activities involving the foot-and-mouth disease virus

1. *Managerial aspects*

The management area of the Institution is responsible for the laboratory biological risk management. It should define and document the institutional policy for biological risk management and make it known to all officers in the institution.

The management is also responsible for:

a) Ensuring the existence of an internal biosafety and biological risk management commission and the implementation of a formal process for biological risk assessment.
b) Implementing a continuous upgrading system to ensure the review of biosafety and biosecurity procedures available.
c) Ensuring that standard operating procedure documents necessary for processes considered critical for biocontainment have been prepared.
d) Ensuring that standard operating procedure documents have been prepared and implemented for FMDV activities and that they comply with current international standards.
e) Approving procedures for emergency situations and contingency plan.
f) Implementing and documenting a system to monitor the access of people to different laboratory areas.

2. *Staff training – competencies*

The institution should ensure that the staff performing activities in environments where the foot-and-mouth disease virus is handled has the required competencies. Competence levels will be defined considering education, training and expertise.

A training program for the staff shall be implemented to keep the workforce trained and updated as regards hazard identification, biosafety and biosecurity procedures/requirements according to risk levels depending on the type of activities performed and biological risk management.

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4. *Staff training – competencies*

The institution should ensure that the staff performing activities in environments where the foot-and-mouth disease virus is handled has the required competencies. Competence levels will be defined considering education, training and expertise.

a) A training program for the staff shall be implemented to keep the workforce trained and updated as regards hazard identification, biosafety and biosecurity procedures/requirements according to risk levels depending on the type of activities performed and biological risk management.
b) The institution will define different types of training according to each activity and will provide details about the organization, monitoring and evaluation of its training program. A record of all the training provided shall be kept.
c) The Biosafety Officer is responsible for providing training and guidelines on how to proceed in the area to all individuals directly or indirectly related with the biocontainment environment and surrounding areas. (This group of people includes laboratory, cleaning and maintenance staff, visitors, etc.).
d) The whole staff must be well informed and regularly trained regarding emergency evacuation procedures.

5. *Laboratory Biosecurity*

The laboratory must implement biosecurity measures in order to protect biological material from misuse or deliberate removal from the laboratory. The measures to be implemented shall include, at least:

a) Adequate security system to detect intruders.
b) A procedure for rapid response to intrusion.
c) A system to control and record the access of people to the laboratory.
d) Procedures to record assets and control biological material taken from the laboratory.

6. *Entry to and exit from the biocontainment area*

a) The institution must have written documents available detailing the procedures to be adopted on entering and exiting the biocontainment area.
b) Any person who has to enter the biocontainment area has to receive training to be familiar with the entry/exit procedures of the area. The biosafety officer is responsible for providing this training.
c) Personnel must enter the biocontainment area solely and exclusively through changing rooms that allow changing clothes and taking a shower on exiting.
d) The access to the changing room will require the use of a personal non-transferable electronic code (or any other electronic means) automatically registered in the laboratory

control system.

e) On entering the outer changing room, all clothes, shoes, accessories (jewelry, piercings, watches, hair brooches, etc.), glasses, contact lenses, cosmetics, make-up, nail polish, must be removed. In case it is necessary to enter wearing glasses, they should undergo decontamination before leaving the biocontainment area.

f) People should enter the internal changing room completely naked. In the internal changing room, they will wear working clothes.

g) Personal protective equipment (PPE) shall be worn in work areas according to the type of activity performed.

h) At exiting the biocontainment area personnel should go into the internal changing room and proceed to remove their work clothes, wash hands and nails thoroughly and rinse the nasal and oro-laringeal mucosa repeatedly (at least 3 times). Personnel will enter the shower box completely naked.

i) Once the procedures mentioned in "h" are completed, a thorough shower shall be taken in the box (both body and hair). Gate opening, user and bath time are operated and recorded by a central automated system.

j) After shower, individual will go into the outer changing room where personal clothes are kept.

k) The access to laboratory areas where foot-and-mouth disease viruses are stored should be limited to the smallest number of people possible. These people should be extremely responsible and very well trained to safekeep the biological material under their responsibility.

l) In order to minimize the risk of unintentional escape of the virus that individuals could carry in their body, the laboratory will define and apply a relevant quarantine period to be observed by people accessing the biocontainment area. This period will vary according to the level of exposure of the individual, within the laboratory, to the viral source. People entering the biocontainment area shall sign a commitment letter that they do not live with animals susceptible to foot-and-mouth disease or have not visited areas or facilities with animals susceptible to foot-and-mouth disease, for a period of at least 3 days (which will be increased according the level of exposure to the viral source).

7. Biocontainment facilities

a) Walls, floors and ceilings: surfaces must be smooth, impervious to water and resistant to chemicals, seamless, and easy to clean. Joints should be sealed with adequate material (silicone, for instance). False (fall) ceilings are not permitted. Angles between walls and floors should be of sanitary design; right angles are not permitted between both surfaces. Entry of service lines (electricity, telephone, internet, etc.) must be totally sealed to adjacent wall, ceiling or floor. The construction should be airtight and strong enough to withstand the gradients of pressure required in biocontainment area for foot-and-mouth disease and, when appropriate, it should observe the building construction codes for seismic areas.

b) Doors: outer doors with active lock operated by an electronic code controlled by a central electronic system. Inner doors may have passive locks except those separating areas considered of higher biological risk (effluent treatment room, antigen quarantine area, cell culture bank, etc.). Doors should be fitted with windows that allow seeing from the outside actions inside, and vice versa. The material used for doors must have a smooth surface, impervious to water and easy to clean, and it should withstand and keep the pressure gradients of the biocontainment area.
c) Windows: totally sealed, double-glazed, and able to withstand internal pressure and major impacts.
d) Access changing rooms: must have access anterooms with double doors with active lock, box with shower for automated baths, and two doors with passive lock.
e) Communication: all areas must have telephone and internet connection to ensure communication between external and internal areas. Some areas require the use of cameras for extra security.
f) Electric generator: an alternative source of electricity is required as a backup for commercial power supply routinely used and, additionally, UPS to secure the provision of energy during the period of time between electricity shutdown and start-up of the alternative source. Critical circuits that must continue operating are the air system, cold chambers, biosafety chambers, safety facilities.

8. Air filtration system

a) Ventilation: The biocontainment laboratory performing activities with FMDV must operate under a negative pressure ventilation system and HEPA filtration of exhaust air. Air filter boxes and ducts will be in a biocontainment area next to the biocontainment laboratory area. Pipes for inlet air supply should be sealed to prevent escape of air from the biocontainment laboratory into the environment. It is necessary to have pressure differentials to ensure the gradient for each step within the biocontainment area, starting with the pressure of the outer area (considered 0) to the most negative pressure in the rooms of higher biological risk. All cases require a system to prevent the occurrence of positive pressure within the rooms where FMDV is handled, due to failures in the ventilation system.
b) Exhaust air filtration system: double and parallel HEPA filtration system is required for exhaust air of biocontainment (diagnosis and vaccine production) laboratories and rooms used as infection rooms for FMDV animals).
c) Inlet air filtration system: a system must be in place to prevent escape of air from the inside of the room via the inlet pipeline in case of ventilation shut-down/inversion. This may be achieved by a single HEPA filter or automatic dampers in the air inlet pipelines.
d) Filters: filters should be incorporated into a preventative maintenance program; the efficiency of HEPA filters should be checked at least once a year to confirm that they meet the manufacturer's specifications. Filters must be replaced when pressure differences exceed the specified limits, or sooner if they fail any of the prescribed efficiency tests (ISO 14644).

e) Manometers: air pressures within different areas should be continuously monitored by manometers. The staff working in these areas shall be trained to read the manometers, and be informed if loss of air pressure occurs and the actions to be taken in this case.
f) Monitoring computerized system: a computerized system shall be in place for online monitoring of pressure gradient of different areas and air treatment equipment. The system should be continuously monitored.

9. Activities involving the foot-and-mouth disease virus

a) Records of active material: all material having or suspected to have FMDV must be properly recorded at entry by a system that enables to track its origin, the studies and results obtained with the material, storage area and final destination.
b) Disposal of contaminated material: in areas handling up to 10 liters/week (supernatant of infected cell culture), suspensions containing FMDV should be inactivated by a validated procedure with disinfectants before disposal into the general liquid waste system of the laboratory. When large quantities of viral suspensions are processed (vaccine production), material shall be treated by chemical methods or other validated procedure, requiring transfer of tanks in a contained system connected by pipes and valves. The equipment used should be decontaminated at the end of the procedure, usually by chemical treatment and steam sterilization.
c) Inoculation, maintenance, sacrifice and disposal of animals: to be elaborated.

10.Material disposal

a) Effluents: effluents from biocontainment laboratories or facilities in which FMDV-infected animals are maintained must be treated using a validated method to eliminate residual active virus. The system for effluent treatment should have enough capacity to store untreated and under treatment effluent and must be installed within a biocontainment area to ensure that there is no possibility of effluent leakage from the system into the environment. Heat or chemical treatments may be used and both must ensure that all waste material is exposed to treatment. The treatment chosen must be validated considering the worst-case scenario within the context of activity of the relevant laboratory. The equipment used for effluent treatment must have an automatic monitoring system with a record of all critical data and an alarm in case failures are detected during treatment process. **Heat treatment**: foot-and-mouth disease virus is sensitive to heat treatment at 100 °C for 1 hour. Treatment must ensure constant homogeneity of the effluent. (The Condition to Eliminate Treated Material will be considered in the definitive version of the document). **Chemical treatment:** foot-and-mouth disease virus is sensitive to acid and alkaline pH conditions. NaOH or N_2CO_3 treatment at pH 12 for at least 10 hours is sufficient to inactivate infectious particles of FMDV in effluent. As with heat treatment, when chemicals are used it is necessary to ensure constant homogeneity of treated material. After treatment, material must be neutralized and pH checked before the effluent is released. The material used for drainage as well as pipelines should be resistant to heat and the action of extreme pH condition. (Containment Dikes for Protection in case of Spillage will be considered in the

definitive version of the document).

b) Solid waste: a validated method should be used to inactivate particles of infectious virus that may be present in the laboratory solid waste. Possible methods of choice are 1) steam treatment in an autoclave in validated temperature, pressure and time conditions. The laboratory shall validate the procedure for different types of material used for the maximum load the autoclave can resist. The autoclave should be double-ended to allow removing material from outside the biocontainment area; 2) incineration shall be environmentally friendly and performed in incinerators that comply with safety standards; 3) hydrolysis of carcasses using validated procedure. In all cases, the laboratory must have emergency procedures to be followed in the event routine treatment of solid waste disposal fails to work.

c) Computerized monitoring system: a computerized system shall be in place for online monitoring of effluent treatment equipment and pass through autoclaves.

11.Equipment and Materials

a) Work surfaces must be smooth, impervious and resistant to chemicals used in the laboratory. Junction between horizontal and vertical surfaces should be radiused.

b) Equipment must be designed so as to control aerosol emissions or be used within biological safety cabinets to ensure that aerosols generated will not escape into the environment.

Equipment must be decontaminated before being removed from the laboratory, according to the type of use, with a previously validated procedure. Equipment that cannot be removed via autoclave shall be removed via air lock. Decontamination by fumigation with formaldehyde 10 g/m^3 at 70% RH for at least 10 minutes or 3 g/m^3 for 24 h or equivalent (aldehydes, ethylene oxide, in proper conditions) should be performed within the airlock chamber. (The use of Filters will be considered in the definite version of the document)

c) For the removal of clothes used within the biocontainment area, they will be subjected to a wet heat treatment (at validated temperature, pressure and time conditions). Laundry of clothes not previously autoclaved is permitted if a laundry is available in the biocontainment area.

d) Documents will be scanned and sent out in electronic format.

e) It may be necessary to send biological material from the biocontainment area to other laboratories. If the material contains infectious foot-and-mouth disease virus, the recipient laboratory must show that the required biosafety measures to handle the virus are in place.

f) All biological material taken out of the laboratory shall be packed in primary containers. Primary containers shall be externally disinfected with a virus-inactivating agent and packed in a secondary packaging. Containers holding these materials will undergo “pass through” fumigation or washing using chemical disinfectants such as: 4% Sodium Carbonate; 0.5% NaOH (caustic soda); 0.2% citric acid; 4% formaldehyde. Other type of product/treatment can be applied. In all cases, method validation records should be available.

12.Suspension of biocontainment conditions and opening of laboratory

a) In certain situations, it will be necessary to perform maintenance activities that will require

to cease biocontainment conditions and opening the laboratory area. It will be first necessary to decontaminate laboratory rooms and equipment in order to reduce the risk of viral escape into the environment. Procedures for the treatment of different areas/rooms should comply with pre-defined international standards.

(Quantification of Biological Risk will be considered in the definite version of the document).

13. Bibliography

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b) EUFMD 40th Session, 2013. Appendix 7. Minimum biorisk management standards for laboratories working with foot-and-mouth disease virus. VERSION GS40/4.2BIS
c) MAPA – Brasil, Secretaria de Defesa Agropecuaria. Instrução Normativa N° 5, 28/03/2012
d) OIE Terrestrial Manual 2012. Chapter 1.1.3 Biosafety and Biosecurity in the veterinary microbiology laboratory and animal facilities
e) OIE Terrestrial Manual on line 2015 (web access). Chapter 1.1.3a. Standard for the management of biorisk in veterinary laboratories and animal facilities. NB: Version adopted by the World Assembly of Delegates of the OIE in May, 2014.
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