



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



**European Union Reference
Laboratory for African Swine Fever
(ASF-EURL)**

Centro de Investigación en Sanidad Animal (CISA-INIA)

Ctra. de Algete a El Casar, s/n

28130 Valdeolmos (Madrid), Spain

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1. GENERAL INFORMATION

1.1. ORDERS FOR REAGENTS AND KITS

All orders must be sent to:

secsgit@inia.es
with copy to
Dr. Carmina Gallardo
(eurl.asf@inia.es; gallardo@inia.es)

ASF European Union Reference Laboratory, EURL-ASF
Centro de Investigación en Sanidad Animal (CISA-)
Ctra. de Algete a El Casar, s/n
28130 Valdeolmos (Madrid), Spain

The **conditions for order** are as follows:

It is necessary to identify all products using the catalogue description, specifying the quantity of each item required. For this item, you should send document of request: *“DESCRIPTION OF REQUEST”* or *“DESCRIPTION OF REQUEST (evaluation of ASF commercial diagnostic kits”* including:

- ⇒ The products to be supplied with the proposed specification.
- ⇒ Full name and address of the client for delivery. Including telephone contact and e-mail.
- ⇒ The billing address, with the fiscal identification number (VAT registration number). In case of countries without VAT number, please provide any other fiscal identity number in order to prepare the corresponding budget.
- ⇒ Transport costs are not included in the catalogue prices, and if necessary will be added in each budget.
- ⇒ Payment shall be made by the client immediately on receipt of the invoice, according it is described in point 1.2.

⇒ To proceed with the delivery:

- As regards European Member States (MS), it is required to receive by e-mail the form “**acceptance biological material shipments from CISA**” signed by the client.
- As regards of Non EU Members States Clients, the form “**acceptance biological material shipments from CISA**” must be accompanied with **all necessary import licences and approvals** or other consents (including import documents for products classified as live and infectious as may be required to enable the products to be imported into the client's country).

1.2. PAYMENT

The price payable by the client and described in the product list is the price prevailing at the date of despatch, in accordance with **Official State Gazette** (“Boletín Oficial del Estado”, Núm, 31 Lunes 6 de febrero de 2017 Sec. III”).

- I. The budget will be sent to the customer by e-mail. Once accepted (signed and stamped), it will be re-sent to us by ordinary mail to the following address:

secsgit@inia.es
with copy to
eurl.asf@inia.es

Besides, we will need the document **acceptance biological material shipments from CISA** signed and stamped too, and for non EU countries the pertinent import permit.

- II. Once we receive the accepted budget and the acceptance form (and the import permit, if is necessary), we will send the reagents and the invoice to the customer, including detailed instructions for payment.
- III. Bank data you will need to make the transfer to INIA are the following ones:

Account number: **0182-2370-46-0200203535**

IBAN code: **ES81-0182-2370-46-0200203535**

SWIFT code: **BBVAESMM**

Account holder: **Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)**

Bank address: **BBV-Argentaria. Paseo de Recoletos, 10. 28001 Madrid, ESPAÑA**

Payment should be made within 30 days of the invoice date. Please, include the invoice number as reference code in order to allow us to identify the income.

2. PRICE LIST: REAGENTS

Transport costs are not included in the catalogue prices, and will be added in each budget.

2.1. ASF REFERENCE REAGENTS

ASF REFERENCE REAGENTS FOR ASF-OIE-ELISA						
Code	Product Description	Price (€)	Presentation		Volume/ Quantity	Determinations*
			Frozen	Lyophilised		
ASF-CP	ASF POSITIVE REFERENCE SERUM	212.07		√	1.5 ml	16,000
		141.38		√	1 ml	8,000
ASF-CN	ASF NEGATIVE REFERENCE SERUM	73.04		√	1.5 ml	16,000
		48.69		√	1 ml	8,000
ASF-CL	ASF LIMIT REFERENCE SERUM	146.09		√	1.5 ml	16,000
		97.39		√	1 ml	8,000
ASFV-Ag	ASFV SEMIPURIFIED ANTIGEN	1,356.22		√	1 ml	16,000
		678.10		√	0.5 ml	8,000

ASF REFERENCE REAGENTS FOR ASF-OIE-IB						
Code	Product Description	Price (€)	Presentation		Volume/ Quantity	Determinations*
			Frozen	Lyophilised		
ASF-CP	ASF POSITIVE REFERENCE SERUM	141.38		√	1 ml	50
		70.69		√	0.5 ml	25
ASF-CN	ASF NEGATIVE REFERENCE SERUM	48.69		√	1 ml	50
		24.35		√	0.5 ml	25
ASF-IB	ASF IMMUNOBLOTTING STRIPS	5.71			1 strip	1

*The number of determinations is referred taking into consideration the working dilutions according are described in the ASF Chapter 3.8.1 of "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals", edition, 2019. World Organisation for Animal Health (OIE) [https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.08.01_ASF.pdf] and in the ASF-URL web page [<http://osf-referencelab.info/asf/en/procedures-diagnosis/sops>]

ASF REFERENCE REAGENTS FOR ASF URL-IPT					
Code	Product Description	Price (€)	Presentation		Volume/ Quantity
			Frozen	Lyophilised	
ASF-CP	ASF POSITIVE REFERENCE SERUM	70.69		√	0.5 ml
ASF-CL	ASF LIMIT REFERENCE SERUM	48.70		√	0.5 ml
ASF-CN	ASF NEGATIVE REFERENCE SERUM	24.35		√	0.5 ml
ASFV-REF _(E70MS-BA71VR)	ASFV ADAPTED VERO OR MS REFERENCE ISOLATE _(P72 GENOTYPE I)	79.59	√		1 ml
ASFV-IPT	COATED 96-WELL PLATES FIXED WITH ASFV ADAPTED ISOLATE _(P72 GENOTYPE I)	115.65	√		1 Plate

ASF REFERENCE REAGENTS FOR ASF VIRUS/GENOME DETECTION					
Code	Product Description	Price (€)	Presentation		Volume/ Quantity
			Frozen	Lyophilised	
ASF-ASFV ISOLATES	ASF REFERENCE VIRAL ISOLATES	79.59	√		1 ml
ASFV-DNAs	ASFV REFERENCE DNAs	51.50	√		100 µl

2.2. MATERIAL AND REAGENTS PROVIDED BY CISA-INIA FOR ASF DIAGNOSIS

CODE	ASF DIAGNOSTIC TEST	REAGENTS INCLUDED	DETERMINATIONS* (Nº)	PRICE (€)
ASF-OIE-ELISA	OIE-ELISA FOR ASF ANTIBODY DETECTION	<ul style="list-style-type: none"> ASF-Ag 0.5 ml ASF-CP 1 ml ASF-CL 1 ml ASF-CN 1 ml HRP labelled Protein A 1 mg ELISA plates 120 plates 	8,000	1,180.47 (0.14 € per determination)
ASF-OIE-IB	IMMUNOBLOTTING FOR ASF ANTIBODY DETECTION	<ul style="list-style-type: none"> ASF-IB (60 strips) ASF-CP 1 ml ASF-CL 1 ml ASF-CN 1 ml HRP labelled Protein A 1 mg 	50	635.49 (12.70 € per determination)
ASF-URL-IPT	INDIRECT IMMUNOPEROXIDASE TEST FOR ASF ANTIBODY DETECTION	<ul style="list-style-type: none"> ASFV- IPT 3 plates ASF-CP 25 µl ASF-CL 25 µl ASF-CN 25 µl HRP labelled Protein A 1 mg 	264	495.11 (1.87€ per determination)

*The number of determinations is referred taking into consideration the working dilutions according are described in the ASF Chapter 3.8.1 of "Manual of Diagnostic Tests and Vaccines for Terrestrial Animals", edition, 2019. World Organisation for Animal Health (OIE) [https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.08.01_ASF.pdf] and in the ASF-URL web page [<http://asf-referencelab.info/asf/en/procedures-diagnosis/sops>].

2.3. REFERENCE MATERIAL FOR VALIDATION AND INTERNAL VERIFICATION OF ASF DIAGNOSTIC TECHNIQUES

ASF REFERENCE PANEL OF SAMPLES					
Code	Product Description*	Price (€)	Presentation		Volume/ Units
			Frozen	Lyophilised	
ASF-Ref-1	SERUM samples for the evaluation, validation and internal verification of ASF ANTIBODY detection techniques	1,131.00		√	1 ml/10 units
ASF-Ref-2	TISSUE AND SERUM samples for the evaluation, validation and internal verification of ASF-PCR techniques.	1,140.45		√	1 ml/16 units
ASF-Ref-3	ASFV DNAs for the evaluation, validation and internal verification of ASF-PCR techniques	1,081.50		√	100 µl/21units

*All reference material has been *inactivated* by heat treatment at 56°C for 70 minutes followed by lyophilization.

3. AFRICAN SWINE FEVER: DIAGNOSTIC SERVICE

3.1. SUBMISSION OF SPECIMENS

To obtain the right diagnosis of a disease, it is essential to select the right samples and to send them to the laboratory under proper conditions. **It is not possible to make a good diagnosis if the samples are not in good conditions.**

3.1.1. Target samples for African swine fever

For ASF antibody detection using ELISA plus confirmatory techniques:

Sera: Draw whole blood collected using sterile tubes without anticoagulant.

Minimum amount recommended: 500 μ l.

For ASF virus detection using PCR and virus isolation:

Sera: Draw whole blood collected using sterile tubes without anticoagulant.

Minimum amount recommended: 1 ml.

Blood: Draw whole blood collected using sterile tubes with anticoagulant (EDTA). *Minimum amount recommended: 1 ml.*

Porcine organs without formalin such as spleen, lung, kidney, heart, tonsils, and lymph nodes (mesenteric, retropharyngeal, renal, gastro-hepatic, etc).
Minimum amount recommended: 5gr.

Organ samples should be obtained during the necropsy of the animal. Blood can be extracted via jugular vein puncture, the inferior vena cava, or the auricular veins (pigs), or during the necropsy.

3.1.2. Sampling materials required

- Containers with hermetically sellable lids, preferable plastic flask. This flask should be used to keep the organs.
- Sterile tubes: some without anticoagulant others with EDTA. Some should be vacutainers and others not.
- Syringes of 10-20 ml. Different sizes of needles that fit the syringe and appropriate for the size of the pig to be sampled and the sampling site.

- Knife, scalpel and scissors to take the organ samples.
- Hermetically sealable flasks for storage of organ sample containers and blood tubes from each animal. These flasks should be clearly labeled.
- Cooler with ice packs or insulated box.
- Dry ice (when frozen sample is required).
- Labels and waterproof markers.

3.1.3. Shipping conditions

Samples should arrive at **INIA-CISA as soon as possible** to avoid their deterioration and **safely** to avoid the possible infection of other animals or persons during transportation as well as to avoid contaminating the samples themselves. Ship specimens must be delivered with adequate amounts of ice packs to prevent deterioration. When delivery setup is delayed beyond 48 hours, samples should be **stored in liquid nitrogen or at -70°C.**

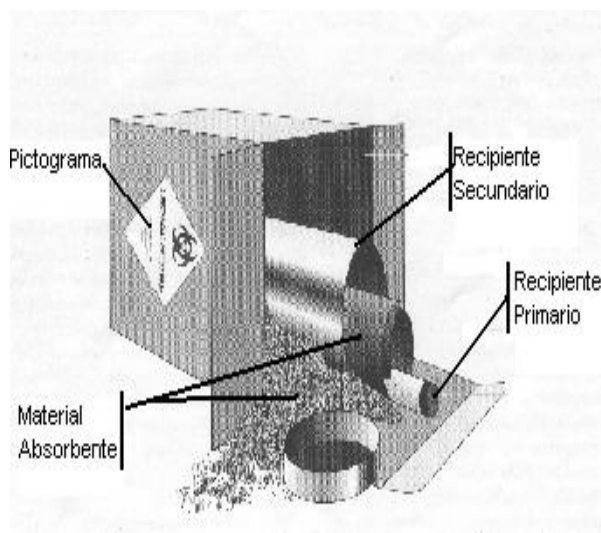
The specimens should be shipped in accordance with **IATA Packing Instruction 650 according is described below.**

Samples should be shipped in water resistant, sterile containers (these should be the primary containers). It is essential to clearly mark each container in order to be able to clearly identify the animals from which the sample has been taken. The lid of each container and the tampon of each tube should be sealed with adhesive tape or Parafilm.

These primary containers should be sealed and then packed individually in shock absorbent material. All of these containers should be placed in a second hermetically sealed water resistant container. The second container can be plastic or metal. Absorbent material should be placed in the interior of the second container to absorb any possible leakage from containers or tubes.

Note: Diagram labels: Upper left: Pictogram, Upper right: Secondary container

Lower Left: Absorbent Material, Lower Right: Primary Container



- Samples that must be shipped at 4 degrees Celsius: These samples should be packed as indicated above. They should be shipped with refrigerants (in sufficient quantity to maintain the desired temperature) within thermally insulated robust boxes. Preferably these boxes should be packed within other shock resistant boxes.
- Samples that must be shipped frozen (-20 degrees Celsius or -70 degrees Celsius): These materials should be packaged according to the directions above, adding enough dry ice to the thermally insulated box to maintain the temperature. It is important to ensure that the second container (described above) is secured within the centre of the box because as the dry ice decomposes the container can become loose and damaged. The carbon dioxide resulting from the decomposition of the dry ice lowers the pH and deactivates the virus; therefore it is absolutely necessary that all the containers be hermetically sealed. Never freeze whole blood or serum that contains coagulant.

3.1.4. Labelling

On the outside of the box the following identification should be placed:

1. Label reading 'biological material'.
2. Label reading 'infectious material for animals', if applicable.
3. Label reading 'dry ice' (if applicable).
4. Label reading 'conserve at 4 degrees Celsius' or 'conserve at -70 degrees Celsius'.
5. Full name and address of sender.
6. Full name and address of addressee.

Dr. Carmina Gallardo

**Centro de Investigación en Sanidad Animal (CISA-INIA)
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Fax: +34 91 620 22 47

SUBMISSION FORM MUST BE INCLUDED WITHIN THE PACKAGE OR SENT BY E-MAIL TO the EURL e-mail (eurl.asf@inia.es) with copy to Dr. Carmina Gallardo (gallardo@inia.es) and Dr. Marisa Arias (arias@inia.es).

The **ASF submission form** is available on the website <http://asf-referencelab.info/asf/> or requesting by email to the ASF reference laboratory department (eurl.asf@inia.es). If

the general submission form is not available, the specimens must be accompanied by a covering letter. This letter should include relevant information concerning the owner, name and district of the farm, animal(s) involved, history, symptoms of illness and postmortem lesions. Test(s) required must be indicated.

It is better to arrange the shipment with a “door to door” service courier that will ensure delivery directly to the Research Centre. The destination should be informed of the shipment by telephone or fax. It should have detailed information and approximate date and time of arrival. If material is sent by air, it should be given the flight number and the airway bill number.

3.2. ASF DIAGNOSTIC SERVICES

The laboratory offers an international diagnostic service for African swine fever, including the following tests:

ASF DIAGNOSIS	TARGET SAMPLES**	TEST DESCRIPTION*	AVERAGE TURNOVER TIME	PRICE(€)
ANTIBODY DETECTION	Serum samples (ELISA+IB+IPT)	<ul style="list-style-type: none"> ELISA test (PNT/CISA/PPA/ELISA/1) (PNT/CISA/PPA/ELISA/2) 	1 working day	15.34/ per determination
	Blood with EDTA and tissues (IPT)	<ul style="list-style-type: none"> Immunoblotting test (PNT/CISA/PPA/IB/1) 	1 working day	25.79 per determination
		<ul style="list-style-type: none"> Immunoperoxidase test (PNT/CISA/PPA/IPT/1) 	1 working day	21.58/ per determination
GENOME DETECTION	Serum samples/ Blood with EDTA/ Tissues	<ul style="list-style-type: none"> Conventional PCR (PNT/CISA/PPA/PCR/1) 	1 working day	29.45/ per determination
		<ul style="list-style-type: none"> Real time PCR (PNT/CISA/PPA/PCR/2) 	1 working day	58.43/ per determination
		<ul style="list-style-type: none"> (PNT/CISA/PPA/PCR/3) 		
VIRUS DETECTION	Serum samples/ Blood with EDTA/ Tissues without formalin	<ul style="list-style-type: none"> Virus isolation and identification by the haemadsorption test (HAD) in primary cultures (PNT/CISA/PPA/VI/1) , (PNT/CISA/PPA/VI/2) 	5 work days (1 PASSAGE) to 15 working days (3 passages)	75.69/ per determination
ASFV GENOTYPING	Serum samples/ Blood with EDTA/ Tissues without formalin	<ul style="list-style-type: none"> Sequencing using EURL standardized procedures (PNT/CISA/PPA/GENOTIPADO/1) 	5 work days	1,975.1 (total study including the corresponding report)

*Standard operating procedures (SOPs) are available in the ASF-URL web page (<http://asf-referencelab.info/asf/en/procedures-diagnosis/sops>)

**See page 8 for more detailed information.

The free service for analysing samples from animals suspected to be infected with ASFV is available for up to 50 samples per country per year (except by prior agreement).

The shipper must be aware that the ASF diagnostic procedures will be selected following the ASF diagnosis schedule established by the European Union Reference Laboratory for ASF.

Results will be provided as soon as is practicably possible following the turnover time described in the table. Senders should be aware that samples will be processed subject to laboratory workload and priority of submission.

3.3. OTHER ASF RELATED SERVICES

SERVICE DESCRIPTION		PRICE(€)
Evaluation of ASF commercial diagnostic kits	STEP 1. Evaluation of ASF diagnostic kits using experimental samples/Assessment report	2,425.73
	STEP 2. Validation of ASF diagnostic kits using field samples/Validation report	5,436.71
	STEP 3. Verification of ASF diagnostic kits/Verification report	970.31
	TOTAL PRICE	8,832.89
Evaluations of the absence of ASFV load in commercial material/Evaluation report		2,334.88

4. TECHNICAL AND SCIENTIFIC ADVICE SERVICES: TRAINING EXPERTISE TO OTHER LABORATORIES

The laboratory offers **ASF scientific and technical individual short and long trainings courses at CISA-INIA** in order to transfer the ASF knowledge to Member States and third countries, with special emphasis to the ASF diagnostic tools available as URL for ASF. These courses aim to create a good coordination and cooperation for ASF disease control measures worldwide throughout ASF diagnostic harmonization.

The schedule of the training courses usually includes **hands-on exercises combined with theoretical lectures** focused on providing a general overview about the disease including history, epidemiology, control, and general aspects of the ASF diagnosis with special attention to the techniques to be performed during the laboratory practices activities. A set of practical information which includes copies of the lectures, laboratory practises results, Standard Operating Procedures (SOPs) and relevant ASF bibliography is provided to all participants in a digital format.

The cost to cover the ASF training courses at CISA-INIA includes:

- ⇒ **TECHNICAL AND SCIENTIFIC ADVISE** (€/hour): 117.05
- ⇒ **TECHNICAL AND SCIENTIFIC TRAINING** (€/per person-day): 123.60

SUBMISSION REQUEST FOR ASF TRAINING COURSES MUST BE SENT BY E-MAIL TO the EURL e-mail (eurl.asf@inia.es) with copy to Dr. Carmina Gallardo (gallardo@inia.es) and Dr. Marisa Arias (arias@inia.es).