Project: Technical assistance to improve implementation of food safety standards and disease crisis preparedness

The sampling of sheep and goats in case of FMD

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Content

- General overview
- The purpose of sampling sheep and goats
- General guidelines
  - Procedures and guidelines for sample collection
  - Biosecurity during sampling
  - Principles for sample packaging
  - Storage and transport
  - Pathogenesis, circumstances and type of sample
  - Samples collecting
    - Blood samples
    - Epithelia and vesicle fluid samples
    - Probang sampling and Oesophageal–pharyngeal fluid
    - Other type of samples

How should use the SOP

This SOP will be used by **member staff** of the CDCC, LDCC and EG and all others involved in the implementation of killing measures in affected establishments.

Where? Whom?

1. ‘central disease control centre’ – CDCC
2. ‘local disease control centre(s)’ – LDCC
3. expert group/s – EG, operational on request of the CDCC or LDCC

Definition of Foot-and-mouth disease (FMD)

Foot-and-mouth disease (FMD) is caused by a virus of the family *Picornaviridae*, genus *Aphthovirus*.

- The virus has seven immunologically distinct serotypes: A, O, C, SAT1, SAT2, SAT3, and Asia1, which do not confer cross immunity.
- FMD cannot be differentiated clinically from other vesicular diseases, such as swine vesicular disease, vesicular stomatitis and vesicular exanthema.
- Laboratory diagnosis of any suspected FMD case is therefore a matter of urgency.
- There have been no reports of FMD cases due to serotype C since 2004 and this serotype is now considered to be extinct.
- For the purposes of this case definition, new FMD virus (FMDV) variants arise due to constant mutation during error-prone viral RNA replication, recombination, and host selection.

The purposes of SOP

The purpose of this document is to provide a guideline for the LDCC and EG ‘officials’ to **ensure the proper identification of samples** to be collected, **correct handling and collecting** of samples, **safe packaging and transport** to the laboratory for diagnostic and **biosecurity measures** that need to be observed in the process.

General Guidelines for sample collecting

The establishment of laboratory diagnosis for FMD is usually a matter of urgency and **samples shall be collected and transmitted without delay and in any case within 12 hours from collection**.

In this regard, the following main factors should be considered.

1. Procedures and guidelines for sample collection
2. Biosecurity during sampling
3. Principles for sample packaging
    a. Packaging
    b. Labelling
4. Storage and transport
5. Pathogenesis, circumstances and type of sample
6. Samples collecting

To be find the details of the main rules for the first 5 items above in the SOP.

More detailed information on "Samples collecting" will be share e.

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Collecting of samples

- As a guide, to reliably confirm diagnosis of FMD in a group of animals take at least 6 epithelium samples and 20 blood samples, or from all animals present if there are less than 20. Epithelium from a fresh lesion is the best sample.
- If fresh lesions are present, take a fingernail-sized piece of lesion epithelium and put in virus isolation buffer (glycerol and 0.04M PBS, 50/50 mix, pH 7.4).
- Take vesicular fluid if available; this can be transported in plain tubes if submission is rapid, but should otherwise be placed in virus isolation buffer. Blood samples should be clotted in a plain tube.
- The virus can be found in organs and tissues. Following table provides an overview of the sample of choice in case of infection with FMD in different stage of the disease.

Collecting of samples

Table samples to take according to the stage of FMD infection

Sample type	Transport/collection	Fresh lesion less than 3-4 days old	Older lesion older than 3-4 days old
Epithelium sample	Virus transport medium	Yes (PCR, Ag ELISA, LFD, Virus Isolation)	No - none present
Serum (clotted blood)	Plain tube, serum (clotted blood)	Yes (PCR for RNA detection)	Yes (Antibody ELISA - NSP, SP)
Saliva/nasal swab	Plain tube with hermetically siled cap	Yes, if facilities available (PCR or LFD)	No
Probang sample	Probang sample buffer	Yes if facilities to handle (PCR, Virus Isolation)	Yes if facilities to handle (PCR, Virus Isolation)

Collecting of samples

Taking and reporting the samples coming to the laboratory from the field by the Sample Acceptance and Reporting Unit".

Serology Laboratory

Source: Şap Enstitüsü, Ankara

Collecting of samples

- Selecting animals to sample is the first and very important part in case of FMD.
- Collection of samples should be based on the clinical examination of the herd and individual animals.
- The samples must be collected from the animals in which clinical sigs have been detected.
- Only in the absence of clinically sick animals, the collection of the samples should be done randomly in apparently healthy animals.
- When sampling sick animals, the principles form for the pathogenesis, circumstances and type of sample should be observed.
- An anticoagulant is not required to obtain serum to be tested for FMD antibodies and the blood is allowed to clot.

Sheep and goat blood-sampling

- Those whose animals are bleeding should use a quick and effective technique.
- This is necessary to minimize trauma to animals and stress to their owners.
- These procedure is for blood collection via the jugular vein in sheep.
- The right and left external jugular veins are large superficial veins that lie within the jugular furrow, a groove on each side of the neck dorsolateral to the trachea.
- Sheep are generally docile in nature and exhibit strong flocking behaviour; they may become very anxious when separated from other sheep.
- Once caught, sheep typically will stand still.
- Handlers should be vigilant at all times so as to avoid injury to animals or themselves.
- The same procedure can be applied for goats as well

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Sheep and goat blood-sampling

- <u>Animal preparation</u>

- Head shall be elevated and jugular vein exposed (Fig. 1);
- Stand sheep with animal's back against your legs. Alternatively, set the sheep on its rump with its back against your legs (tipping or "set-up") (Fig. 2);
- old the head of the sheep at about a 30° angle to the side to extend the neck and expose jugular (Fig. 3);
- Blood collection is most easily performed with two handlers – one to restrain and one to collect blood;
- Wear gloves, wash hands.

Figure 1. Pull Head to the Side and Occlude Jugular to Visualize Vein

Figure 2. Insert Needle

Figure 3. Collect Blood

Source: The University Veterinarian and reviewed by Virginia Tech IACUC

Sheep and goat blood sampling

- Jugular Vein Bleed 1

- With bevel up, insert the needle through the skin and into the vein at a 20° angle;
- Use a gauge of needle smaller than the vein;
- Using the vacutainer method - once needle inserted, stabilize needle and push the vacutainer tube into the hub;
- If you have hit the vein, blood will flow freely into the tube. Multiple tubes can be filled by removing the filled tube and replacing it with a fresh tube;
- Using needle and syringe method / monovette – clear air, and with the needle attached to the syringe, insert the needle at a 20° angle, and aspirate syringe to confirm insertion and collect blood;
- If you have missed the vein, you can carefully reposition the needle until the vessel is penetrated;

Sheep and goat blood-sampling

- Jugular Vein Bleed 2

- The vessel is fairly big and relatively apparent;
- Typically, no more than two to three attempts should be made at a time to minimize distress to the animal and potential damage to the vein;
- Collect required quantity of blood. One frequent reason for unfitness of serum for serological testing is inadequate quantity i.e., too little sera after contraction of the clot;
- Apply pressure until bleeding has stopped (1+ minutes)
- Serial samples can be taken by alternating sides, and by moving insertion sites caudally, as long as there is no hematoma formation.

NB: When using vacutainer, do not pull the needle out of the skin with a vacutainer tube attached, as this will cause the vacuum to be lost

Epithelia and vesicle fluid samples

Epithelium or fluid from vesicular lesions are the preferred samples to confirm foot-and-mouth disease virus (FMDV) infection in livestock. Ideally, at least 1 g of epithelial tissue should be collected from an unruptured or recently ruptured vesicle, usually from the tongue, buccal mucosa or feet (Fig.4).

Epithelial samples should be placed in a transport medium composed of equal amounts of glycerol and 0.04 M phosphate buffer, pH 7.2–7.6, preferably with added antibiotics (penicillin [1000 International Units (IU)], neomycin sulphate [100 IU], polymyxin B sulphate [50 IU], mycostatin [100 IU]). If 0.04 M phosphate buffer is not available, tissue culture medium or phosphate-buffered saline (PBS) can be used instead, but it is important that the final pH of the glycerol/buffer mixture be in the range pH 7.2–7.6.

Fig 4. – epithelium or vesicular fluid

(source: EUFMD)

Epithelia and vesicle fluid samples

Collecting epithelial tissue

- Use scissors and forceps to collect about 2cm^2 (approx. 1 - 2g) of epithelium from an unruptured or freshly ruptured vesicle;
- Cut the epithelium into smaller pieces and put them into the wide neck vial with leak proved lid;

Collecting vesicular fluid

- Aspirate with the syringe and needle or use the cotton bud to collect about 500µl (12 drops) of fluid from the vesicle,
- If you use the syringe, put the liquid into a sterile collecting tube;
- If you use the cotton bud, insert it into the sterile collecting tube

Probang sampling and Oesophageal - pharyngeal fluid collection

Indication for collecting of OP fluid is where epithelial lesion samples are not available from ruminant animals (**in advanced or convalescent cases**), or where infection is suspected in the absence of clinical signs (**carrier animals**) or very early infection (**preclinical stage**).

Procedure 1:

- OP fluids should be collected before animal feeding. If possible, it may be better to collect OP fluids 12 hours after feeding time;
- Restrain animals properly;
- Hold the mouth of the animal open by gentle pressure on the tongue with four fingers of the left/right hand in a gap between the lower right incisor teeth and the premolar teeth. Push the probang cup into the mouth, ensuring that the concave arm of the probang cup wire handle is towards the ground. Push the cup over the back of the tongue into the pharynx and then into the upper oesophagus. Judge its position by palpation of the upper oesophagus;

Probang sampling and Oesophageal-pharyngeal fluid collection

Procedure 2:

- Move probang up and down within the pharynx/oesophagus over a distance of 5-10 cm for 30 seconds or 5-6 times. Gently remove the cup from the animal's mouth and try to retain as much material as possible in the probang cup;
- After collection, pour OP fluids containing cellular materials into the previously prepared tube containing equal volume of the transport medium and thoroughly mix by gentle shaking. Can pour the transport medium to the probang cup to rinse the OP fluids and pour back to the container tuber if OP fluids stick;
- Examine visually for quality. If samples are heavily contaminated with ruminal contents, discard and flush the animal's mouth with water or PBS before repeated sampling;

Probang sampling and Oesophageal–pharyngeal fluid collection

Procedure 3:

- Samples from sheep tend to be small, mucoid and difficult to detach from probang cup, if so, insert the probang cup directly into a disposable universal 20 ml tubes or similar container into which has been dispensed 3 ml of buffer solution and gently mix, pour the sample and buffer into a previously labelled tubes for transport;
- Tightly close and properly label tubes and disinfect and clean the outside of the tubes;
- Keep OP fluid samples in ice boxes or ice pack (4°C) immediately to maintain the quality of the sample before delivery to the laboratory;
- Between collections from each animal, disinfect and wash probang cup thoroughly. Wash probang cup between animals using three buckets system (Fig.5): First bucket: tap water (Fig.6); Second bucket: disinfectant (Fig.7); Third bucket: tap water (Fig.8).

Probang sampling and Oesophageal–pharyngeal fluid collection

Probang sampling and Oesophageal–pharyngeal fluid collection

Special attention is needed for the following

- If disinfectant is not flushed off the probang, then it will kill the virus from the next animal;
- Care must be taken not to introduce the probang too far down the oesophagus. This will cause the animal to regurgitate, and rumen fluid (pH <7) will ruin the sample;
- In these cases, sampling should be repeated once the mouth of the animal has been rinsed with water or PBS;
- Samples seen to contain blood are not desirable but may be suitable for molecular testing;

Probang cup

For goats and sheep: the cup is about 1-2 cm full diameter and 2-2.5 cm deep, attached to a curved metal wire (43 cm in length) (Fig.9).

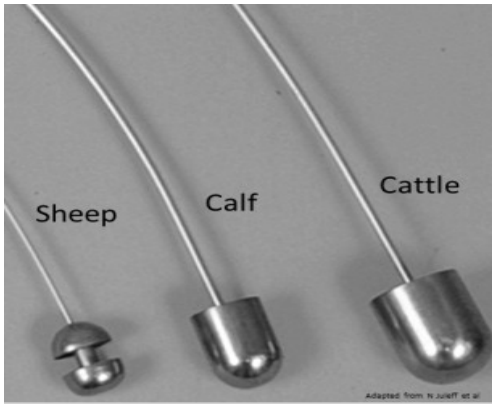

Fig 9. - probang cup

Other types of samples

Nasal swabs: May be collected on occasion to attempt virus isolation from animals which may be in the incubation period (e.g., following known contact with infected animals, personnel or equipment).

Throat swab: For the collection of throat swabs from sheep or goats, the animal should be held on its back in a wooden cradle with the neck extended. Holding a swab in a suitable instrument, such as an artery forceps, the swab is pushed to the back of the mouth and into the pharynx.

Saliva from acutely infected animals: Saliva may be collected, diluted about 1:10 with phosphate-buffered saline (to lower the pH for ruminant saliva in particular, and to dilute antiviral factors present in the saliva), and maintained chilled (4-10 ℃) for transport to the laboratory. Useful if fresh epithelial samples are not available. If saliva is not readily available, the mouth of an affected animal may be rinsed with phosphate buffered saline and this sample submitted to the laboratory.

Other types of samples

Milk: May contain virus for several days before and after the onset of clinical signs. Collect milk into a plain glass tube. Keep at 4 °C until arrival at the laboratory. Freeze if there is likely to be a delay in testing, to avoid a fall in pH which may inactivate the virus

Post-mortem samples: Lymph nodes, thyroid, heart muscle from a freshly dead juvenile animal can be used. Place in phosphate buffered saline/glycerol transport medium.

Serum Samples: Serum, rather than whole blood, must be submitted. A minimum of 4 ml is essential. It is essential that sterile containers should be used. If sterile, samples can be submitted without refrigeration, but refrigeration or freezing is an added safeguard against spoilage

- Collect under sterile conditions into plain sterile glass tube without anticoagulant.
- Allow to clot.
- Centrifuge then decant serum into sterile tube.

Reference materials 1

Local legal texts:

1. Legal text on Animal Health 41-2012 http://veteriner.gov.ct.tr/Mevzuat
2. Instruction for the animal diseases control program http://veteriner.gov.ct.tr/Mevzuat

EU legislation:

3. Regulation (EU) 2016/429 of the European Parliament and of the Council of 9 March 2016 on transmissible animal diseases and amending and repealing certain acts in the area of animal health ('Animal Health Law') (Text with EEA relevance) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32016R0429&qid=1657187827264

4. COMMISSION DELEGATED REGULATION (EU) 2020/687 of 17 December 2019 supplementing Regulation (EU) 2016/429 of the European Parliament and the Council, as regards rules for the prevention and control of certain listed diseases https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32020R0687

FMD SAMPLE COLLECTION VIDEOS

FMD collecting diagnostic samples

https://www.youtube.com/watch?v=bVwgS5USvic

FMD Collecting a probang sample

https://www.youtube.com/watch?v=bVwgS5USvic

Reference materials 2

Other resources:

1. FAO
   https://eufmdlearning.works/pluginfile.php/8030/mod_page/content/5/sampling_onepager.pdf

2. OIE Terrestrial Manual - Chapter 3.1.8 Foot and mouth disease (infection with the FMD virus
   https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.08_FMD.pdf

3. OIE Terrestrial Manual - Chapter 1.1.3. Transport of biological material,
   https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/1.01.03_TRANSPORT.pdf

Annex 2 Materials - check list 1

No	Type of sampling	Quantity	Check box
	Collecting of blood and vesicle fluids		
1	Disposable gloves	1 pack	
2	Gauze	20 pieces	
3	70% Alcohol	1 bottle	
4	Syringes 5 ml	10 pieces	
5	Syringes 10 ml	10 pieces	
6	Vacutainer/monovets	25 pieces	
7	Vacutainer holder	5 pieces	
8	Needles 18 - 20 gauge	1 box	
9	Needles 21 -23 gauge	1 box	
	Collecting of epithelial tissues		
1	Disposable gloves	1 pack	
2	Curved surgical scissors	2 pieces	
3	Forceps	2 pieces	
4	Scalpel handle	2 pieces	
5	Scalpel blades	10 pieces	
6	Wide opening screw lid containers (20 – 50 cm^3)	10 pieces	

Annex 2 Materials - check list 2

No	Type of sampling	Quantity	Check box
	Collecting oesophageal samples		
1	Probang cups (of suitable size for large and small ruminants)	Na*	
2	Sterile plastic tubes with screw cap	20 pieces	
3	Ice and ice box (preferably liquid nitrogen)	1 box	
4	Disinfectants, buckets (10-litre each for disinfection of probang cup	3 pieces	
5	Transport medium	500 ml	
6	Disinfectant (Approved by the VD)	500 ml	
	Packaging (secondary and outer)		
1	Ziploc lock bags – multiple sizes in which to collect primary receptacles (packaging)	25 pieces	
2	Outer packaging - Sampling containers/insulated box – multiple sizes	5 Pieces	

Annex 2 Materials - check list 3

No	Type of sampling	Quantity	Check box
	General equipment		
1	Transport medium/buffer (glycerol / phosphate buffered saline with antibiotics)	500 ml	
2	Sampling swabs	25 pieces	
3	Sharps container	2 pieces	
4	Disposable biohazard bag	5 pieces	
5	Indelible pen for writing on sample bottles	3 pieces	
6	Sampling forms	5 copies	
7	Paper towels	3 pieces	
8	Small brush	1 piece	
9	Torch	2 pieces	
10	Eye protection (recommended)	2 pieces	
11	Batteries	2 sets	
12	Restraining equipment or portable restraining box	1/2 sets	
	*depend on the type of animal, at least one per susceptible species present on the holding		

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