Diagnostic and Sampling procedures for FMD
Diagnostic windows

1. Rapid confirmation of clinical signs
2. Active surveillance for infected animals (including pre-clinical cases)
3. Sero-surveillance for FMDV exposed animals

- FMD virus in blood
- Clinical lesions
- Antibody response

Representative “in contact” cattle data from Alexandersen et al., 2003 and unpublished data from IAH
Laboratory diagnosis of FMD

Confirms clinical diagnosis

Supports but does not replace the need for accurate clinical diagnosis

The quality of the laboratory diagnosis depends on the selection and quality of the samples submitted

Requires full epidemiological information on samples submitted for rational interpretation
Principals of FMD Diagnosis

Uninfected

Live Virus by virus isolation in cell cultures
1-4 days

FMD Virus infected

Lesion, swab, probang or clotted blood samples
Viral Proteins by LFD or double antibody sandwich ELISA
10-30 minutes (LFD)
4 hours (ELISA)
Viral Nucleic Acid by RT-PCR
Within a day

Recovered (or vaccinated)

Clotted blood samples (saliva)
Anti-viral antibodies can be detected
5-18 hours (ELISA)
2-3 days (VNT)

Probang samples

Clotted blood samples

Anti-FMD antibodies can be detected in serum by ELISA or VNT
FMDV diagnosis: Window of detection by different techniques/tissues

clinical signs of FMD

1 2 3 4 5 6 7 8 9 10 11 12 14 15

Cell culture / Ag ELISA
- Epithelium
- Serum
- Milk

RT-PCR
- Epithelium
- Serum
- Milk

ELISA Structural

ELISA Non structural

VNT
Objectives of the Clinical investigation

• to confirm the presence of clinical signs of FMD
• to collect suitable samples to confirm FMD infection has occurred
  – search for fresh/most recent cases, less than 6 days age!
• to identify the oldest lesions in the unit, to estimate the timing of entry of infection
  – search for the oldest lesions! use serology if animals recovered and lesions healed
Sampling from lesions

- Lesion material is the richest source of FMDV and the sample of choice for diagnosis.
- Samples from ~ 4 animals with obvious (and early) lesions should be sufficient to confirm a diagnosis.
- The most suitable materials are:
  - Vesicular epithelium, vesicular fluid, heart muscle from myocarditis cases.
  - For tissues:
    - At least 2 cm\(^2\) of epithelium from unruptured or freshly ruptured vesicles.
    - Transport medium - equal amounts of glycerine and 0.04 M phosphate buffer pH 7.2-7.6.
  - For vesicular fluids:
    - Plain, small volume tube.
Virological Samples

- **Urgent**
  - send as soon as possible, by most direct route
  - always give advance warning to lab and ETA
  - correct external package label to identify urgency
  - do not package together with other samples of less urgency
- **Hazardous (unless inactivated – for PCR)**
  - package and label properly (UN/IATA dangerous goods standards)
- **Fragile**
  - keep cool but not frozen, except by prior arrangement, if long delay
  - avoid extremes of pH therefore use buffered media
- **Adequate quantities**
- **Separate tube for each animal**
- **Correct forms**
Sampling in absence of lesions – incubation period or recovered

- Incubation period:
  - For diagnosis select ~ 6 animals, prioritizing those with suspicious clinical signs
    - Fever, depression, lameness, hot feet

- Collect clotted blood samples to obtain serum to detect viraemia

- Recovery period:
  - Clotted blood samples for antibodies
  - Oronasal swabs and/or probangs may be of value, but need to take account of laboratory capacity for processing (VI and PCR based methods)
Time needed for current assays for FMDV detection

- **Virus isolation (CTY or IBRS2)**: 1-4 days
- **Ag ELISA**: ~4 hours
- **Automated TaqMan® RT-PCR**: ~5 hours
Probang samples

- Aliquot 2-3 ml 0.08 M PBS with 0.01% BSA, 0.002% phenol red and antibiotics, adjusted to pH 7.2 per animal to be sampled.
- Cattle: pour probang sample from the cup into a wide-necked bottle & examine visually for quality. Add 2ml, including visible cellular material, to equal volume of buffer and mix. The final pH of a normal sample should be ~pH 7.6.
- Sheep: insert probang cup directly into a disposable container into which has been dispensed 3 ml of buffer solution and gently mix
- Samples taken from some animals may be heavily contaminated with ruminal contents - these should be discarded and the animal's mouth should be flushed with water before repeat sampling

Different sizes:
Sheep
Calf
Cow

3 bucket “system”
- Water
- 4% Na₂CO₃ or 0.2% citric acid
- Water
WRL procedure for FMDV antigen detection

Original epithelium/vesicular fluid

- Virus isolation in tissue culture
  - 48 hours
  - First blind passage
    - 48 hours
    - Second blind passage

- Antigen detection ELISA
  - Confirmation
    - No Virus Detected
      - FMDV detected & serotyped
‘Pen-side’ test for antigen detection

Lateral Flow Device (LFD)

- Not serotype specific
- Based on technology used in pregnancy test kits
- Similar sensitivity to Ag-ELISA
- High specificity
- Used to test epithelium or vesicular fluid
- Result within minutes
- Used on-farm in UK 2007
- Used in regional lab in Turkey in 2009
- Relatively low cost per test
Antigen detection ELISA

- Increased sensitivity over the Complement fixation test
- Similar sensitivity to LFD but can serotype (A, O, ...)
- Often used after virus isolation as cell culture amplifies virus and enables detection/typing
- Takes 8h but with initial VI prolonged to 4 days
FMDV molecular diagnostics

**Multiplex RT-PCR**
- Very sensitive
- Simple
- Takes 4h
- Ability to serotype

**Automated RT-PCR**
- 2 pan-serotype assays in routine use
- Automated RNA extraction
- 84 samples ~5 hours
- Highest demand: 311 samples/day
Rapid detection of FMDV in the field: Portable PCR platform

- Non-specialist user
  - Nucleic acid extraction
  - PCR set-up
  - Analysis
- 5 independent modules
- Battery operated
- Decontaminate by immersion
- Field trial (Turkey)
- Platform for other livestock diseases

Smiths Bio-Seeq™
Serology

- Clotted blood – one tube per animal
- Do not need refrigeration unless delayed/ very hot weather
- Separate forms and packaging from virological samples
Tests for antibodies to structural proteins of FMDV (SP tests)

- Detect antibodies to the virus capsid or shell
- SP antibodies are induced by both infection and vaccination
  - But usually stronger and more long-lasting antibody response to infection
- Relatively serotype specific
- SP antibodies appear around 5 days after infection and usually within 2-3 days of appearance of lesions
• Include
  – Virus Neutralisation Tests (VNT)
  – Various ELISAs
    • Solid Phase Competition ELISA
    • Liquid Phase Blocking ELISA
    • Ceditest FMDV type O (Prionics)
    • Isotype-specific tests for IgM and IgA
Tests for antibodies to non-structural proteins of FMDV (NSP tests)

- Detect antibodies to the non-structural proteins of FMDV involved in virus replication, e.g. 3ABC
- NSP antibodies are induced by infection but not by immunisation with purified vaccines
  - Multiple vaccination increases the likelihood of inducing NSP antibodies
- Pan serotype specific
- Several commercial ELISA test kits, some of which are species-specific and some work for all species
  - Ceditest FMDV-NS (Prionics)
  - Bommeli
  - SVANOVIR™ FMDV 3ABC-Ab ELISA, Svanova Biotech AB
  - UBI
- NSP antibodies appear around 7 days after infection and usually within 3-4 days of appearance of lesions
- NSP response may be reduced and delayed in case of subclinical or mild clinical infection following vaccination
**NSP antibody detection - ELISA**

- Commercial kit based systems; several options are available
- Detect Ab to NSPs of FMDV
- The best option for discrimination infection from vaccination
- Mainly used for serosurveillance activities, but also possible used for ag detection indirect
- Simple and practical
- Takes 4h or 5h (2days)

Naci BULUT, 01 June 2009
Sampling in Erzurum

- Epithelium samples: gly-iso buffer
- Vesicular fluid: collect using a syringe and needle. No buffer.
- Blood: whole blood in EDTA (or Trizol – for PCR), clotted blood in plain vacutainer
- Probang samples: buffer
- Saliva: saliva kit

Erzurum lab
- NSP test
- Antigen ELISA – serotype A, O, Asia 1
- Lateral flow device

Central (SAP)
- SP (LPB-ELISA)
- Virus isolation
- Multiplex PCR
- Real-time PCR
- Sequencing