



**ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DELLA LOMBARDIA E DELL'EMILIA ROMAGNA**

Via Bianchi 9 - 25124 Brescia

CENTRO NAZIONALE DI REFERENZA PER LE MALATTIE VESCICOLARI (CERVES)



WOAH/ FAO REFERENCE LABORATORY FOR FOOT-AND-MOUTH DISEASE



LABORATORY TRAINING ON FMD DIAGNOSIS

VENUE: IZSLER, Brescia, 10 working days

AGENDA

Day 1: Introduction and theory - FMDV Antigen detection and serotyping ELISA

- Welcome address
- Introduction of trainees
- Invitation of participants to present their Laboratory capacity
- Presentation of the work programme
- Lecture: Overview of epidemiology and Laboratory Diagnosis of FMD

Introduction to testing for Day 2

- Concepts for the differentiation between FMDV vaccinated and infected animals (DIVA tests)
- DIVA tests available for FMD and summary of performances
- Principle and SOP of the IZSLER 3ABC-trapping ELISA
- Principle and SOP of the ID Vet NS-ELISA

Day 2: FMDV NSP-ELISAs for anti-NSP Antibody detection

Wet work

- Execution of 3ABC-trapping ELISA by each participant: testing of a serum panel
- Execution of ID Vet NS-ELISA by each participant: testing of a serum panel
- Interpretation and joint discussion of results

Introduction to testing for Day 3

- Principle of the Solid Phase Competitive ELISA (SPCE) for the detection and serotyping of Antibodies to FMDV Structural Proteins
- Examples of SP-serology applications and interpretation
- Test procedure according to the IZSLER kit

Day 3 and 4: Detection of Antibodies to FMDV Structural Proteins (SP) by Solid Phase Competitive ELISA (SPCE)

Wet work

- Execution of the test using ready-to-use kits (each participant): testing of a serum panel for Antibodies against FMDV types O, A, Asia 1 (screening and titration)
- Explanation of basic concepts for expression of results (screening or end-point titration) and relevant calculations

- Joint discussion of results and interpretation of the FMDV status, also in relation to results of NSP-ELISAs

Introduction to testing for Day 5

- Principle and SOP of the sandwich ELISA for FMDV detection and typing
- Development and validation of the IZSLER monoclonal antibodies based ELISA
- Principle of the test: Lateral Flow Chromatography
- Data available on LFD validation
- Suggested use of penside tests in FMD diagnosis: advantages and limits

Day 5: Penside test for FMD diagnosis and Ag detection ELISA

Wet work

- Sample homogenisation using the FMDV-Ag extraction kit
- Parallel testing of the same samples by FMDV Antigen detection ELISA and LFD
- Interpretation and joint discussion of results

Lecture: Concepts of Biosecurity for FMD Laboratory

- Presentation of requisites and videos of the high containment laboratories dedicated to work with infectious FMDV

Introduction to testing for Day 6

- Principle and SOP of the Virus Neutralization Test (VNT)
- Introduction to FMDV-specific biomolecular tests

Day 6: Work in the high containment laboratory BSL3

- Demonstration of VNT
- VNT reading of Virus titration type O and A

Day 7: Work in the high containment laboratory BSL3+

- Demonstration of FMDV RNA extraction by affinity columns and FMDV pan Real-time RT-PCR based on 3D gene

Day 8: Work in the high containment laboratory BSL3+

- Execution of FMDV RNA extraction and Real-time RT-PCR (2 trainees)
 - VNT reading and interpretation (from Day 6)
- Guided visit to the structures of the BSL3+ labs

Day 9: Work in the high containment laboratory BSL3+

- Execution of FMDV RNA extraction and Real-time RT-PCR (2 trainees)
- Solid Phase Competitive ELISA (SPCE) type O and A on samples tested by VNT on Wednesday ((2 trainees)
- Discussion of the results obtained from the Real-time RT-PCR
- Introduction to FMDV sequencing and phylogenetic analysis
- Visit of IZSLER Virology Unit

Day 10: Take-home-messages

- Round table, General discussion, suggestions/inputs by participants