Food and Agriculture Organization of the United Nations

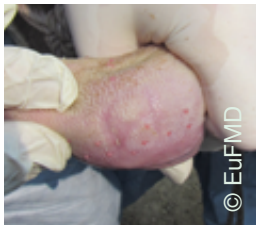
European Commission for the Control of Foot-and-Mouth disease

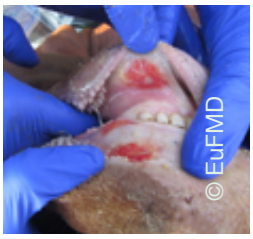
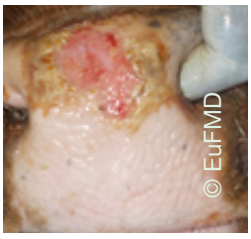
JobAid

Foot-and-Mouth Disease (FMD)

Sampling collection and laboratory diagnosis based on the stage of the disease process

Lesion ageing and sample type

Stage of the disease process	Lesion appearance	Viral antigen, nucleid acid or virus / antobody presence	Sample type
2 days before	--		• Vesicular epithelium. • Vesicular fluid. • Anticoagulated blood. • Swab. • Oropharyngeal fluid (probang sample). • Milk. • Myocardium.
Day 0	--		
Day 1 **Unruptured fluid-filled vesicle.** In some cases, blanching of the epithelium may be noticed before the formation of the vesicles.	 	 	
Day 2 **Freshly ruptured vesicle**. Clear edge, epithelial tags, raw red erosion base, no fibrin.	 		

Stage of the disease process	Lesion appearance	Viral antigen, nucleid acid or virus / antobody presence	Sample type
Day 3-4 **Edges less sharp, epithelial tags lost, less bright colour to underlying base.** Fibrin deposition starts.	 	 	• Anticoagulated blood. • Clotted blood. • Oropharyngeal fluid (probang sample).
Day 5-7 Fibrin deposition. **Epithelial regrowth at the edges of the lesion** forming a shoulder and from the base leading to greying.	 	 	
Day 8-11 Fibrin deposition. **The erosion is covered by new epithelium** but it is thinner than surrounding tissue and papillae have not regrown.	 	 	
Over 11 days Scar tissue.	 	 	

What to sample

Sample type	Aiming to detect	Suggested container and preservative	Diagnostic test (and average testing time)	Notes
Vesicular epithelium		Place at least 2cm² (fingernail size) tissue in a suitably sized container containing neutral buffered viral transport medium (VTM), preferably 50:50 glycerol-phosphate buffered saline plus antibiotic mixture (pH 7.2 - 7.6).	• RT-PCR[1] (4-5 hours) . • Ag ELISA[2] (4 hours). • Penside lateral flow device (LFD)[3] (10 – 30 minutes). • Virus isolation[4] (1-4 days).	• Sample of choice. • The richest source of virus. Always collect if there are unruptured or freshly ruptured vesicles. • Refrigerated/ kept on ice (4°C)[1]
Vesicular fluid		5 mL plain sterile containerplus antibiotic mixture (pH 7.2 - 7.6).	• RT-PCR (4-5 hours). • Ag ELISA (4 hours). • Penside Lateral flow device (LFD) (10 – 30 minutes). • Virus isolation (1-4 days).	
Anti-coagulated blood		EDTA vacutainer (purple top) tube.	• RT-PCR (4-5 hours). • Virus isolation (1-4 days).	• Always collect from some animals whether or not lesions are present in a suspected farm[6]. • EDTA anti-coagulated blood can be used in active surveillance for pre-clinical cases. • Refrigerated/ kept on ice (4°C)[1]

Sample type	Aiming to detect	Suggested container and preservative	Diagnostic test (and average testing time)	Notes
Clotted blood		Plain vacutainer (red top) tube.	• Antibody ELISA (1 day). • Virus neutralization test (VNT)[5].	• Always collect from some animals whether or not lesions are present in a suspected farm. Clotted blood is used for the detection of antibodies in recovering/ immune animals. • Clotted blood can be maintained at ambient temperature unless delayed/ very hot weather. • Do not freeze.
Saliva, oral, nasal or tonsillar swab		5 mL plain sterile container. Swabs are best snapped into a small volume viral transport medium (VTM) plus antibiotic mixture (pH 7.2 - 7.6), without glycerol.	• RT-PCR (4-5 hours). • Virus isolation (1-4 days).	• May be of value (especially if vesicular epithelium or fluid is not available). • Refrigerated/ kept on ice (4°)[1].
Milk		5 mL plain sterile container.	• RT-PCR (4-5 hours). • Virus isolation (1-4 days).	• Refrigerated/ kept on ice (4°)[1].

Sample type	Aiming to detect	Suggested container and preservative	Diagnostic test (and average testing time)	Notes
Miocar-dium		Place tissue in a suitably sized container containing viral transport medium (VTM): 10 mL glycerol-phosphate buffered saline plus antibiotic mixture (pH 7.2 - 7.6).	• RT-PCR (4-5 hours). • Ag ELISA (4 hours). • Virus isolation (1-4 days).	• From dead animals, also collect lymph nodes thyroid, adrenal gland, kidney, spleen, and any other macroscopic lesions. • Refrigerated/ kept on ice (4°C)[1] and in neutral buffered formalin fo histo-pathology.
Oropha-ryngeal fluid (probang sample)		To take the sample: • Ensure adequate restraint of the animal. • Introduce the probang into the mouth centrally. • Palpate the outside of the larynx and upper esophagus, do not introduce too far into the esophagus. • Gently move the probang backwards and forwards five times in this region. • Carefully withdraw the probang. • Place fluid in a suitably sized container containing equal volume viral transport medium (VTM) plus antibiotic mixture (pH 7.2 - 7.6), without glycerol.	• RT-PCR (4-5 hours). • Virus isolation (1-4 days).	• May be of value (especially if vesicular epithelium or fluid is not available). • Refrigerated/ kept on ice (4°)[1].

Sample type	Aiming to detect	Suggested container and preservative	Diagnostic test (and average testing time)	Notes
Interdigital and coronary band lesions When suitable oral epithelium is no longer present		Place tissue in a suitably sized container containing viral transport medium (VTM): 10 mL glycerol-phosphate buffered saline plus antibiotic mixture (pH 7.2 - 7.6).	• RT-PCR (4-5 hours). • Ag ELISA (4 hours).	• Least reliable diagnosic samples. Prone to secondary contamination. • Refrigerated/ kept on ice (4°C)[1].

1. FMD samples are hazardous; they must be packaged and labeled correctly to prevent virus release and to ensure that there is no contamination of the outside of the package. Please check your local rules for exact packaging guidelines.

If you are taking samples to confirm or rule out a suspicion of FMD in a free country, samples must be sent to the laboratory as an emergency, via the most rapid route possible, and the laboratory must be notified as soon as possible that samples are sent to them, with an expected time of arrival.

Sample collection - Guidelines

General principles

- Contact the laboratory before collecting or sending any samples.
- Obtain a full range of samples (independent of lesion ageing assessment).
- Vesicular fluid, epithelium and blood are the diagnostic samples of choice in live animals (but also collect blood and where appropriate saliva, oral, nasal and tonsillar swabs may also be taken).
- During necropsy, duplicate samples of heart muscle, lymph nodes (especially around the head), thyroid, adrenal gland, kidney, spleen, and any other macroscopic lesions including from the gastrointestinal tract.
- Maintain an aseptic sample collection technique.
- Tubes should be carefully sealed and correctly labelled (use indelible ink).
- Samples should be kept on ice immediately (4°C) until received by the laboratory.
- Examine sufficient numbers of animals, find the most recent lesions, collect representative samples from both acutely ill and recovered animals, and sample from all relevant epidemiological units.
- Take good quality samples from at least 5 animals with obvious lesions; sample at least 10 animals if no obvious lesions are present, prioritizing those animals with suspicious clinical signs.
- More intensive sampling may be appropriate in a first case then once FMD has been confirmed in a given region or country .

Sample collection in the field

- Samples are collected and placed in primary containers (e.g. vacutainers, swab containers, vials) and then in a secondary container (resealable bag).
- Sample records are bagged separately in resealable bags, unfolded, so that they can be read without opening the bag.
- Samples are all carried in a cool box to maintain samples at or near 4°C. Local rules should stipulate whether it is sufficient to clean and disinfect the outside of the cool box or if the exterior of individual samples must also be decontaminated, depending upon how and where the samples will be unpacked.
- Disposable sampling items are placed in a biohazard bag prior to exiting the premises.
- Used reusable items are placed in a container for decontamination at the premises exit.
- Unused, reusable items may remain unopened in the container in which they were carried onto the premises.

Sample handling when exiting the premises

- All contaminated samples or equipment that cannot be appropriately decontaminated must be left on the premises.
- Rinse your gloves in a suitable disinfectant as a precautionary measure before handing samples.

- Disinfect and place all disposable sampling equipment in a biohazard bag on the “dirty” side.

Sample cleaning

- Remove samples and record bags from the on-site cooler without opening the secondary container.
- Carefully clean any visible debris from the outside of the secondary container with detergent solution, then spray with suitable disinfectant solution.
- Clean the inside of the cool box.
- Place the samples (in secondary containers) back in the cool box.
- Clean the outside of the cool box.
- Now seal the cool box with duct tape.
- Place the Specimen Advice Sheet (SAS) in a resealable bag secured (taped) to the top of the cool box– not inside it.
- Spray cool box with a suitable disinfectant.

Sample packaging and transport

Ensure you check your national guidelines before proceeding with packaging and sending any samples.

- It is important to package specimens so that their quality is maintained, whilst minimising biosecurity risks.
- Packaging for all samples should follow a systematic procedure and consist of three components (triple packed) as follows:
  - The primary container must be watertight:
    - This is placed into watertight secondary packaging with absorbent material sufficient to absorb the entire contents.
    - The secondary packaging must be placed into outer packaging with a secure closure.
- The laboratory sample submission form should be placed between the secondary packaging and the outermost packaging.
- Ensure correct sample classification, packaging, labeling and supporting documentation are carried out. The outer packaging should be labeled to indicate the nature of the contents.
- The transport of samples should be done by the fastest possible route.
- Where Dangerous Goods (which also include dry ice or liquid nitrogen) are to be transported by air, packages must be prepared by individuals with the appropriate IATA training and accreditation (contact the transport company for further information).

Equipment checklist

Instruments	
Rat tooth forceps.	
Fine needle nosed forceps or needle drivers.	
Small pair of curved surgical scissors.	
Scalpel handle and blades.	
Needles and syringes (for vacutainers and syringe sampling)	
½ - ¾ inch hypodermic needles (21-23G).	
1½ inch needles (18G).	
2 - 3, 5 and 10 ml syringes.	
Sharps container.	
Sample containers and associated equipment	
Vacutainers: plain (red top) and EDTA (purple top) tubes - 10 mL capacity.	
Vacutainer holders.	
Small (5ml) plain screw top vials.	
Small (5 -10 ml) screw top containers filled with FMD viral transport media including 50:50 glycerol/ phospate buffered saline with antibiotics.	
Resealable bags – multiple sizes in which to collect primary containers, e.g. vacutainers.	
Sampling containers (e.g. Bio-Bottles or equivalent) – multiple sizes.	
Indelible pen for writing on sample containers.	
Additional equipment to assist sample collection and labelling	
Waterproof torch (may be provided by mobile phone).	
Data recording system - clipboard, paper, forms and pen and/or digital app.	
Disposable bags (for biohazard and for garbage).	
Camera or mobile device with camera (waterproof or in a waterproof case).	
Global positioning system (GPS) (optional – most mobile devices now include a GPS).	
Paper towels.	
Thermometers.	
Digital voice recorder.	
Bucket and brush for cleaning feet and a small brush for finer cleaning – e.g. toothbrush.	

Move FAST
Foot-and-mouth And Similar Transboundary animal diseases

Sustainable Development Goals, UN-SDGs.
EuFMD´s programme focus

Funded by the European Union

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