Foot and Mouth Disease (FMD) Sampling Guidelines

Laboratory diagnosis of FMD

Laboratory diagnosis is necessary to confirm or exclude a clinical suspicion of FMD. It also provides critical information such as the serotype and strain of virus present, both of which are important for selection of an optimal vaccine and may assist epidemiological investigations.

Laboratory testing does not replace the need for a thorough clinical investigation; however it is important to note that FMD cannot be differentiated from other vesicular diseases on clinical grounds alone. Laboratory testing also contributes to identifying an alternative diagnosis, and the exclusion of other vesicular diseases which are also exotic.

The specific diagnostic tests applied are dependent on the stage of disease and available samples and may be used to detect the virus, virus genome and/or antibodies. It is therefore important to provide a good clinical history with sample submission and to collect a range of samples.

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. Virus can be detected in an epithelium sample or vesicular fluid when clinical signs first appear.

Antibodies may be detected approximately 3-4 days after the start of clinical signs.

This document summarises information on the collection of samples from all ruminant species and pigs.

Samples for FMD diagnostic testing

Where oral, foot or teat lesions are present these are the preferred sample for diagnostic testing. Lesions may be sampled by collecting tissue, vesicular fluid and swabs of the lesions, as described below. Blood should always be collected in addition to these samples. Where lesions are not available for sample collection, then oral swabs should also be collected as an additional or alternative sample.

1. Epithelium

Up to 2cm² or 1g of epithelium is ideal. However, if sufficient lesion is not available, collect as much as possible. Ensure the animal is adequately restrained (Photo 1) before taking samples as it can be difficult collecting samples from a painful mouth. Samples from fresh lesions may rub off or you may need to gently grasp the epithelium with forceps before cutting a section away.

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.

Epithelium can be transported in phosphate buffered gelatin saline (PBGS) or in an emergency, sterile phosphate buffered saline (PBS).

2. Vesicular Fluid
Vesicular fluid can be withdrawn with a narrow gauge needle. It can be transported in a plain tube such as a sterile specimen container. Ensure that the vial is proportionate in size to the volume of the sample to allow recovery of the sample. Samples should be no more than 5 ml volume.

Photo 1. Collecting epithelial samples from cattle

Photo courtesy of Eu-FMD

3. Vesicular swab
Swabs of vesicular lesions should be placed in phosphate buffered gelatin saline (PBGS) for transport. This is particularly useful for older, ruptured lesions that no longer contain vesicular fluid.

4. Oral and nasal swabs
Vigorously swab the buccal mucosa, as far into the mouth as practical or well into the nares with sterile cotton swabs and place in PBGS. Nasal swabs are less valuable than oral swabs.

5. Blood sample
Serum should be collected from all affected animals and/or in contact animals for detection of both virus and antibodies. Collect blood from up to 10-12 animals and identify any that are showing clinical signs.

6. Post-mortem Tissues
Samples from dead animals should include the above fresh samples as well as tissue samples such as tonsils, mandibular and submaxillary lymph nodes, thyroid, spleen, kidneys and epithelial tissue from between the digits. Heart muscle should be collected where there are signs of myocarditis, as may particularly occur in young animals infected with FMD. Duplicate samples should be taken, with one sample of each tissue collected as fresh tissue in phosphate buffered gelatin saline (PBGS) and one fixed in 10% formalin.

Which animals to sample?
If there are plenty of animals with fresh lesions, samples from approximately 5 affected animals should be sufficient for a diagnosis. If there are animals with a mixture of lesion ages, then take samples from the freshest lesions and oral and nasal swabs from other animals that have lesions that are healing or healed.

What if there are no suitable lesions?
In some situations there may be no suitable lesions to sample, but you still suspect FMD. This may be the case when:

1) Lesions are typical of older infection but there is no longer epithelium or vesicular fluid to sample. Collect swabs of old lesions, clotted blood, oral and nasal swabs
2) No obvious lesions present - collect clotted blood, oral and nasal swabs

In these situations at least 10 animals should be sampled, prioritising those with symptoms such as fever or drop in production or those showing signs of healed lesions.

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

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Transport/Collection</th>
<th>Tests which can be performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium/Vesicular Fluid/Vesicular Swabs</td>
<td>Phosphate buffered gelatin saline (PBGS)</td>
<td>PCR, Antigen ELISA, Virus Isolation</td>
</tr>
<tr>
<td>Serum (Clotted blood)</td>
<td>Plain evacuated tube</td>
<td>PCR, Antibody ELISA</td>
</tr>
<tr>
<td>Oral &amp; Nasal Swabs</td>
<td>Phosphate buffered gelatin saline (PBGS)</td>
<td>PCR, Antigen ELISA, Virus Isolation</td>
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</table>

Labelling of samples

Carefully label all the samples and cross reference them to your paperwork so they can be linked both to the animal and premises. Differentiate samples from affected and other animals.

Ideally take photos of the lesions and send them with the submission form.

Transport of FMD samples

Swabs should be placed in virus transport medium i.e. phosphate buffered gelatin saline (PBGS) and submitted chilled to the State Veterinary Diagnostic Laboratory (SVDL), Elizabeth Macarthur Agricultural Institute (EMAI), Woodbridge Road, Menangle NSW 2568. A specimen submission form must accompany specimens submitted to the laboratory. Forms are available at NSW Department of Primary Industries.

PBGS is available free of charge from the Virology Laboratory at EMAI. Orders can be placed by sending requests to virology.enquiries@dpi.nsw.gov.au. The media can be stored in the freezer indefinitely and is also suitable as a transport medium for most other virus investigations.

In an emergency situation, and if no PBGS is available, swabs can be placed in 1 ml of sterile saline if they are delivered to the SVDL on the same day.

Samples must be marked URGENT. It is important to contact the SVDL and the Virology Laboratory to advise samples are being sent. The Emergency Animal Disease Hotline (ph. 1800 675 888) should also be contacted.

Samples will be forwarded to the Australian Animal Health Laboratory (AAHL) for confirmation of FMD and other vesicular diseases. Testing will be undertaken at EMAI to confirm or exclude other diseases.

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

Unpreserved tissue, swabs and blood samples should be sent chilled with ice packs (NOT frozen). Samples must be packed as a category B diagnostic specimen. See the vet lab manual at http://www.dpi.nsw.gov.au/agriculture/vetmanual/submission for details on:

- completing the specimen submission form
- packaging of specimens

Laboratory testing scenarios
The recommendations in this Primefact are for collection of samples when investigating a suspect case of FMD or other vesicular disease.

Laboratory testing would also play an important role at various stages during the management of an FMD outbreak. This would include, for example, testing of samples from suspect infected properties, sequencing of selected isolates to inform molecular epidemiology studies and proof of freedom testing.

**Notification of Suspected FMD cases**

If samples are being sent to SVDL at EMAI for investigation of possible FMD or any other vesicular disease, then it is important to notify the NSW Chief Veterinary Officer. This can be done by calling the emergency disease hotline (1800 675 888), a NSW DPI Veterinarian or your Local Land services District Veterinarian.

**More information**
Sarah Britton, NSW Biosecurity and Food Safety

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