FREQUENTLY ASKED QUESTIONS

Diagnosis

During the EuFMD online *FMD Emergency Preparation courses (FEPC)* and *FMD Investigation Training courses (FITC), a number of* questions are raised by the trainees, discussed in the course forum and answered by the different experts working for EuFMD.

This document summarizes some of these **frequently asked questions** with the answers given during our courses related to **FMD diagnosis**.

What transport medium should be used for epithelial samples, swabs and probang samples taken to test for FMD virus?

Quoting from the FMD Chapter of the ***OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals:***

"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. FMDV is extremely labile in low pH and buffering of the transport media is critical for successful sample collection. Samples should be kept refrigerated or on ice until received by the laboratory".

In the UK, the lab supplies stocks of PBS glycerol ready made up. It should be stable for some time at room temperature if prepared aseptically as it contains both antibacterial and anti-fungal agents. Swabs and Oro-pharyngeal (OP) fluids are normally collected into tissue culture medium (~VTM) or PBS with the pH indicator phenol red that exhibits a red colour at neutral pH, turning orange and then yellow as the pH falls and pink as it rises. Vesicular fluid could be collected into a plain tube or in case of a very small volume sample into a small volume of medium/PBS.

Regarding probangs, again quoting from the OIE Diagnostic Manual:

"After collection of oro-pharyngeal fluid by probang, the contents of the cup should be poured into a wide-necked transparent bottle of around 20 ml capacity. The fluid is examined, and should contain some visible cellular material. This fluid is then added to an approximately equal volume of transport fluid, ensuring that cellular material is transferred; the mixture is shaken gently and should have a final pH of around pH 7.6. Samples contaminated with ruminal contents may be unsuitable for culture. Samples seen to contain blood are not entirely satisfactory. Repeat sampling can be done after the mouth and throat of the animal have been rinsed with water or PBS. Where several animals are to be sampled, the probang must be cleaned and disinfected between each animal. This is done by washing the probang in tap water, immersing it in a suitable disinfectant (e.g. 0.5% [w/v] citric acid in tap water) and then rinsing off all the disinfectant with water before sampling the next animal. OP samples from small ruminants are collected by putting 2 ml of transport fluid into a wide-necked bottle of about 20 ml capacity and, after collection, rinsing the probang cup in this transport fluid to discharge the OP sample. This is then transferred to a container of about 5 ml capacity for transport. The small container should be capable of withstanding freezing above dry ice or liquid nitrogen (Kitching & Donaldson, 1987). Samples of OP fluid should be refrigerated or frozen immediately after collection. If they are to remain in transit for more than a few hours, they should preferably be frozen by being placed either above dry ice or liquid nitrogen. Before freezing, the containers should be carefully sealed using airtight screw caps or silicone. This is particularly important when using dry ice, as introduction of CO2 into the OP sample will lower its pH, inactivating

any FMDV that may be in the samples. Glass containers should not be used because there is a risk that they will explode on defrosting in the event of liquid nitrogen leaking into them. Samples should reach the laboratory in a frozen state or, if this is not feasible, maintained under reliable cold chain conditions during transit."

Instead of dry ice, dry shippers can be used to keep probang samples frozen in hot climates (Dry shippers are large vacuum flasks that contain a porous material. They are designed for the safe shipment of specimens at liquid nitrogen temperatures without the risk of spilling liquid nitrogen).

Reference

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.1.5. FMD. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.05_FMD.pdf

Based on David Paton's answer during FEPC_AU1.

How many animals should be examined during an FMD outbreak investigation?

Background information
When there is a suspicion of FMD in a farm, animals need to be examined to check the clinical signs that they show and a very important part of the outbreak investigation will be the ageing of the lesions: It is possible to estimate the "age" of an FMD lesion, and therefore estimate the likely time that clinical signs first appeared.

Knowing how old a lesion is allows you to establish when clinical signs probably first appeared, and from this, the likely time period animals first became infected. This is important for tracing possible sources of infection. Similarly, you can also estimate when viral shedding would have begun, allowing tracing of further spread of the virus.

*When carrying out lesion ageing, it is important **to examine all animals in affected groups**, looking for the **oldest lesions**. This will allow identification of the approximate time period that the first animal in the group became infected.*

Nevertheless, in very big farms, examining all the animals could in practice be very challenging,…

Statistics can help with some situations related to 'how many animals do I have to examine', namely:

1) if you want to estimate the prevalence of animals with lesions or

2) if you are examining a herd/flock and want to be confident that there are NO lesions (ie to be able to say you are "95% certain that the flock is free of clinical lesions")

Unfortunately, however, statistics cannot provide us with a number to examine to be sure you will find the oldest lesion. In some situations, there might just be a single animal that was introduced to the herd that was infected and introduced the disease...this one animal will have older lesions than the rest.

If you cannot examine all of the animals due to very large numbers, you'll have to rely on common sense in order to decide which ones to examine. Some things to consider are:

- make sure to examine some animals from every separate 'group' on the farm that is housed or grazing together
- how disease was thought to be introduced might guide you...be sure to examine newly introduced animals, for example

Note also that the search for oldest lesion can take place after the animals have been culled, which makes it possible to examine many animals relatively quickly.

Based on Melissa McLaws' answers during FEPC_AU1 and on the content from EuFMD training courses.

What is the sensitivity/specificity for each of the FMD lab tests?

For virus detection tests, the diagnostic sensitivity (Se) is very dependent upon what samples are collected, when in the course of disease and how they are shipped. Specificity (Sp) of some tests, such as RT-PCR is also largely determined by laboratory practice. Therefore it is difficult to give a definitive figure for diagnostic Se/Sp in practice. However, there are important differences in the analytical Se of the different tests that dictate when they should be used.

RT-PCR is extremely sensitive and if done properly is also very specific. It can be used on virtually any sample type and it can detect non-infectious virus, for example complexed by antibody or cooked in transit.

Virus isolation is also very sensitive as long as you use the right cell system, but it only detects live virus and some types of sample are too toxic to the cells to be tested. For a high Sp, cytopathic agents identified in the test must be confirmed as FMDv by Antigen ELISA or RT-PCR.

Antigen detection ELISA (AgELISA) is much less sensitive than these other tests as it relies on recognition of the virus by antibodies without amplification of the agent. It is therefore only suitable for tissues and especially vesicular epithelia or fluids (and to confirm virus isolation tests). Non-specific reactions are also possible, so intrinsic Sp is slightly less. A big advantage is that is specifies the serotype.

Both AgELISA and RT-PCR can occasionally also miss viruses that have critical mutations in genes / altered coat proteins.

A **very important point** is to remember that the Se and Sp for a test system involving one sample and one test can be quite enhanced by testing many samples with different tests. This is why it is important to collect a range of samples and then examine them with multiple tests of different types when there is a suspected index case of FMD. The resulting corroboration should give an extremely reliable result. In contrast, in the middle of an epidemic, with limited resources, more reliance can reasonably be placed on diagnoses made on clinical signs (if clear-cut) complemented by fewer tests.

It is more difficult to give a simple answer to this Se/Sp question for serology, as there are several different tests used in different situations.

Serology for FMD measures antibodies against either structural or non-structural proteins (SP/NSP). As tests to detect animals that have been infected, only NSP tests can be used on vaccinated animals, but both types can be used on unvaccinated animals. The NSP test has the advantage of detecting antibody to any serotype, but the concomitant disadvantage that you cannot use it to distinguish which serotype is the cause of any detected antibodies.

The Se of the tests should be >95%, but may be affected by a number of factors including: (1) the time after infection; (2) in case of NSP tests, the protective effect of vaccination, as this can reduce virus replication and antibody response; (3) in case of SP tests the antigenic relationship between the strain of virus that has infected the animal and the strain of virus used in the test.

The Sp of most FMD serology tests is quite high (often 99% or higher), but small differences can be important when testing large numbers of animals to prove freedom. In this respect, NSP tests used after vaccination have reduced specificity if samples are collected from animals given impure vaccines (i.e. not all of the NSP removed during manufacture) or from animals that have been vaccinated many times (boosts up antibody to residual NSP in the vaccine). In an Australian emergency, these problems should be mitigated by use of high quality purified vaccines given as a single shot. As with virus detection tests, a combination of assays may be used to increase Se or more frequently Sp.

References

OIE Manual of Diagnostic Tests and Vaccines.
http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.05_FMD.pdf

OIE Terrestrial Animal Health Code. http://www.oie.int/international-standard-setting/terrestrial-code/access-online/

Based on David Paton's answer during FEPC_AU1.