Appendix 1: Foot-and-Mouth Disease Laboratory Diagnosis

Note that the CO₂ emanating from the dry ice can create acidic conditions which can impact FMD virus survival. **Only use wet ice or ice packs for shipping FMD specimens to a diagnostic laboratory**.

SUBMISSION DURING AN FMD OUTBREAK

- o If possible, vesicular fluid aspirated with a syringe (at least 2 ml) in a separate sterile tube
- Two gm of affected epithelial tissue taken as aseptically as possible and placed in 5 ml of phosphate buffered glycerin virus transport medium (pH 7.6).
- O Scrapings of foot lesions in separate buffered glycerin transport medium.
- o Sera (10 ml) from clinically affected and recovered animals.
- Tissues from vesicular lesions of animals being destroyed or from animals being slaughtered in abattoirs may also be submitted for histology. If only a carcass is available, submit pre-scapular lymph node, adrenal gland, kidney, and thyroid gland for (viral) culture.

SUBMISSION DURING ROUTINE DISEASE INVESTIGATION

TO MAXIMIZE THE LIKELYHOOD OF ARRIVING AT A DIAGNOSIS, ALWAYS CONDUCT A FULL POST-MORTEM WHERE POSSIBLE and submit fresh or frozen tissues for culture and fixed tissues for histological examinations.

Tissue samples should include (teat skin; tonsil (P)*;lymph nodes - submandibular (P), prescapular (B), pleural (O/C), mesenteric; spleen; liver; kidney; lung; heart; piece of terminal ileum and ileo-coecal valve (5cm long and tied off at both ends); half brain (sagitally cut);Mammary (O/C) and any observed gross lesion.

Blood samples should include 10 ml serum; 10 ml EDTA and 6 air dried smears fixed in 70% alcohol. Other samples include nasal and tracheal swabs in transport media (B,O/C); body cavity fluids; infected joints fluid (P)

LABORATORY TESTS

Diagnosis of FMD for the index case is by demonstration of FMD viral antigen (double antibody sandwich ELISA) and/or virus isolation or nucleic acid specific to FMD in samples of tissue or fluid. Detection of specific humoral antibody can also be used for diagnosis, but results should be interpreted carefully for the index case.

For cases subsequent to the index case, the DAS-ELISA and real time or conventional PCR will be the main diagnostic methods. Virus isolation will be important for subsequent epidemiological investigation.

^{* (}B=bovine; P=porcine; O/C=ovine/caprine).

The laboratory tests currently available at NC-FAD are shown in Table 1 and include the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), virus isolation, real time (kinetic) reverse transcription-polymerase chain reaction (RT-PCR). The serological tests are the solid phase competitive ELISA and the serum neutralization test.

The time needed to confirm the diagnosis of the index case will depend on the quantity, the quality and the type of sample received by the laboratory. Sample types include: vesicular fluid, epithelial tissues and oro-pharyngeal fluid (probang). Good quality fluid collected from vesicles is ideal, but from a practical point of view difficult to obtain. Epithelial tags collected from the edges of ruptured vesicles are the more likely sample the field staff will submit. Ideally these tissues should be clean and relatively fresh and the oral tissues should be separated from the feet lesions.

The preferred procedure for the detection of FMD viral antigen and identification of viral serotype is the DAS-ELISA. However, a negative test result may be obtained when a specimen is from an old lesion, or is too small, or contains too little antigen to be detected. The method used to determine whether any infectious virus is present is to attempt virus isolation by inoculating and passaging the original tissue suspension through susceptible cell cultures, and looking for a cytopathic effect.

Detection of specific sequences of the virus nucleic acid can be attempted by PCR by using generic primers targeting the 3D gene (RNA polymerase) of the virus.

FMD virus infection can be diagnosed by the detection of a specific antibody response. Virus neutralization (VN) and ELISA are used as serotype specific serological tests. VN tests depend on cell culture systems and are therefore more prone to variability than ELISAs. Generally, antibodies to the whole virus appear in serum 7-10 days after infection.

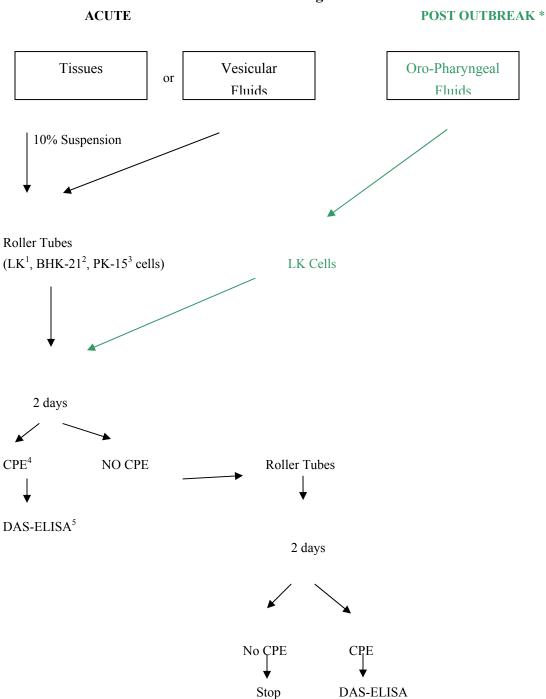
Once the NC-FAD has isolated a FMDV, it will be forwarded to the FMD World Reference Laboratory at the Institute for Animal Health, Pirbright Laboratory, UK for conducting molecular typing and providing advice on the selection of the antigen in the vaccine bank or from another source. Molecular typing is not required to initiate the control measures.

Table 1 Diagnostic test currently available at NC-FAD for FMD

Test	Specimen required	Test detects	Time taken
			to obtain result *
Virus isolation	Tissues	Virus	2-4 days
DAS-ELISA	Epithelial tissue or vesicular fluids	Antigen and serotype identification	4-5 hr
Real time RT-PCR	Tissues	Viral RNA	6-8 hr
Solid-phase competitive ELISA	Serum	Specific antibody	5-6 hr
Virus neutralization	Serum	Specific antibody	2-3 days

^{*} Represents only the actual test time. Time in sampling processing, repeat and reporting is not included. A complete turn around time (TAT) is available in the CFIA laboratory test TAT.

Flow Chart of Diagnostic Procedure



- 1) LK = Lamb Kidney
- 2) BHK-21 = Baby Hamster Kidney
- 3) PK-15 = Pig Kidney
- 4) CPE = Cytopathic Effects
- 5) DAS-ELISA = Double Anitbody Sandwich Enzyme-Linked Immundsorbent Assay

^{*} Print in colour

Appendix 2: Foot-and-Mouth Disease Disinfectant

Note: To be verified by AERT C&D supervisors.

Varying the pH of the environment of the virus outside of the range pH 5-10 is a practical method of destroying the FMD virus. Virus sensitivity is marked in the acid range and for this reason weak acids can be used in many situations.

(Adapted from AUSVETPLAN - Tables 2.8 and 4.0. See www.aahc.com.au/ausvetplan/index.html.)

Note: Dilution rates for use against FMD relate to effectiveness when applied to a *clean surface*. A dirty surface must be cleaned before it can be satisfactorily disinfected because the dirt may make the disinfectant useless. Ensure that the surface or material is:

- o thoroughly cleaned, ensuring that dung, litter, etc. is removed
- o thoroughly washed or sprayed with a disinfectant

DISINFECT- ANT GROUP	FORM (usual)	STRENGTH OF USUAL DILUTION (final conc.)	STRENGTH OF FINAL DILUTION weight/volume	CONTACT TIME	APPLICATIONS
ACIDS:					
Citric acid	powder	2 g/litre	0.2% (w/v)	30 min	Safe for clothes & body decontamination. Especially useful for FMD virus decontamination.
Acetic Acid (household vinegar)	liquid	4%	2%	30 min	Similar uses to above but mildly corrosive.
Hydrochloric acid	Concent acid (10 Mol)	1:50	2%(v/v)	10 min	Used only when better disinfectants not available. Corrosive for many metals and concrete.
OXIDIZING AGENTS:					
Virkon*	powder	20 g/litre	2% (w/v)	10 min	Excellent disinfectant for use on animals, human housing, machinery, vehicles, aircraft and clothing

DISINFECT- ANT GROUP	FORM (usual)	STRENGTH OF USUAL DILUTION (final conc.)	STRENGTH OF FINAL DILUTION weight/volume	CONTACT TIME	APPLICATIONS
Sodium hypochlorite NaOCl (household bleach)	conc. liquid (5.25% available chlorine)	1:10	5.25% available chlorine (52,500 ppm)	10-30 min	Effective for animals and clothing, except when in the presence of organic material. Less stable in warm, sunny conditions above 15°C.
Calcium hypochlorite Ca(OCl) ₂	solid	30 g/litre	2-3% available chlorine (20,000 - 30,000 ppm)	10-30 min	Effective for animals and clothing, except when in the presence of organic material. Less stable in warm, sunny conditions above 15°C.
ALKALIS: Do n	ot use in the	presence of alumir	nium and derived al	loys.	
Sodium hydroxide	pellets	20 g/litre	2% (w/v) ¹	10 min	Very effective for use in the environment, on animals, water tanks, dams
Sodium carbonate anhydrous (Na ₂ CO ₃)	powder	40 g/litre	4% (w/v)	10 min	Recommended for use in the presence of high concentrations of organic material
washing soda (Na ₂ CO ₃ .10H ₂ 0)	crystals	100 g/litre	10% (w/v)	30 min	
ALDEHYDES:					
Formalin	40% formal- dehyde	0.05	8% (v/v)	10-30 min	Use on feed contaminated with FMD Disinfectant releases irritating, toxic gas.
Formaldehyde gas	Paraforma Idehyde powder*	1 gm/cubic foot		15-24 hours	Toxic gas, recommended only if other methods of decontamination cannot be used.

Notes:

- Products effective for decontamination of viruses on the hands and the skin are limited. Virkon is reported to have low toxicity and to be effective. Citric acid may be added to washing water to induce antiviral conditions by lowering the pH.
- Vinegar and household bleach are both common disinfectants and effective when used for the purposes and concentrations recommended see table.

- A list of disinfectants and suppliers for North America is currently under development.
- * Requires a specialized generator and a lot of electricity. Usually, the formaldehyde gas emitted in a confined area will either have to be neutralized with ammonium carbonate or the area will have to be well ventilated before being used again. Bacterial indicators are required to control the effectiveness of the treatment.

Appendix 3: Foot-and-Mouth Disease Virus Inactivation Procedures

(From OIE Terrestrial Animal Health Code 2005), Appendix 3.6.2

Inactivation of the virus in meat

Article 3.6.2.1

- 1. Canning Meat is subjected to heat treatment in hermetically sealed container to reach an internal core temperature of at least 70°C for a minimum of 30 minutes.
- 2. Thorough cooking Meat previously deboned and defatted shall be subjected to heating so that an internal temperature of 70°C or greater is maintained for a minimum of 30 minutes. After cooking, it shall be packed and handled in such a way that it cannot be exposed to a source of virus.
- 3. Drying after salting When *rigor mortis* is complete, the meat must be deboned, salted with cooking salt (NaCl) and completely dried. It must not deteriorate at ambient temperature. "Drying" is defined in terms of ratio between water and protein which must not be greater than 2.25:1

Inactivation of the virus in animal products

Wool & hair (Article 3.6.2.2)

For inactivation of viruses present in wool and hair for industrial use, one of the following procedures should be used:

- 1.- industrial washing, which consists in the immersion of the wool in a series of baths of water, soap and sodium hydroxide (NaOH or soda) or potassium hydroxide (KOH or potash);
- 2. chemical depilation by means of slaked lime or sodium sulphide;
- 3. fumigation in formaldehyde in a hermetically sealed chamber for 24 hours. A common practical method of accomplishing this objective is to add commercial formalin (53 ml) to potassium permanganate (35g per cubic metre). Warning: the reaction between formalin and potassium permanganate is quite violent and generates a considerable amount of heat. For that reason, plastic and polyethylene containers should not be used and protective clothing, including goggles, are essential. The operator should also ensure a rapid exit from the room is possible. Good ventilation of the room is also essential after the fumigation. A safer fumigation method is to use the automatic formalin generators (Certek) from paraformaldehyde with neutralization with ammonium carbonate.
- 4. industrial scouring which consists in the immersion of wool in a water-soluble detergent held at $60-70^{\circ}$ C.
- 5. storage of wool at 18°C for 4 weeks, or 4°C for 4 months or 37°C for 8 days.

Bristles (Article 3.6.2.3)

For inactivation of viruses present in bristles for industrial use, one of the following procedures should be used:

- 1. -boiling for at least 1 hour.
- 2. –immersion for at least 24 hours in a 1% solution of formaldehyde prepared from 30 ml of commercial formalin per litre of water.

Raw hides and skins

Article 3.6.2.4

For the inactivation of viruses present in raw hide and skins for industrialized use, the following procedure should be used: salting for at least 28 days in sea salt containing 2% sodium carbonate.

Milk or cream for human consumption (Article 3.6.2.5)

For inactivation of viruses present in milk and cream for human consumption, one of the following procedures should be used:

- 1. A sterilisation process applying a minimum temperature of 132 0 C for at least 1 second (Ultra High Temperature [UHT]
- 2. If the milk has a pH less than 7.0, a sterilisation process applying a minimum temperature of 72° C for at least 15 seconds (high temperature-short time pasteurization simple HTST) or
- 3. If the milk has a pH of 7.0 or over, the HTST process is applied twice.

Milk for animal consumption (Article 3.6.2.6)

For the inactivation of viruses present in milk for animal consumption, one of the following procedures should be used:

- 1. Double High Temperature Short Time (HTST) pasteurisation (72°C for at least 15 seconds);
- 2. HTST combined with another physical treatment e.g. maintaining a pH 6 for at least one hour or additional heating to at least 72°C combined with desiccation;
- 3. Ultra-high temperature (UHT) combined with another physical treatment referred to in point 2 above.

Disinfection of Skins and Trophies (Article 3.6.2.7)

For the inactivation of viruses present in skins and trophies from wild animals susceptible to FMD, one of the following procedures should be used prior to complete taxidermal treatment:

- 1. boiling in water for an appropriate time to ensure that any matter other than bone, horns, hooves, claws, antlers or teeth is removed
- 2.- gamma irradiation at a dose at least 20 kilogray at room temperature (20°C or higher);
- 3. soaking with agitation in 4% (w/v) solution of washing soda (sodium carbonate Na_2CO_3) maintained at pH 11.5 or above for at least 48 hours;
- 4. soaking with agitation, in a formic acid solution (100 kg of salt [NaCl] and 12 kg of formic acid per 1000 litres of water) maintained at below pH 3.0 for at least 48 hours; wetting and dressing agents may be added.
- 5. for raw hides, salting for at least 28 days with sea salt containing 2% washing soda (sodium carbonate Na $_2$ CO₃).

Practical Field Inactivation (not in OIE Code)

Acid Treatment

The addition of acid to reduce the pH below 4:

- o 3 parts glacial acetic acid to 97 parts milk;
- o 500 gm citric acid or sulphamic acid to 240 litres milk, or;
- o 1.5 litres of ortho-phosphoric acid technical grade to 500 litres milk).

Because the milk will curd, do not undertake this activity while milk is in the bulk tank. Open tanks or containers will facilitate the disposal.

Other Disinfectants

To add another disinfectant, see Appendix 2.

When acid treatment is employed, the resulting mix of milk and acid will be agitated and held for one hour. Milk to which acid has been added can be pH verified using pH assay strips.

Appendix 4: Summary FMD Movement Control

Movement control is described in the Procedures in the Control Area (4.6) and the *Control Area Regulations Section 80* (under preparation). Transmission can be effected directly by animal movement or indirectly by fomites or things such as animal products, waste, animals, people etc. Means of transmission can be placed into logical groupings and assigned a risk (low, medium, or high) according to the innate ability to contain, sustain and transmit FMD virus. Movements are classified for epidemiological purposes based on the origin and destination, risk category of item, direction of movement, effectiveness of treatment, welfare and fate of risk good once moved.

Table I: Definition of risk categories

High risk	Medium risk	Low risk	
 Susceptible animals Genetic material from susceptible animals 	 Non-susceptible animals Susceptible animal products Fomites (susceptible animal contact) Vehicles incl. dairy tankers, feed, farm trucks 	 Diagnostic specimens (TDG packaged) People Vehicles (non-farm) 	
	 Non-animal products i.e. feed, some crops Animal service industries 	3	

Table II: Table of permit decision rules based on the source of the movement, the conveyor type, and the direction of the intended movement.

Risk	Destination	Source of requested movement		
		Suspect Infected Places & INFECTED ZONE	Other Premises in SURVEILLANCE ZONE	Premises outside CONTROL AREA
High	Within CA	Prohibited except slaughter	Permitted with conditions	Not applicable
	Into CA	Not applicable	Not applicable	Permit- not to suspect IPs
	Out of CA	Prohibited	Prohibited except to slaughter	Not applicable
Medium	Within CA	Permitted with conditions	Permitted with conditions	Not applicable
	Into CA	Not applicable	Not applicable	Permit- not to suspect IPs
	Out of CA	Prohibited	Permitted with conditions	Not applicable
Low	Within CA	Permitted with conditions	No permit required	Not applicable
	Into CA	Not applicable	Not applicable	No permit
	Out of CA	Permitted with conditions	No permit required	Not applicable

^{*} Note all places in the INFECTED ZONE are suspect infected places.

Infected Zone	Surveillance Zone			
1. ANIMALS				
1.1 Movement <u>out</u> of susceptible animals				
- prohibited - only susceptible animals from premises which have no epidemiological links to known infected premises and/or high risk premises may be allowed to move by permit to abattoirs within the infected zone or the surveillance zone provided that: no animal in herd of origin has shown clinical signs of FMD within 14 days (21 days for sheep & goats) no additions to herd of origin for 14 days (21 d. S & G) no clinical FMD within 10 km for 14 days (21 d. S & G) transport conveyances meet C&D requirements of zone veterinarian inspection within 2 days of movement	- from non-quarantined premises permitted: i) to abattoirs within the BUFFER SURVEILLANCE ZONE; ii) to other premises within the BUFFER SURVEILLANCE ZONE with the same owner; Movement permit conditions will require: no animal in herd of origin has shown clinical signs of FMD within 14 days (surveillance) 21 days (vaccinated premises and sheep & goat) no additions to herd of origin for 14 days (surveillance) 21days (vaccinated premises and S & G) no clinical FMD within 10 km for 14 days (surveillance) 21days (vaccinated premises and S & G) a vaccinated animal may only move to another vaccinated premises			
1.2 Movement <u>in</u> of susceptible animals - prohibited except to immediate slaughter under permit	transport conveyances meet C&D requirements of zone veterinarian inspection within 2 days of movement - allowed by permit to non-quarantined premises (see			
	1.1)			
1.3 Movement of other non-susceptible livestock/Control of confine to property; may function as mechanical vectors, - movement by permit to slaughter after appropriate C&D of animals and conveyance, i.e. egg cases or other containers must be C&D.	- from non-quarantined premises may move without restriction; - where vaccinated animals on same premises under permit.			
1.4 Movement out of dead animals				
-dispose on site (submit if clinical signs). - if disposal on the premises is not possible carcasses may be moved under permit to a landfill site, available crown land (pending environmental assessment) for burial/ burning within the CONTROL AREA or sent to a rendering plant (4.3). -compliance with provincial/municipal.	Allowed by permit within CONTROL AREA			
1.5 Movement out of animals to slaughter (no abattoir in in	nfected/surveillance zone)			

In the absence of an abattoir in the CONTROL AREA, live FMD susceptible animals can be transported under permit to the nearest abattoir in a free zone for immediate slaughter only under the following conditions:

No animal in the farm of origin has shown clinical signs of FMD for at least 30 days prior to movement. The animals were kept in the farm of origin for at least 3 months prior to movement.

FMD has not occurred within a 10 km radius of the farm of origin for at least 3 months prior to movement.

The animals must be transported under the supervision of the CFIA in a vehicle which was cleaned and disinfected before loading, directly from the farm of origin to the abattoir without coming into contact with other susceptible animals.

This abattoir is not approved for export.

All products obtained from the animals must be considered infected and treated in such a way as to destroy any residual virus.

Vehicles and the abattoir must be subjected to thorough cleaning and disinfection immediately after use.

2. PEOPLE AND VEHICLES

2.1 Movement of people - in & out

- subject to strict disinfection & no contact with susceptible species
- no contact with susceptible species outside of CONTROL AREA

2.2 Movement of vehicles - in & out

- wheels, wheel wells & specialized equipment of the vehicle are suitably cleaned and disinfected prior to leaving premises
- trucks that have been used to transport susceptible animals or animal products within the INFECTED ZONE require cleaning and disinfection at an approved C&D station prior to leaving the zone
- trucks subject to C&D if transporting susceptible animals at origin & destination
- trucks must be C&D if leaving CONTROL AREA

3. ANIMAL SERVICE INDUSTRIES

3.1 Movement of Services-veterinarians/ AI inseminators/feed companies - in & out

- restrict service to INFECTED ZONE
- C&D protocol strictly followed.
- -restrict multiple dose vials to one premises;
- -use disposable equipment and leave such equipment on the premises;
- -carry a minimum of equipment / supplies
- -clean and immerse non-disposable equipment in an appropriate disinfectant;
- -leave vehicles outside livestock area
- -disinfect vehicles upon leaving premises
- -use disposable clothing including boots

- restrict service to SURVEILLANCE ZONE
- C&D protocol strictly followed.
- -restrict multiple dose vials to one premises;
- -use disposable equipment and leave such equipment on the premises;
- -carry a minimum of equipment / supplies
- -clean and immerse non-disposable equipment in an appropriate disinfectant;
- -leave vehicles outside livestock area
- -disinfect vehicles upon leaving premises
- -use disposable clothing including boots

4. ABATTOIRS & ANIMAL PRODUCTS INCLUDING MILK

4.1 Abattoir Operation

- -abattoir not approved for export
- only susceptible animals licensed for slaughter;
- receive only enough susceptible animals for one day's operation;
- veterinary ante mortem inspection immediately upon arrival
- all carcasses chilled (not frozen) & held for 3 days
- no plant or CFIA employee should have contact with any susceptible animals operation;
- an approved cleaning & disinfection protocol for all personnel and equipment;
- sewage must be disinfected;

- receive only susceptible animals licensed for slaughter
- no plant or CFIA employee should have contact with any susceptible animals operation;
- an approved cleaning & disinfection protocol for all personnel and equipment;
- meat products from plants must be marketed within CONTROL AREA, unless de-boned and heat processed. (see Appendix 3)

4.2 Movement of fresh/frozen meat from susceptible animals

- meat products from plants must be marketed for human consumption only within CONTROL AREA, unless de-boned and heat processed (Appendix 3)

4.3 Movement of abattoir waste/rendering

- offal and waste products for rendering ($\ge 69^{\circ}$ C) must be moved under permit in a closed, leak-proof sealed vehicle which is C&D before leaving premise;
- -Rendering plants should be inspected and approved
- the rendering process must be monitored.
- rendering equipment must be thoroughly cleaned and disinfected after use.
- rendered product is not permitted to be used in susceptible animals feed

4.4 Movement out of susceptible animal by-products (hides etc.)

By-products from susceptible animals originating within the INFECTED ZONE must be treated (Appendix 3) can move under permit

Allowed within CONTROL AREA unless treated (Appendix 3) then may move out under permit.

4.5 Movement of Milk

- collected under permit on truck route confined <u>only</u> to premises in INFECTED ZONE
- adequate C&D of personnel & conveyance.
- products subject to treatment (Appendix 3) and marketed within CONTROL AREA

-milk collected under permit with suspect infected places collected separately or at the end of the run

-Allowed within CONTROL AREA unless treated (Appendix 3) then can move under permit.

5. GERMPLASM

5.1 Artificial insemination, embryo collection and transfer centres

- operate under license & under quarantine
- all semen collected from susceptible animals within 14 days of leaving an infected premises and any possible cross-contaminated semen must be disposed.
- semen, ova and embryo collection may continue for freezing and storage provided each lot is identified, maintained separately, the donors tested for FMD virus before being moved from the premises under permit
- -domestic movement in INFECTED ZONE &/or export as per the latest OIE Terrestrial Animal Health Code.
- -Personnel follow approved personal C&D upon entering and leaving the establishment and must not have contact with susceptible animals operations.

- operate under license
- all semen collected from susceptible animals within 14 days of leaving an infected premises and any possible cross-contaminated semen must be disposed.
- -all "clean" semen must be held pending a risk assessment.
- -domestic movement in CONTROL AREA &/or export as per the latest OIE Terrestrial Animal Health Code.
- -Personnel follow approved personal C&D upon entering and leaving the establishment and must not have contact with susceptible animals operations.

6. ANIMAL BY-PRODUCTS

6.1 Movement of litter & manure -prohibited unless treated then by permit in a closed, -prohibited; spread or treat on suspect premises leak-proof sealed vehicle which is C&D before leaving - may be composted at least 100 meters from livestock premise buildings for 42 days before it is spread on fields and - destination only within CONTROL AREA with no ploughed under. The compost pile will be covered with susceptible animal contact and immediately ploughed heavy impervious plastic. Spray with disinfectant in the surrounding of the compost pile. under - must be moved under permit in a closed, leak-proof sealed vehicle which is C&D before leaving premise and only moved to fields within the INFECTED ZONE with no susceptible animal contact and immediately ploughed under 7. FEED & EQUIPMENT 7.1 Movement of Feed & Equipment -prohibited except unexposed feed or cleaned & -by permit (i.e., unexposed feed or cleaned & disinfected equipment may move by permit disinfected equipment may move) -straw/forage if treated as per the latest OIE Terrestrial -straw/forage if treated as per the latest OIE terrestrial Animal Health Code. Animal Health Code. 8. SPECIAL PREMISES 8.1 Susceptible animals Assembly points- stockyards, auction markets, sales, fairs, zoos -assembly of susceptible animals prohibited; all assembly points must be cleaned & disinfected - assembly points with susceptible animals at the time of closure should -if swine are known to be infected, destroy and dispose of by burying, burning or rendering; if swine are known to have been directly exposed either destroy as above or ship directly to slaughter, according to circumstances; all other susceptible animals may move to destination under permit 8.2 Edible Residual Material Feeders (Swill Feeders) -prohibited -prohibited 9. TRANSPORTATION THROUGH ZONE 9.1 Transportation of susceptible animals through zone -prohibited; re-route around zone -direct movement allowed if origin & destination outside SURVEILLANCE ZONE and vehicles sealed by **CFIA** 10. SUSCEPTIBLE WILDLIFE 10.1 Control of susceptible wildlife - campaign to monitor the disease status. - Monitor for disease status - Exclude from premises - Exclude from premises

Appendix 5 – Epidemiological sampling of herds/flocks undergoing depopulation

Sampling requirements to meet the statistical significance suggested by international standards will need to be added for transmittal to the field staff. It is recommended that this Appendix be written by the team of epidemiologists that will build the rationale for recognition of FMD freedom by the international trading partners.

Appendix 6 – Surveillance sampling protocol for the Control Area and the disease-free zone

The sampling protocols for surveillance during and after the outbreak within the CONTROL AREA as well as in the FMD-free zone need to be added to build the argument for trade from the unaffected parts of the country. When to initiate surveillance both within the CONTROL AREA as well as in the FMD free zone, determine the confidence and the prevalence criteria if different from 95% and 1%. The OIE standard calls for 95% level of confidence at a 20% prevalence. The European Commission states 95% / 2%. It is recommended that this Appendix by written by the team of epidemiologists who will be building the rationale in support of a recognition of freedom from the trading partners.