African Swine Fever (ASF) diagnosis and molecular characterization

PRELIMINARY REPORT ISSUED BY THE EUROPEAN UNION REFERENCE LABORATORY FOR AFRICAN SWINE FEVER (EURL-ASF), INIA-CISA

DATE: August 11th 2025

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RESPONSIBLE FOR SUBMISSION:

Name: Imbi Nurmoja
Intitution/Lab: National Centre for Laboratory Research and Risk Assessment (LABRIS)
Address: Fr. R. Kreutzwaldi 30, 51006 Tartu
Country: Estonia
Tel. number: : +372 738 6111
E-mail address: imbi.Nurmoja@labris.agri.ee

Num. ARRIVAL REGISTER CISA: REG. 57/2025

TEST REQUESTED: AFRICAN SWINE FEVER (ASF) CONFIRMATORY DIAGNOSIS

Num. SAMPLES RECEIVED: 14 samples, 7 from domestic pig and 7 from wild boar (see table 1).

Table 1 → Identification of the samples tested.

ID CISA		SAMPLE IDENTIFICATION RECEIVED FROM NRL			
ID Tube	Sample Type	Animal ID No.	Tube No.	Animal species	Sample Type
1	TISSUE	TA2514779-1	DP1	DOMESTIC PIG	ORGANS
2	TISSUE	TA2516154-1	DP2	DOMESTIC PIG	ORGANS
3	TISSUE	TA2516563-1	DP3	DOMESTIC PIG	ORGANS
4	TISSUE	TA2516633-7	DP4	DOMESTIC PIG	ORGANS
5	BLOOD	TA2516633-6	DP5	DOMESTIC PIG	SERUM
6	TISSUE	TA2516723	DP6	DOMESTIC PIG	ORGANS
7	TISSUE	TA2517123-2	DP7	DOMESTIC PIG	BONE MARROW
8	TISSUE	TA2515299	WB1	WILD BOAR	ORGANS
9	TISSUE	TA2515741	WB2	WILD BOAR	BONE MARROW
10	TISSUE	TA2516595	WB3	WILD BOAR	ORGANS
11	TISSUE	TA2516699	WB4	WILD BOAR	BONE MARROW
12	TISSUE	TA2516738	WB5	WILD BOAR	BONE MARROW
13	BLOOD	TA2517025	WB6	WILD BOAR	SERUM
14	BLOOD	TA2517114	WB7	WILD BOAR	SERUM

EXECUTION DATE: 11th August 2025

Ctra. de Algete a El Casar, s/n
28130 Valdeolmos
MADRID
TEL: 91 620 23 00

Email:
eurl.asf@inia.csic.es

ASF DIAGNOSTIC TESTS PERFORMED:

1. **ASF virological diagnosis:**

1.1. **ASFV genome detection:** 10% (W/v) clarified homogenized tissue´s suspensions have been prepared in phosphate-buffered saline (PBS) from 11 tissues [SOP/CISA/ASF/SAMPLES/1]. The DNA was extracted from **the sera samples** and the **tissue homogenates,** using the High Pure PCR Template Preparation Kit [Ref. 11796828001 (ROCHE)] following the standardised procedure [SOP/CISA/ASF/DNA EXTRACTION/1]. For amplification of the ASFV genomic DNA the UPL real-time PCR (Fernández-Pinero *et al*., 2013; WOAH 2021) [SOP/CISA/ASF/PCR/3] was carried out using undiluted DNAs, except for samples WB6 and WB7, which were tested at 1:10 dilution.

1.1. **ASF virus isolation and haemadsorption (HAD) assay*** [SOP/CISA/ASF/VI/1] in progress.

2. **ASF serological diagnosis** in progress.

3. ***ASFV molecular characterization*** in progress.

RESULTS

1. **ASF virus detection** → All samples received have been positive by UPL real-time PCR. The results obtained in virus detection are summarized in the **table 2.**

Table 2 → ASFV virus detection

ID CISA		SAMPLE IDENTIFICATION RECEIVED FROM NRL		UPL-real time PCR[(a)]	
ID Tube	Sample Type	Tube No.	Sample Type	1st PCR Ct value	Result
DP1	TISSUE	DP1	ORGANS	16.77	POSITIVE
DP2	TISSUE	DP2	ORGANS	18.47	POSITIVE
DP3	TISSUE	DP3	ORGANS	21.61	POSITIVE
DP4	TISSUE	DP4	ORGANS	19.31	POSITIVE
DP5	BLOOD	DP5	SERUM	27.89	POSITIVE
DP6	TISSUE	DP6	ORGANS	22.30	POSITIVE
DP7	TISSUE	DP7	BONE MARROW	25.98	POSITIVE
WB1	TISSUE	WB1	ORGANS	24.33	POSITIVE
WB2	TISSUE	WB2	BONE MARROW	18.21	POSITIVE
WB3	TISSUE	WB3	ORGANS	23.36	POSITIVE
WB4	TISSUE	WB4	BONE MARROW	24.51	POSITIVE
WB5	TISSUE	WB5	BONE MARROW	15.85	POSITIVE
WB6	BLOOD	WB6	SERUM	27.03	POSITIVE
WB7	BLOOD	WB7	SERUM	31.29	POSITIVE

Ctra. de Algete a El Casar, s/n
28130 Valdeolmos
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(a) ***UPL-real time PCR*** → *Real time PCR test described by Fernández et al., 2013 based on the Universal Probe Library (UPL) and described in the WOAH Manual of diagnosis for ASF (Chapter 3.9.1. WOAH edition 2021).*

(1) *Extracted at 1:10 dilution*

CONCLUSION

1. The **presence of ASFV has been confirmed** by ASFV genome detection in **all samples received from Estonia** from wild boar and domestic pigs.

Virus isolation, serological diagnosis, and molecular characterization is in progress.

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In Valdeolmos, Madrid (Spain) 11th August, 2025

ARIAS NEIRA M.LUISA - DNI 00683346Q

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Fecha: 2025.08.11 14:16:15 +02'00'

Marisa Arias
Technical Director
EU Reference Laboratory for ASF
CISA-INIA/CSIC

Ctra. de Algete a El Casar, s/n
28130 Valdeolmos
MADRID
TEL: 91 620 23 00

Email:
eurl.asf@inia.csic.es

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Ctra. de Algete a El Casar, s/n
28130 Valdeolmos
MADRID
TEL: 91 620 23 00
FAX: 91 620 22 47