VIRUS ISOLATION

1. PURPOSE AND SCOPE

This test is used as a first-line test during clinical suspicions of foot-and-mouth disease for the isolation of FMDV.

2. NORMATIVE REFERENCES

Official analytical method of the World Organisation for Animal Health (WOAH), described in Chapter 3.1.8. of the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 2022.

3. DEFINITIONS

Matrix: epithelial homogenate supernatant from lesions, vesicular fluid, oropharyngeal samples, organ homogenate

Analyte: infectious foot-and-mouth disease virus.

Abbreviations:
- FBS: foetal bovine serum
- Ab: antibiotic
- NC: negative control for virus isolation

4. PRINCIPLE OF THE METHOD

The test relies on the *in vitro* detection of the infectivity of foot-and-mouth disease virus through the observation of a cytopathic effect (CPE) following the inoculation of susceptible cells.
If there is a clinical suspicion of foot-and-mouth disease in pigs, a differential diagnosis with swine vesicular disease is undertaken directly using two different cell types.

If a CPE is observed, the culture supernatant is collected and additional tests should be carried out to confirm FMDV.

5. EQUIPMENT AND MATERIALS

- Class II microbiological safety cabinet (MSC): the use of live FMD virus makes it necessary to work in a high containment laboratory complying with the EuFMD standards
- Refrigerated chambers: -20°C (± 5°C) and +5°C (± 3°C)
- Thermostatic chambers: + 37°C (± 2°C)
- Refrigerated centrifuge
- Water bath at +37°C (optional)
- Inverted microscope
- Microplate shaker
- Weighing scale (± 0.1 g)
- (Sterile) small standard laboratory equipment: Erlenmeyer flasks with caps, beakers, graduated cylinders, tubes with caps, Eppendorf tubes, cryotubes, pipettes, etc.
- Cell-culture dishes or microplates
- Single-channel and multi-channel micropipettes, suited to the various volumes to be collected
- Sterilised tips suited to the micropipettes used
- Vortex vibration shaker
- Timer

6. REAGENTS AND PRODUCTS

6.1. LIST

- Susceptible cells; IB-RS-2 (porcine kidney) and ZZ-R-127 (goat tongue) cell lines. Cell cultures are prepared in 24-well plates and used at 80-100% confluence
  Note: Unlike IB-RS-2 cells, ZZ-R-127 cells are not susceptible to swine vesicular disease virus
- MEM, IMDM, DMEM-F-12 culture media
- HEPES
- Lactalbumin hydrolysate
- Antibiotic mixture (Invitrogen) (components of the mixture = Penicillin G Sodium 10,000 units per ml and Streptomycin 10,000 µg per ml)

6.2. PREPARATION OF THE COMPLETE CULTURE MEDIUM

It is a cell culture medium supplemented with antibiotics used for the preparation of samples and the recovery of cell monolayers following viral inoculation:

- For the preparation of samples: MEM + 2% Ab
- For IB-RS-2: MEM + 1% Ab + 2.5% HEPES + 1.5% Lactalbumin hydrolysate
- For ZZ-R-127: IMDM / DMEM-F-12 (vol/vol) + 1% Ab

The media are stored at 5°C (± 3°C) and brought to ambient temperature before use.

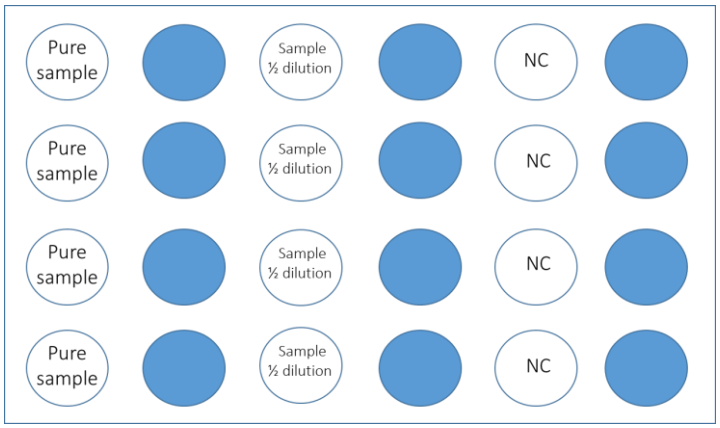
7. PREPARATION OF SAMPLES

The preparation of samples is described in Document "**Sample preparation"**.

8. PROCEDURE

8.1. INOCULATION AND INCUBATION

For each sample to be processed, use one 24-well plate for ZZ-R-127 and one 24-well plate for IB-RS-2.
At least 1200 µl of homogenate supernatant are necessary per sample. If the volume of homogenate is not sufficient, adapt the number of wells and the dilutions (maximum 1:10) to be inoculated.
Prepare a 1:2 dilution of the homogenate supernatant (400 µl of pure homogenate + 400 µl of MEM + 2% Ab).
Empty the cell culture medium from the wells and wash the cells once with culture medium, heated to +37°C (± 2°C).
Empty the rinsing medium.
Add 100 µl of homogenate supernatant, either pure or diluted to each well according to the following plate sheet. For NC (cell control) add 100µl of MEM + 2% Ab for the NC. Identify the plates: "*Samp X - P0 - cell line - date - time*".



Incubate the plates for one hour at 37°C (± 2°C) in the incubator.
Inoculum can be collected and stored at < -70°C. Add 500 µl of complete cell culture medium (without FBS) per well

- For ZZ-R-127 = DMEM-F-12 / IMDM (vol/vol) + 1% Ab
- For IB-RS-2 = MEM +1% Ab +2.5% HEPES + 1.5% Lactalbumin hydrolysate

Incubate the plates at 37°C (± 2°C) in the incubator and regularely observe the cell monolayers for the development of a potential CPE, up to 48 hpi.

8.2. OBSERVATION OF THE CELL MONOLAYERS

Observation for 48 hpi	Interpretation	Next steps to be taken
IB-RS-2 CPE++ ZZ CPE++	High suspicion of FMD	RNA extraction (RT-qPCR) Ag ELISA
IB-RS-2 CPE++ ZZ no CPE	High suspicion of SVD	RNA extraction (RT-qPCR) Ag ELISA
IB-RS-2 no CPE ZZ CPE++	Possibility of FMD	RNA extraction (RT-qPCR) Ag ELISA
IB-RS-2 no CPE ZZ no CPE	Possibility of non-infection	Perform a 2nd passage (P0+1)

8.2.1. If a CPE develops

Collect the supernatants, pool them, and freeze them at < -70°C (for at least 30 minutes). After thawing, centrifuge at 5000 xg for five minutes, at +5°C (± 3°C), and collect the supernatant in a sterile tube.

This supernatant can be used as a matrix for virological analyses (Ag ELISA, viral production in flasks if necessary) and for molecular analyses (RT-PCR and RT-qPCR).

Store at +5°C (± 3°C) if used on the same day (otherwise at < -70°C).

8.2.2. If no CPE develops within 48 hours

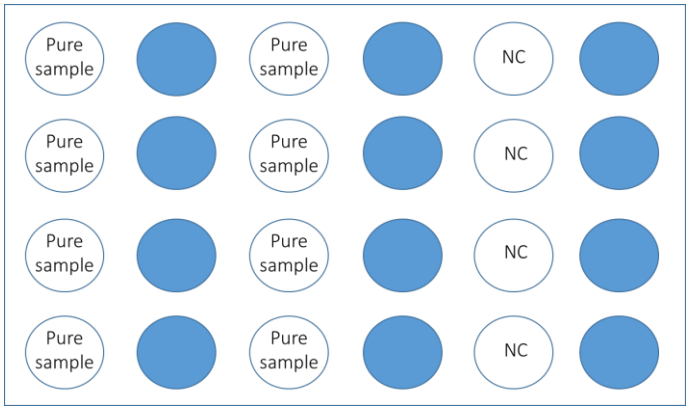
For each cell type (ZZ-R-127 and IB-RS-2) a second passage is necessary. Use the most concentrated inoculated dilutions (if there are not enough, use the diluted supernatants).

To do so, freeze the 24-well plates at < -70°C (for at least 30 minutes). After thawing, collect the content of the wells concerned, pool them (by cell type and to have the same sample dilutions), and centrifuge at 5000 xg for five minutes at +5°C (± 3°C). Collect the supernatant in a sterile tube.

Use one 24-well plate for ZZ-R-127 and one 24-well plate for IB-RS-2. Empty the cell culture medium from the wells and wash the cells once with culture medium, maintained at ambient temperature or heated to +37°C (± 2°C).

Empty the rinsing medium. Add 100 µl of P0 supernatant per well according to the following plate sheet.

Identify the plates: "*Samp X -**P0+1** - cell line - date - time*".



Incubate the 24-well plates for one hour at 37°C (± 2°C) in the incubator.

Add 500 µl of complete cell culture medium (without FBS) per well

- For ZZ-R-127 = DMEM-F-12 / IMDM (vol/vol) + 1% Ab
- For IB-RS-2 = MEM +1% Ab +2.5% HEPES + 1.5% Lactalbumin hydrolysate

Incubate the 24-well plates at 37°C (± 2°C) in the incubator and regularely observe the cell monolayers for the development of a potential CPE, up to 48 hpi.

If a CPE develops, proceed as described above (Section 8.2.1).

If no CPE develops after this second passage, virus isolation is considered negative.

9. EXPRESSION OF RESULTS

9.1. READING

- Principle: cells are observed under inverted microscope.
- Observe the negative control (NC). The cells should be in good condition and should not be lysed.

9.2. EXPRESSION OF RESULTS

For each cell type:

- If a CPE is observed during the 1st or 2nd passage: **positive virus isolation**
- If no CPE is observed after the 2nd passage: **negative virus isolation**

Note: The presence of foot-and-mouth disease virus can only be confirmed after the virus or one of its components are detected by a different method (Ag ELISA, RT-PCR).