Foot-and-mouth disease virus detection based on a diagnostic method or an integrated quality controlled strategy

Kris De Clercq
CODA-VAR, Section Development of Diagnostic Tools for epizootic diseases, Ukkel, Belgium


Virus isolation

Virus isolation (VI) remains the ultimate proof of the presence of live FMDV. Primary cells like bovine thyroid cells (Snowdon, 1966) or lamb kidney cells (House and House, 1989) are very sensitive but laborious to maintain. The quality can differ with each cell batch and requires therefore a well established quality assurance/quality control (QA/QC) system to guarantee a 24/24h availability of cells with the same sensitivity. Cell lines are easier to cultivate. Mostly pig cell lines are used such as IB-RS-2, PK15 or SK6 or a baby hamster kidney cell line (BHK-21). The cell line used, must be sensitive enough to isolate FMDV from samples coming from different species. Pig cell lines were not always suitable for isolation of FMDV coming from goats or sheep excreting sometimes very low amounts of virus (Bouma et al., 2001). The BHK-21T cell line seems to be less species dependant (Ahl, 2000).

Well established low positive controls are essential within a QA/QC system to proof that each cell batch is highly sensitive. These low positive controls must be calibrated to international accepted standards which are unfortunately not available. An international ring test is urgently needed for virus detection. This would increase the confidence and allow mutual recognition of results. It would also fill in a big gap in the framework of accreditation.

A small proportion of samples may give negative results for infectivity but positive results by ELISA or RT-PCR (Alexandersen et al., 2003). Exact figures for sensitivity and specificity can hardly or not be found, although years of experience with VI. However, these figures are essential to determine the positive predictive value of the test result, should be use to calculate the sample rate in surveillance campaigns and brought into account for calculating the true prevalence of FMDV.

Complement fixation test

The CFT has been of great value in the past in many FMD laboratories. The CFT is serotype dependant and requires a good practical knowledge of anti-complement reactions. Near Europe it is still used nowadays in some Transcaucasian countries and e.g. in regional laboratories in Iran (Mahmoudan et al., 2003). CFT could be replaced by the more sensitive antigen-ELISA (Roeder and LeBlanc Smith, 1987) but the availability of an ELISA reader,
ELISA plates and reagents are essential factors. The necessity of using a more sensitive test depends on the phase of the FMD control campaign.

**Antigen detection**

*The antigen (Ag)-ELISA* as described by Ferris and Dawson (1988) will identify the serotype of the FMDV present. In some laboratories however the test is limited to some serotypes (classical A/O/C and sometimes Asia1). A pan-serotype antibody would be very useful.

As for all tests the quality of the result depends in the first place on the quality of the sample. Although the sensitivity of the test is lower than VI, the success rate is high for samples from cattle and pigs. For sheep the percentage of samples directly yielding a positive ELISA result is lower (Alexandersen et al., 2003). A sample with a negative ELISA result requires further examination in highly susceptible cell cultures.

Each ELISA plate should contain the necessary low positive controls. As for VI there is a lack of international standards and the same remarks accounts. Also for Ag-ELISA it is difficult to find figures concerning the test performance. As for many tests the validation stopped with determining the analytical sensitivity (until which dilution the virus can be found) and specificity (cross reactivity). Although more and more laboratories claim to work within an accreditation system, hardly anything is known about the number of false positives and negatives based on a reliable number of samples. It must be stressed that the require numbers of positives needed for validation is very difficult to get for diagnostics dealing with exotic diseases.

To check the presence of FMDV in sheep flocks or in vaccinated cattle with low excretion of virus the number of samples must be relatively high to be statistical meaningful. Probang sampling is highly unpractical on large scale. Mouth or nose swabs are an alternative (Callens et al., 1998) and could provide a large number of samples (which in turn could be the bottle neck). A high throughput test such as ELISA is then acquired but the sensitivity of the ELISA should be improved as the amount of virus in those swabs will be too low. This increase can be obtained by using rolling circle amplification (RCA) ELISA (Schweitzer et al., 2000).

*A pen-side test* is in fact based on the same reaction between antibody-antigen and often revealed by chromatography technology and maybe in future by biosensors. For antibody detection a pen-side test was already brought on the market before the UK outbreak in 2001 by Genesis Diagnostics. Several companies and the WRL (Reid et al., 2001) are working on this kind of tests. The latter was based on a pan-serotype monoclonal antibody for capture of the antigen.

Pen-side tests do not acquire a well organized and safe sample transport, have the advantage of the short time before a result is known and can easily be repeated. They must be very robust and therefore a full validation is absolutely essential.

Pen-side tests bring in the problem of the user and the interpretation of the results. Will all potential users be aware of the nature of the samples to be taken and how? What about a negative result: case closed? What about a positive result: will all users declare their results or will the animal transport activity the night before an outbreak (high risk period 1 – Crauwels et al., 2001) increase even more than is already the case now. If pens-side tests are used by
accredited people: will permanent training be assured even in absence of an FMD outbreak for many years? How will continuous validation be organized?

*The Ab Microarray* is designed to be used as a screening tool. It is a clear high-throughput bench method. A wide range of different Mabs is immobilized on glass surface. In the field of screening proteomics the technique already exist to immobilize over 500 Mabs on a surface of 1 cm². The result is read by a fluorescent image reader. The most difficult point in the microarray platform is the data analysis. Often software is needed for the interpretation of the result. A full validation of the test and the data analysis is of the utmost importance. For FMD virus-screening, an Ab microarray using Mabs developed for Ag profiling and reacting each a little bit different and covering a very wide range of FMDVs could be developed.

**Nucleic acid recognition methods**

*Polymerase chain reaction (PCR).*

During the recent (2003) avian influenza outbreaks in Belgium the presence of the virus was confirmed and an outbreak declared on the basis of a PCR result only. Also during the classical swine fever epidemic in the UK in 2000 PCR declaration of outbreaks was done on the basis of RT-PCR results (D. Paton, personal communication, 2002). In the field of FMD the discussion is still going on whether a positive RT-PCR really means that live virus is present. Thanks to this discussion the possibility exists for a country of not declaring an outbreak and meanwhile clearing out the possible dangerous suspect.

Various procedures are developed: conventional RT-PCR (Rodriguez et al, 1992), RT-PCR ELISA (Callens et al., 1998), nested RT-PCR (Moss and Haas, 1999), real time RT-PCR (Reid et al., 2002). Some developed methods could distinguish among the serotypes (Vangrysperre and De Clercq, 1996; Callens and De Clercq, 1997; Reid et al, 1998). These tests are at least as sensitive as VI and clearly faster. But what is the added value if the result is only taking into account when it is complemented by VI. Of course also here we must admit that there is not one method for which the number of false positives and negatives is described. Reid et al. (2002) claim that the tests could be used to avoid a second passage for secondary herds. To use RT-PCR as high throughput expensive automates are necessary (Reid et al., 2003) and even then ‘only’ 90 samples a day could be managed which is too little for a statistical virus screening.

A portable PCR is developed as a kind of pen-side test (Hearps et al., 2002). The same remarks made for the pen-side antigen detection could be made here.

The question of including valuable controls is very relevant. Some teams use plasmids, others synthetic cDNA or RNA. The latter is the only one covering the whole chain from RNA isolation till PCR product. International agreement and standardization would be very welcome.

**DNA microarrays**

DNA microarrays are microscopic glass slides on which cDNA fragments are spotted. The cDNA fragments can hybridize with viral genome if present in the sample. It has been developed for subtyping of influenza virus (Li et al., 2001). These results open interesting
perspectives in the generalization of the use of this high throughput technology of microarrays to the study of FMDV epidemiology.

**Integrated quality controlled strategy**

All above mentioned diagnostics are often evaluated as a pure laboratory stand alone method. Seldom FMDV detection is seen as a complex of different elements in which each step is important. The weakest element in the chain determines the strength of the chain. Whether new diagnostic methods brings in an added value or not depends on what exactly we want to achieve and if that new method fill in the gap. One of the main gaps is full validation of tests with determining the test performance and an accompanying continuous QA/QC system. Although very important for reliability and sampling strategies it is rarely studied. The majority of institutes performing FMDV diagnosis are research institutes. Research money is not available for validation activities and the research boards of these institutes do not consider these activities as high level research. Researcher themselves find it often boring and certainly not innovating. It demands a lot of work before it can be published and is often not accepted by high qualified scientific journals. Money must be made available for validation studies.

FMD diagnosis should be seen as a complete system (contingency plan) that has to be managed very well. First the strategy has to be defined with the goals on short and long terms, with the weaknesses and the strong elements. It should be clearly defined where investments are needed to obtain the goals and which elements can be left out. Some new developments are nice to have but are not essential to the strategy. Once the strategy is clearly defined it should be applied but must be continuously checked. A quality control of the strategy must be implemented. When elements are out of line actions must be taken and if necessary the strategy adapted.

**References**


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