



EU Reference Laboratory for ASF  
Animal Health Research Centre  
(CISA), INIA  
Ctra Algete-El Casar s/n  
28130, Valdeolmos, Spain



# Training on Diagnosis on African Swine fever (ASF)

Organized by the EU reference laboratory for African swine fever (ASF)

Centro de Investigación en Sanidad Animal  
INIA-CISA/CSIC, Valdeolmos, Madrid, España



## Contact people:

Dr. Carmina Gallardo: [gallardo@inia.csic.es](mailto:gallardo@inia.csic.es)

Raquel Nieto: [nieto.raquel@inia.csic.es](mailto:nieto.raquel@inia.csic.es)

## Venue:

EU Reference Laboratory for African Swine Fever  
Centro de Investigación en Sanidad Animal  
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA-CSIC)  
Valdeolmos, 28130, Madrid, SPAIN.  
Telf.: +34 916202300



## Day I.

**Time Schedule: 9:00 -16:15**

- 9.00- 9.30** Welcome, organizational details and opening of the ASF training course by the Laboratory Coordinator of the EURL for ASF.
- 9.30- 11:00** Lectures focusing on the latest updates regarding ASF and advancements in diagnostic techniques.
- ❖ **Lecture I:** African swine fever (ASF): a general overview.
  - ❖ **Lecture II:** African swine fever (ASF) diagnostic techniques; key points.
- 11.00– 16.00** **LABORATORY PRACTICES AT THE INIA-CISA/CSIC, BSL-3 Laboratory Facilities. Simulation exercises on Laboratory Diagnosis.** Coordinated by Dr. Carmina Gallardo and Ms. Raquel Nieto.

For the execution of the laboratory practices, the participants will be divided into different Working Groups (WGs), with each group receiving a panel of blind samples. These samples will be used for conducting ASF serological and virological diagnosis, considering different epidemiological situations of ASF.

**Laboratory practice I: Sample distribution and preparation.** Each WG will propose a diagnosis workflow to be done on the samples received according each epidemiological situation.

- **Target samples.**
- **Sample preparation.**
- **Storage of clinical material.**

**Laboratory practice II: ASFV genome detection by real time PCR**

- **Preparation of PCR controls for the extraction**→ Preparation of the adequate dilution for the extraction control
- **DNA extraction method.**

**Location:** CISA-INIA, BSL-3 Laboratory Facilities

## Day II

**Time Schedule: 9:00 -16:15**

**Laboratory practice III: ASFV genome detection by real time PCR.**

- **Preparation of PCR controls for the amplification**→ Preparation of the adequate dilution for the amplification control.
- **Real time PCR set up.**
- **Interpretation of the results.**

**Laboratory practice IV: Virus isolation\***

- **Haemadsorption (HAD) test in porcine blood monocytes (PBMs)** → Extraction and culture procedure of peripheral blood monocytes from donor domestic pig.
- **Inoculation** with the ASFV PCR positive samples.

\* The plates will be read every day for checking the presence of cytopathic effect (CPE) and haemadsorption (HAD).



**Location:** CISA-INIA, BSL-3 Laboratory Facilities

### Day III

**Time Schedule:** 9:00 -16:15

#### Laboratory practice V: Antibody detection by ELISA test

- **Target samples for ELISA test.**
- **Blocking ELISA:** commercial INGENASA (INGEZIM PPA COMPAC K3) ELISA set up/ analysis of the results

#### Laboratory practice VI: Antibody confirmatory test: Immunoblotting (IB)

- **Target samples for IB test.**
- **IB set up/ analysis of the results**

**Location:** CISA-INIA, BSL-3 Laboratory Facilities

### Day IV

**Time Schedule:** 9:00 -16:15

#### Laboratory practice VI: Antibody detection by Indirect Immunoperoxidase test (IPT)

- **Target samples for IPT test.**
- **IPT set up/ analysis of the results**

**Location:** CISA-INIA, BSL-3 Laboratory Facilities

### Day V

**Time Schedule:** 9:00 -14:30

**Location:** CISA-INIA, BSL-3 Laboratory Facilities

**Closing session:** Quality issues; ASF hands-on diagnostic simulation results; Interpretation of the results; Discussion, Conclusions and Recommendations on ASF diagnosis.

**Location:** CISA-INIA, BSL-3 Laboratory Facilities