

## SECURITY STANDARDS FOR FMD LABORATORIES

### FOREWORD

In 1985 the European Commission for the Control of Foot-and-Mouth Disease, FAO, adopted a document entitled "*Minimum Standards for Laboratories working with FMDV in vitro and in vivo*" describing 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.

Although the above document dealt with all important aspects of FMD containment, it has been found necessary to review it with special reference to the need for more specific technical and general requirements as a consequence of the recent change in Europe to a policy of non-vaccination. As a result the present document has been prepared.

The security standards as specified herein should be considered as minimum requirements for FMD laboratories located in FMD-free countries with or without systematic prophylactic vaccination. Even in countries where FMD is present it is important to avoid the escape of FMD virus from laboratories so the standards in this document are recommended as the minimum for FMD laboratories, regardless of the prevailing disease situation.

### INTRODUCTION

Foot-and-mouth disease is one of the most contagious diseases known and manipulating the virus in the laboratory without adequate precautions is a hazard. The escape of a single infectious unit of FMD virus from a laboratory could potentially cause an outbreak.

The main sources of virus or infectious RNA (in increasing risk of hazard) are:

1. Infected tissue cultures.
2. Infected baby mice, guinea pigs, rabbits etc.
3. Physical and chemical processing of large quantities of virus outside closed vessels (e.g. concentration, purification, inactivation etc.).
4. Infected pigs, cattle, sheep, goats and other susceptible animals.

Ways by which the virus or infectious RNA may escape or be carried out from laboratories include:

Personnel  
 Air  
 Effluent and other waste  
 Equipment

Therefore all laboratories manipulating FMD virus must work under high containment conditions. The safety precautions must preclude every kind of escape of virus and special attention must be given to:

- the prevention of illegal entry into the restricted area
- the presence of changing and showering facilities
- the responsible behaviour of personnel within and when they leave the laboratory
- application of rules for primary containment
- the use of inactivated virus where possible
- the maintenance of negative air pressure where virus is manipulated and decontamination 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

To achieve this containment a variety of technical installations and a comprehensive set of disease security regulations are required under the supervision of a Disease Security Officer.

The Disease Security Officer must regularly receive technical reports about the various installations and monitor their performance. On the basis of day-to-day records he prepares an annual report on security (incidents, improvements etc.) to the director.

FMD laboratories may be authorised to manipulate live FMD virus for:

DIAGNOSIS  
 and/or  
 LARGE SCALE VIRUS PRODUCTION,  
 VACCINE TESTING INCLUDING INFECTION OF LARGE ANIMALS, and  
 EXPERIMENTAL INFECTION OF LARGE ANIMALS.

## MINIMUM REQUIREMENTS

### I. Personnel

1. Control of access to the premises - prevention of illegal entry to the restricted area<sup>1</sup>.
2. Personnel must be appropriately trained for the position held.
3. Entry into - and exit from - the restricted area must take place only through changing and showering facilities. This means a complete change from private to working clothes on entry - and another complete change as well as a shower on exit.
4. Personnel must be regularly trained in disease security.  
A code of disease security practice, including instructions for entry into - and exit from - restricted areas, must be available for all employees on site and for visitors.  
The disease security regulations must have been read and signed by each employee at the beginning of their employment.
5. 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.
6. Personnel must contractually agree not to keep any animals which are susceptible to FMD, nor reside on premises where such animals are kept, nor in the same household as other persons working with such animals 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. The same applies to visitors.  
  
Special care should be taken to ensure that visitors are instructed in decontamination procedures and that these procedures are properly followed.
7. Regular supply of appropriate laboratory clothing for use within the restricted area.

### II. BUILDINGS

8. General construction of buildings and their surfaces, including ducting of the air conditioning system:
  - well maintained condition with a high standard of airtightness
  - insect, rodent and bird proof

---

<sup>1</sup> Restricted area: Area where virus is manipulated and rooms in direct and indirect contact therewith.

9. Windows:
- non-opening and able to withstand operating pressures
  - shock proof in animal rooms
10. Doors:
- warning sign emblem at entrances:

**ACCESS FOR AUTHORISED PERSONNEL ONLY  
BIOLOGICAL HAZARD**

- access restricted by locked doors
  - airlocks provided with self-closing doors or with airtight doors
  - doors fitted with windows where appropriate
11. Walls, floors, ceilings:
- appropriate surfaces easily cleaned
  - sealed (airtight) entry of service lines

### III. AIR

#### Ventilation systems

12. Air is removed from virus manipulating areas through a HEPA filtration system which guarantees a negative pressure to atmosphere of at least 35 pascals (3.5 mm water) for laboratory rooms and rooms for small experimental animals and 50 pascals (5 mm water) for large scale virus production rooms and for large animal rooms.
13. The exhaust air from rooms where experiments in large animals are carried out must pass through two HEPA filters in series.
14. The filter installations must allow for their testing and safe changing *in situ*.
15. Manometers measuring the negative pressure in facilities and, where appropriate, the pressure drop across filters must be installed. They must be monitored and recorded regularly and, where appropriate, incorporate alarms. Every effort should be made to prevent a positive pressure within the building when either shutting off or turning on the ventilation system. Input and extract fans can be interlocked so that the failure of an extract fan shuts off input air and prevents reflux of contaminated air to the exterior, e.g. by means of a flap valve in the air intake duct.
16. The laboratory power supply should be equipped with a back-up source of electricity which starts without a delay of more than a few minutes in the event of power failure, or the commercial power supplier must guarantee a supply from an alternative source within a few minutes.

17. Vertical flow safety cabinets (Class 2), approved and checked, with absolute filtration of exhaust air must be available for the handling of FMD virus outside closed vessels.

#### Monitoring of ventilation systems

18. Ventilation systems should be continuously monitored to ensure proper function.
19. Before installation all filters must have passed an overall test for efficiency (normally done by the manufacturer).
20. When HEPA filters are installed or replaced an efficiency test must be carried out to ensure proper installation and function of the filters. This testing must be done at least once every two years, or when needed, e.g. when there is a sudden change in pressure over the filters. Testing of filters should be carried out by properly trained staff.
21. Among the methods acceptable for testing efficiency of filtration are:
  - A. A smoke challenge and photometric detection system using either DOP (dioctyl phthalate) or Shell Ondina (ANNEX 1 & 4).
  - B. A lithium flame photometer test for highly efficient filters (ref.: Hans Flyger and H.C. Rosenbaum, in American Industrial Hygiene Association Journal, Vol. 26, 409-412, 1965 (ANNEX 2).
  - C. Electronic particle counting (ANNEX 3).

#### Change of HEPA filters

22. Filters must be changed when the pressure difference exceeds certain limits in accordance with the instructions given by the manufacturer, or whenever needed.

Replacement of both pre- and absolute (HEPA) filters must be safe and must take place in accordance with an authorised procedure. Strict precautions must be taken to prevent spread of virus from filters or via contaminated air. Replacement of filters from outside must take place after decontamination *in situ* or in "safe change" air-handling units.

Decontamination of filters after use: wet autoclaving or incineration.

23. Filters in safety cabinets must be tested following installation and thereafter on a regular basis using the same or equivalent methods as used for testing of the general HEPA filtration system. When changed, filters from safety cabinets must be autoclaved or otherwise decontaminated before removal from the restricted area.

#### IV. EFFLUENT

24. Effluent from laboratories and from facilities holding animals should be treated in a manner which ensures that the inactivation of FMD virus has been achieved. For this purpose heat or chemical treatment can be used in a system which ensures that all the material is exposed to the specific treatment. The entire effluent treatment system, including the system for transport of effluent to the treatment unit, must comply with high containment conditions. There must be sufficient storage capacity (tanks) for the storage of untreated effluent.
25. The equipment should have automatic monitoring systems to ensure proper function. It must be ensured that the required temperature/pH is reached, and that the installation will stop automatically when required limits are reached, e.g. sterilisation temperature, time and maximum temperature or pH for discharge of the effluent.

#### Liquids

(slurry, waste water)

26. Heat treatment:  
100<sup>0</sup> C for 1 hour or an equivalent heat effect.
27. Monitoring:  
Automatic and continuous temperature/time/flow rate recording at different stages of the process.
28. Chemical treatment:  
NaOH or Na<sub>2</sub>CO<sub>3</sub> or other alkaline treatment at pH 12 for at least 10 hours. Thorough mixing of the materials must be ensured. After treatment the mixture must be neutralized and the pH must be checked before the effluent is released.
29. Monitoring:  
Automatic and continuous control and regulation of pH.

#### Solid waste

(animal carcasses, feedstuffs etc.)

30. Wet heat treatment (autoclave, 115<sup>0</sup> C in the center of all material for 30 min. or an equivalent heat effect, e.g. in a rendering process) on site.
31. Incineration on site. The incinerators must comply with current safety standards and be fitted with afterburners.
32. The system must exclude the possibility of re-contamination.

33. Monitoring:  
The heating systems should be continuously monitored and recorded as part of a fail/safe system. In the event of failure the system must be protected as far as possible against the release of potentially infectious material.

## V. EQUIPMENT AND MATERIALS

### Laboratory fittings

34. Benches: - impervious surfaces
35. Centrifuges, sonicators, homogenizers etc.: - must be designed so as to contain aerosols
36. Laboratory facilities and equipment must be cleaned and appropriately disinfected at regular intervals. Cleansing and disinfection must be supervised and recorded.

### Handling of FMD virus

37. Primary containment must be given proper attention. Handling of FMD virus outside closed vessels must be done in approved (Class 2) safety cabinets.
38. Processing of large quantities of virus must take place in closed systems. Proper attention should be paid to the decontamination of effluent air from vessels and pipe work and to the decontamination of the processing systems before opening for cleaning/maintenance/repair etc.
39. Inoculation of animals and the keeping of infected animals must take place within the restricted area under negative pressure. Personnel must wear appropriate protective clothing when handling virus suspensions and when inoculating or handling infected animals. On exit from animal rooms protective clothes and footwear must be left inside the room or appropriately decontaminated before being taken out.

### Removal of equipment and materials

40. Before removal from restricted areas equipment must be decontaminated according to the size and use of the equipment:
41. either by: heat (wet autoclave), if possible, at 115<sup>0</sup> C for 30 min. or an equivalent heat effect.
42. or dry heat at 50<sup>0</sup> C for 48 hours, in special situations, e.g. for certain sensitive instruments.

43. 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 for 1.5 hrs. at 50<sup>0</sup> C)
44. or thorough wash in an appropriate chemical disinfectant such as:
- 4 % washing soda (Na<sub>2</sub>CO<sub>3</sub>)
  - 0.5 % caustic soda (NaOH)
  - 0.2 % citric acid
  - 4 % formaldehyde or equivalent with other aldehydes. e.g. glutaraldehyde
  - or other disinfectant officially approved for the purpose

Note: The efficiency of these chemical disinfectants is considerably improved by the addition of a non-ionic detergent (concentration 0.005 %).

45. Decontamination of clothing before removal from the restricted area for laundry: heat (wet autoclave) at 115<sup>0</sup> C for 30 min. or equivalent heat effect. The laundry process must involve at least a hot (80<sup>0</sup> C) standard detergent wash at some stage of the cycle. Laundering must be done on site.
46. Treatment of papers before removal: 50<sup>0</sup> C for 48 hours, or an equivalent heat effect, or, for single sheets, fumigation. Removal of books should be exceptional and under the control of the Disease Security Officer. Fax or photocopy barrier systems should preferably be used.
47. All contaminated materials (including dead laboratory animals) must be placed in sealed bags inside leak-proof containers and decontaminated (e.g. by autoclaving) or transported for incineration or rendering on site. The surface of these containers should be disinfected before removal from the restricted area.
48. Before sending non-FMD biological material to another laboratory the necessary precautions must be taken to ensure that the material does not contain FMD virus. The recipient laboratory must be informed about the potential risk of material coming from a laboratory manipulating FMD virus. The recipient laboratory must further sign a statement that it is prepared to receive the material and that it will take the necessary precautions.

### Air Filtration Systems at the Institute for Animal Health, Pirbright

At Pirbright air filtration systems have been installed in all buildings in which viruses are used or experimental animals housed. The Buildings are maintained under negative pressure in relation to the atmosphere and the extracted air is passed out through two H.E.P.A. filters arranged in series. There are now 25 such units covering all laboratories and animal units.

#### Filter Leak Testing

H.E.P.A. filters are tested by producing an oil/smoke aerosol by blowing (CO<sub>2</sub>) carbon dioxide through a hot liquid plasticiser (Ondina Oil) which will produce particles of 0.3 microns in diameter.

A high concentration of "smoke" particles are introduced into the air trunking up-stream of the H.E.P.A. filters. The concentration of challenge particles is determined by the forward light scattering photometer (J.M. 8000). When a photometer reading of between 4.0 and 4.5 (Log scale) has been reached this will indicate that there is a concentration of 80 - 100 micrograms of smoke per litre of air. This is considered to be a satisfactory challenge as it is  $1 \times 10^4$  times above the minimum sensitivity of the photometer.

Holding the meter probe approximately 2.5 cm from the filter face on the downstream side, the entire surface area including the gasket, is traversed using slightly overlapping strokes at a rate of not more than 3 metres per minute. A significant leak is described as 0.01% or more of the challenge concentration.

#### Frequency of H.E.P.A. filter testing.

1. Filters must be tested when installed in the air handling unit.
2. When there is a sudden change in pressure over the filters.
3. Every six months in the large animal compounds.
4. If the air filtration system has been inoperative for more than one week.

**Appendix 6 (ii) Annex 2**Air Filtration Systems at the State Veterinary Institute for Virus Research, Lindholm

At Lindholm air filtration systems have been installed in buildings in which FMD virus is handled or experimental animals are housed. Facilities for virus/vaccine preparation and houses for experimental animals are maintained under negative pressure in relation to atmosphere and the extracted air is passed out through two H.E.P.A. filters arranged in series. There are at present 16 such units and 9 units with single H.E.P.A. filters.

Testing of filtration efficiency

H.E.P.A. filters are tested by producing an aerosol of lithium sulphate microcrystals with a particle size of 0.3 microns.

This is achieved by atomizing a 2% aqueous solution of lithium sulphate monohydrate by a specially constructed apparatus equipped with 3 Collision type atomizers. The aerosol is introduced into the exhaust air channels in such a way that even distribution of particles over the whole filter area is obtained. In the animal units, where most of the air is recirculated, the aerosol is introduced into the recirculation system, which in fact fills up the whole room with particles.

With the aerosol generator running air is sampled by controlled flow through 50 mm  $\varnothing$  cellulose nitrate membrane before and after H.E.P.A. filters. The filters are transferred to measuring flasks, dissolved in nitric acid, diluted, and lithium is assayed by atomic absorption spectrophotometry. A retention of more than 99.95% of Li is considered as correct performance.

Contrary to the sodium flame test, which may be seriously influenced by common dust particles, this lithium assay takes advantage of the fact that lithium is a very rare metal in nature.

An inconvenience is the relatively long sampling time (1 - 8 hrs) required to make sure that a sufficient load of particles has reached the filters in relation to the sensitivity of the meter.

The method was recommended by the Danish Atomic Energy Commission originally for use in monitoring exhaust air from nuclear reactors.

Frequency of H.E.P.A. filter testing

Filters must be tested

- when installed in the air handling unit,
- when there is a sudden change in pressure over the filters,
- at least once every 12 months in the large animal compounds,
- if the air filtration system has been inoperative for more than one week.

Air Filtration Systems at the Federal Research Centre  
for Virus Diseases of Animals, Tübingen

At Tübingen exhaust air filtration systems are in function in buildings in which FMD virus is handled or in which experimental animals are housed. These parts of the Research Centre are maintained under negative pressure in relation to atmosphere. Extract air is passed through a combination of prefilters and HEPA filters. For the laboratory building, a two chambers system is used. When filters have to be replaced air stream through one chamber is uncoupled. After removal of the filters from the isolated side, the chamber is disinfected. Subsequently, new filters are installed from "outside". During this procedure the other chamber remains in function, and air stream is not interrupted.

When filters have to be exchanged in animal houses a bypass system for the extract air flow is applied.

Filter Leak Testing

HEPA filters are tested for leak proof and efficiency with the aid of an electronic particle counter, which detects particles of  $\geq 300$  nm (0,3 micron) (exclusion size). This apparatus is equipped with an aspirating unit formed like a funnel and can be used for testing both the airtight site of the frame and efficiency of the filter area. The detector is equipped with an optical unit to detect passing particles, and an electronic regulation system assuring a high signal to noise ratio. Results are continuously shown on a display and printed out upon release.

Air Filtration Systems at the Centraal Diergeneeskundig Instituut, (CDI), Lelystad

At the CDI high containment building in Lelystad air filtrating systems have been installed in all buildings in which viruses are used or experimental animals are housed.

Different levels of negative pressure in relation to the atmosphere are maintained in these buildings. The exhaust air passes a combination of prefilters and HEPA-EU 13 filters. The exhaust air from the rooms that may contain high concentrations of FMDV passes two HEPA filters arranged in series. At present there are 272 filter units in use.

**Gasket testing:**

Most of the airhandling units are equipped with a rill for testing the gaskets of the filters. A filter is discarded if a leak of more than 2 l/h at a pressure of 200 Pa is detected.

**Efficiency testing:**

The efficiency of the filters is measured in situ by generating a DOP-oil aerosol (ATI TDA4A). It contains particles of 0.3  $\mu\text{m}$ . A high concentration of those particles is introduced into the air upstream of the HEPA filter. The concentration of particles in the downstream air is measured by a ATI TDA2E photometer. A filter is discarded if the efficiency is less than 99.995% of the challenge concentration (equivalent to a hole of 1 mm in diameter in a filter surface of 18  $\text{m}^2$ ). The tests are performed by a member of the technical staff in collaboration with someone from the Safety and Quality Assurance Department.

**Frequency of testing:**

Filters are tested when installed in the airhandling units and every two years if they are not discarded previously. Prior to incinerating, the filters are fumigated with formaldehyde in situ.