Department of Primary Industries
Department of Regional NSW

DPI Primefact
Foot and Mouth Disease (FMD) field investigation

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Author: **Animal Biosecurity**

If you suspect FMD you should immediately phone:

- **your Local Land Services on 1300 795 299 (during business hours), or**
- **a NSW Department of Primary Industries veterinarian or authorised officer, or**
- **the Animal Biosecurity Emergency Hotline – 1800 675 888 –monitored 24 hours a day.**

Laboratory testing for FMD

FMD cannot be differentiated from other vesicular diseases on clinical grounds alone. This document summarises information on the collection of samples from all ruminant species and pigs. Diagnostic tests may be used to detect virus, virus genome and/or antibodies.

Laboratory testing is used to confirm or exclude a clinical suspicion of FMD. It also provides information on the serotype and strain of FMD virus, which assists in the selection of an optimal vaccine and may assist in epidemiological investigations. Laboratory testing is also used to exclude other exotic vesicular diseases and may help to identify an alternative diagnosis.

It is important to conduct a good clinical examination of affected animals and at-risk animals, document the clinical history and findings and collect a range of samples. Record the temperature of each animal that is examined and sampled.

FMD virus is likely to be present in the serum, oral fluids or nasal secretions 1-2 days prior to the appearance of clinical signs. Viral genome can be detected in oral swabs collected from 1-2 days prior to the appearance of clinical signs until at least 21 days later. FMD virus can be detected in epithelial samples or vesicular fluid from animals with acute clinical signs and antibodies may be detected from approximately 3-4 days after the start of clinical signs.

Biosecurity for field visits

There are no public health implications for FMD. People can harbour FMD virus sub-clinically, in the nasal passages and throat for up to 28 hours. During FMD outbreaks people should avoid moving between premises with susceptible animals that are not of an equivalent risk status, for at least 28 hours.

People conducting field visits should follow the standard operating procedures:

- Personal decontamination and
- Decontamination of vehicles and equipment

Samples for FMD diagnostic testing

1. **Collect blood and oral swabs from every animal** that is sampled.
2. **Sample oral, foot or teat lesions if present** by collecting tissue, vesicular fluid and swabs of the lesions, as described below.

Epithelium

Up to 2cm2 or 1g of epithelium is ideal. If enough lesion is not available, collect as much as possible. Samples from fresh lesions may rub off or alternatively, gently grasp the epithelium with forceps before cutting a section away. Epithelium can be transported in dry sterile screw topped vials.

Do not mistake a large fibrin clot for epithelium - test the texture before submitting it. Fibrin crumbles more easily than epithelium and fibrin will be less likely to contain virus.

Photo 1. Collecting epithelial samples from cattle

Photo courtesy of Eu-FMD

Vesicular Fluid

Withdraw up to 5ml of vesicular fluid with a narrow-gauge needle. Place in a plain sterile specimen tube. Ensure that the vial is proportionate in size to the volume of the sample to allow recovery of the sample, but total vial volume should not exceed 5mL.

Vesicular swab

Swab vesicular lesions and place in phosphate buffered gelatin saline (PBGS) for transport.

If no PBGS is available, place swabs in 2-3 mL of sterile saline in a screw topped vial.

Oral and nasal swabs

Vigorously swab the inside of the cheeks with sterile cotton swabs, as far into the mouth as practical and place in PBGS.

If it is impractical to collect swabs from the mouth, collect nasal swabs by vigorously rubbing the swab on the lining well inside the nose.

Blood sample

Collect 8-10ml of blood into a plain (red topped) tube, for detection of both virus and antibodies.

Be sure to avoid contamination of the container and stopper and remove all blood and faecal matter prior to packing and dispatch.

Use a separate sterile needle for each animal to avoid mechanically transmitting infectious agents from one animal to another.

Note samples may not be suitable for testing if blood cells are haemolysed. Common causes of haemolysis include:

- use of non-sterile containers for collection or storage
- contamination by water, faeces and other dirt etc
- a slow flow from the needle due to obstruction of the needle or failure to insert into mid-vein
- forcibly expelling blood through a needle
- heating of samples, usually in car boots or through back windows of cars, or after prolonged
- exposure to direct sunlight during collection
- freezing.

Post-mortem Tissues

Samples from dead animals should include the above fresh samples and duplicate tissue samples collected from each site into dry sterile screw topped vials and 10% formalin. Tissues to be sampled include tonsils, mandibular and submaxillary lymph nodes, thyroid, spleen, kidneys and epithelial tissue from between the digits. Heart muscle should be collected if there are signs of myocarditis, such as in young animals.

NOTE: Place fresh samples and samples in PGBS into well sealed 'ziplock' plastic bags (preferably double bagged) and fixed (10%) formalin samples into a different sealed bag. Samples can be transported in the same polystyrene box. Place specimen submission forms in a separate bag to specimens. Ensure all specimens are correctly labelled.

Selecting animals to sample

Sampling animals with classic signs of disease

If there are multiple animals with fresh lesions, collect a full set of samples (lesion, oral swabs and bloods) from approximately five affected animals.

If there are animals with a mixture of lesion ages, then take samples from the animal with the freshest lesions and oral and nasal swabs and bloods from at least five additional animals that have lesions that are healing or healed.

Sampling animals with healed lesions or without classic signs of disease

In some situations, there may be no suitable lesions to sample, but you still suspect or want to exclude FMD.

For example, when:

1. Lesions are typical of older infection. Collect swabs of old lesions, clotted blood, oral and nasal swabs

Or:

2. No obvious lesions present - collect clotted blood, oral and nasal swabs.

Sample at least 10 to 12 animals. Prioritise those with symptoms such as fever or drop in production or those with healed or healing lesions.

Swab transport media

- PBGS transport media is available free of charge from EMAI
- Orders can be placed by sending requests to laboratory.services@dpi.nsw.gov.au using the form at: https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0006/680586/Media-request-form.pdf
- The media can be stored in the freezer indefinitely and can be held chilled for several weeks after thawing.
- If no PBGS is available, place swabs in 2-3 mL of sterile saline in a screw topped vial.
- Swabs and PBGS are also held at all Local Lands Services offices.

Labelling of samples

Samples must be labelled serially (e.g. from 1 to 30) with a waterproof pen, preferably on an adhesive label. **Do not label the stopper or lid**, it is removed during testing. Carefully cross reference each sample to information provided on the submission form so they can be linked to an animal, unit within the premises (e.g. shed) and the premises.

Record which samples are from affected or non-affected animals.

Take photos of the lesions and email them to laboratory.services@dpi.nsw.gov.au and animal.biosecurity@dpi.nsw.gov.au.

Transport of FMD samples

A specimen submission form must accompany specimens submitted to the laboratory. Forms are available at https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0007/680425/Vet-specimen-advice-form-Feb2018.pdf.

Blood samples must be allowed to clot before transporting them over any distance. Once the clot has retracted by holding at room temperature, blood samples must be held chilled (but not frozen) to reduce contamination, haemolysis and autolysis.

All specimens must be clearly labelled and sent in leak-proof containers (not a syringe). Check that screw caps are tight. Place all containers in clean, sealed (e.g. “Ziplock) plastic bags, and smaller bags in an outer larger bag if necessary.

Fresh tissue, swabs and blood samples should be sent chilled with ice packs (NOT frozen). Pack in insulated containers with enough ice bricks to ensure that they are still cold when received at the laboratory. Prevent direct contact between coolant bricks and specimens, which may otherwise become frozen.

Fixed tissues in vials should be sent in a separate plastic bag(s) to the fresh tissues but can be included in the same package.

Samples must be packed as a category B diagnostic specimen. See Video 1 - Collecting, packaging and shipping samples at https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/veterinary/veterinary-test-list/collecting-and-submitting-samples-for-veterinary-testing for information on packing samples.

Send samples to the NSW Animal and Plant Health Laboratories, Elizabeth Macarthur Agricultural Institute (EMAI), Woodbridge Road, Menangle NSW 2568.

Booking a courier

Samples may be sent with Metrostate Couriers and StarTrack. Ring the laboratory customer service unit on 1800 675 623 8:30am-4:30pm Monday-Friday (excluding public holidays) or the Emergency Animal Disease Hotline on ph. 1800 675 888 to arrange urgent deliveries after hours.

DPI will pay for the cost of the courier and the laboratory testing for notifiable diseases.

Summary of samples and testing

Table 1. Samples to be collected and tests which can be performed

Sample type	Transport/collection	Test which can be performed
Epithelium/Vesicular Fluid	Sterile dry 5mL screw topped vial	PCR, Antigen ELISA, Virus Isolation
Vesicular Swabs	Phosphate buffered gelatin saline (PBGS)	PCR, Antigen ELISA, Virus Isolation
Serum (clotted blood)	Plain evacuated tube	PCR, Antigen ELISA
Oral or nasal swabs	Phosphate buffered gelatin saline (PBGS)	PCR, Antigen ELISA, Virus Isolation
Oral fluids – pigs ('Rope chews')	Sterile dry 20mL screw topped vial	PCR, Antigen ELISA, Virus Isolation
Fresh tissue samples	Sterile dry 5mL screw topped vial	PCR, Antigen ELISA, Virus Isolation
Fixed tissue samples	10% formalin	Histopathology
Bulk milk	Sterile 5mL screw topped vial containing bronopol	PCR
Probang	Phosphate buffered gelatin saline (PBGS)	PCR, Antigen ELISA, Virus Isolation

Laboratory testing arrangements

Preliminary testing for FMD will be conducted at EMAI. Samples will be forwarded to the Australian Centre for Disease Preparedness (ACDP) for confirmation of FMD status and to test for other vesicular diseases. Testing to confirm or exclude endemic diseases will be conducted at EMAI.

Additional information

- The Emergency animal diseases: A field guide for Australian veterinarians chapter 3.10 on page 120
- https://www.dpi.nsw.gov.au/animals-and-livestock/beef-cattle/health-and-disease/viral-diseases/fmd

Acknowledgments

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