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SOP/CISA/ASF/SAMPLES/1

STANDARD OPERATING PROCEDURE FOR SAMPLES PROCESSING FOR AFRICAN SWINE FEVER (ASF) DIAGNOSIS.

CONTENTS

1. PURPOSE

The main goal of this procedure is to describe the work methodology to prepare the samples for the African swine fever (ASF) diagnosis.

Note: *The samples must be previously registered with a laboratory register identification number as well as an identification number per each sample.*

2. SCOPE

This procedure is applicable to the following samples:

1. **Samples for ASF serological diagnosis:** EDTA-blood, serum, porcine fluids (i.e, articular fluids, pericardium fluid, ascites, etc) and exudate of pig´s tissues.
2. **Samples for ASF virological diagnosis:** EDTA-blood, serum, porcine fluids (i.e, articular fluids, pericardium fluid, ascites, etc) and any kind of pig´s tissues or organs. Also it can be tested soft ticks and culture supernatants.

3. REFERENCES

3.1. DOCUMENTS USED IN THE PROCEDURE REDACTION

1. AFRICAN SWINE FEVER. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees). CHAPTER 2.8.1. OIE, 2012 [http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.01_ASF.pdf]
2. COLLECTION AND SHIPMENT OF DIAGNOSTIC SPECIMENS. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees). CHAPTER 1.1.1. OIE, 2008 http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/1.01.01_COLLECTION.pdf
3. Food and Agriculture Organization of the United Nations (FAO). AFRICAN SWINE FEVER: DETECTION AND DIAGNOSIS. A manual for veterinarians. FAO 2017 http://www.fao.org/3/a-i7228e.pdf

ASF REVIEWS:

1. Arias, M., Sánchez-Vizcaíno, J.M. (2012). African swine fever. In: Zimmerman, J., Karriker, L.A., Ramirez, A., Schwartz, K.J, Stevenson, G.W. (Eds), Diseases of swine, 10th Edition. John Wiley and Sons, United States of America, pp. 396-404.
2. Arias, M.; Sánchez, C.; González, M.A.; Carrasco, L. y Sánchez-Vizcaíno, J.M. (2002). "Peste porcina Africana" In "Curso digital de enfermedades infecciosas porcinas". [http://www.sanidadanimal.info/cursos/curso/7/7-ppa.htm] on line, July, 2002.
3. Gallardo C, Nieto R, Soler A, Pelayo V, Fernández-Pinero J, Markowska-Daniel, Pridotkas G, Nurmoja I, Granta R, Simón A, Pérez C, Martín E, Fernández-Pacheco P, Arias M. Assessment of African Swine Fever Diagnostic Techniques as a Response to the Epidemic Outbreaks in Eastern European Union Countries: How To Improve Surveillance and Control Programs. J Clin Microbiol. 2015 Aug;53(8):2555-65.

3.2. COMPLEMENTARY DOCUMENTS (SOPs) TO BE USED.

- Procedure for the extraction of African Swine Fever Virus (ASFV) DNA **(SOP/CISA/ASF/DNA EXTRACTION/1)**
- Procedure for the detection of African Swine Fever Virus (ASFV) by conventional polymerase chain reaction (PCR) **(SOP/CISA/ASF/PCR/1)**
- Procedure for the detection of African Swine Fever Virus (ASFV) by real time polymerase chain reaction (PCR) **(SOP/CISA/ASF/PCR/2)**
- Procedure for African Swine Fever Virus (ASFV) isolation on porcine leucocytes **(SOP/CISA/ASF/VI/1)**
- Procedure for African Swine Fever Virus (ASFV) isolation on porcine alveolar macrophages **(SOP/CISA/ASF/VI/2)**
- Procedure for the detection of antibodies against African swine fever (ASF) by indirect ELISA **(SOP/CISA/ASF/ELISA/1)**
- Procedure for the detection of antibodies against African swine fever (ASF) by Immunoblotting test (IB) **(SOP/CISA/ASF/IB/1)**
- Procedure for the detection of antibodies against African swine fever by indirect immunoperoxidase test (IPT) **(SOP/CISA/ASF/IPT/1)**

4. BACKGROUND INFORMATION

4.1. ABBREVIATION

ASF: African swine fever
ASFV: African swine fever virus
r.p.m.: revolutions per minute

4.2. BACKGROUND

The starting point for the laboratory investigation of ASF is the sampling collection taking into consideration the purposes of the study such as disease diagnosis, disease surveillance or health certification.

The ASF diagnostic laboratories require the submission of appropriate samples that arrive at the laboratory in good condition. **It is not possible to make a good diagnosis if the samples are not in good conditions**.

The target samples for ASF diagnosis are:

1. Domestic and wild porcine samples:

⇒ *For ASF antibody detection by ELISA*:

- **Sera:** Draw whole blood extracted via jugular vein puncture, the inferior vena cava, or the auricular veins or during the necropsy using sterile tubes without anticoagulant. *Minimum amount recommended: 500* µl
- The **decanted plasma** separated from the EDTA-blood can be used for antibody detection with the indirect immunoperoxidase test (IPT) [SOP/CISA/ASF/IPT/1] or indirect fluorescent antibody (IFA) test.
- **Exudate tissue samples**, mainly obtained from the spleen, liver, and lungs, are also very useful to check for the presence of antibodies using IPT [SOP/CISA/ASF/IPT/1] and IFA.
- **Corporal fluids** such as abdominal, articular, pericardia, etc. can be also tested by IPT or IFA

⇒ *For ASF virus detection*:

- **Sera:** Draw whole blood extracted via jugular vein puncture, the inferior vena cava, or the auricular veins, or during the necropsy using sterile tubes using sterile tubes without anticoagulant. *Minimum amount recommended: 1 ml.*
- **Blood:** Draw whole blood extracted via jugular vein puncture, the inferior vena cava, or the auricular veins, or during the necropsy using sterile tubes using sterile tubes with anticoagulant (EDTA). *Minimum amount recommended: 1 ml.*
- **Organs without formalin:** Although all porcine organs and tissues can be used to check for the presence of ASFV (mainly in the acute and subacute forms of the disease), the target organs are spleen, lymph nodes, liver, tonsil, heart, lung, and kidney. Of these, spleen and lymph nodes are the most important as they usually contain the highest amounts of virus. Bone marrow is also useful in incidents involving dead wild animals, as it might be the only tissue that is comparatively well preserved if an animal has been dead for some time. Intra-articular tissues of joints can be examined to check for the presence of low virulent isolates. *Minimum amount recommended: 5gr.*

2. Soft tick's samples: *Ornithodoros* soft ticks that are involved in ASFV transmission can be collected from warthog burrows, crevices/holes in pigsties, and sometimes from rodent burrows that may open inside pigsties using three different techniques such as the manual collection, carbon dioxide trapping and vacuum aspiration. After collection, ticks should be kept alive or directly stored in liquid nitrogen to ensure best conservation of the virus inside ticks and to avoid DNA degradation. **Soft ticks are used for ASFV virus and genome detection.**

As a general rule, the samples should be taken with care, to avoid undue stress or injury to the animal or danger to the operator. Where appropriate, samples should be collected aseptically, and care should be taken to avoid cross-contamination between samples. The samples should be carefully packaged, labelled, and transmitted to the laboratory by the fastest practicable method, with the appropriate temperature control. There are specific requirements for the packaging and shipping of ASF samples that

must be followed [http://asf-referencelab.info/asf/files/envio-muestras/ASF_URL_PRODUCT_PRICE_LIST_CISA_INIA.pdf].

5. PROCEDURE DESCRIPTION

5.1. EQUIPMENT AND MATERIALS

MATERIALS.

- Analytical Balance.
- Absorbent paper.
- Chamber 37±3ºC.
- Chronometer.
- Eppendorff tubes of 1.5 ml.
- Freezer <-10ºC.
- Freezer <-70ºC.
- Fridge 4±3ºC.
- Glass or plastic pipettes for volume of 1-25 ml.
- MINISART filters [Ref.: 16555K (Sartorius) or similar characteristics] of 0.45 microns.
- Latex or nitrile gloves.
- Pipetboy acu or equivalent.
- Ph meter (0.01 UpH).
- Single channel pipettes 1-10 µl.
- Single channel pipettes 10-100 µl.
- Single channel pipettes 10-200µl.
- Single channel pipettes 200-1000µl.
- Sterile disposable tips (1-10 µl, 1-200µl, 100-1000 µl).
- Sterile plastic tubes (5ml, 10ml, 50 ml).
- Table centrifuge Megafuge 1.0R [rotor Heraeus #7570 or similar characteristics].
- Tissue homogenator (mechanical or manual).

REAGENTS.

- **Gentamicine sulphate** (50mg/ml) 1% [Ref.: 17-518Z (BioWhittaker) or similar characteristics]. *Store at* 4±3ºC.
- **Phosphate buffered saline** (PBS 1x) pH 7.2 (±0.2 UpH)→ The PBS could be obtained in tablets [Ref.: 524650-1 (CALBIOCHEM) or similar characteristics] or could be prepared as follows:

ClNa [Ref. 1.06404.1000 (MERCK) or similar characteristics]	------	8.0 gr (±0.1)
ClK [Ref. 1.04936.0500 (MERCK) or similar characteristics]	------	0.2 gr (±0.01)
PO_4H_2K [Ref. 1.04873.1000 (MERCK) or similar characteristics]	------	0.2 gr (±0.01)
PO_4HNa_2 [Ref. 1.06586.0500 (MERCK) or similar characteristics]	------	1.15 gr (±0.05)
Distilled water	------	1,000 ml

Store at room temperature. Expiry date: 1 year.

5.2. METHODS

5.2.1 SAMPLE PROCESSING FOR ASF ANTIBODY DETECTION TECHNIQUES.

To prepare clotted blood for ASF antibody detection techniques, [SOP/CISA/ASF/ELISA/1, SOP/CISA/ASF/ELISA/2, SOP/CISA/ASF/IB/1, SOP/CISA/ASF/IPT/1] the blood samples should be treated as follows **to obtain the serum**:

a. Incubate 1 hour at 37±2ºC and later for 14-18 hours at 4±3ºC for the separation of the coagulum.
b. Discard the coagulum and centrifuge in microfuge at 780g (1,500 r.p.m) during 10 minutes.
c. Recover the supernatant **(serum sample)** in a 1.5ml eppendorf labelled with the ID registration number and use immediately in the antibody detection techniques or *store at <-70ºC until further use.*

The **decanted plasma** separated from the EDTA-blood can be used for antibody detection with the indirect immunoperoxidase test (IPT) SOP/CISA/ASF/IPT/1] or indirect fluorescent antibody (IFA) test. **Exudate tissue samples**, mainly obtained from the spleen, liver, and lungs, are also very useful to check for the presence of antibodies

using IPT [SOP/CISA/ASF/IPT/1] and IFA. **Corporal fluids** such as abdominal, articular, pericardia, etc. can be also tested by IPT or IFA

5.2.2 SAMPLE PROCESSING FOR ASF VIRUS DETECTION TECHNIQUES.

For ASF virus detection techniques, [SOP/CISA/ASF/DNA EXTRACTION/1, SOP/CISA/ASF/VI/1, SOP/CISA/ASF/VI/2] the samples should be prepared as follows:

Clotted blood:

a. Incubate 1 hour at 37±2ºC and later for 14-18 hours at 4±3ºC for the separation of the coagulum.
b. Discard the coagulum and centrifuge in microfuge at 780g (1,500 r.p.m) during 10 minutes.
c. Recover the supernatant **(serum sample)** in a 1.5ml eppendorf and treated with 0.1 % of antibiotic (*gentamicin sulphate*) during 1h at 4±3ºC. The treated serum labelled with the ID registration number can be used immediately for ASF virus and genome detection or *store at <-70ºC until further use.*

Organ and tissue samples.

a. Manual homogeneization → Prepare a 1/10 homogenate of the tissue in sterile PBS 1x, pH 7.2(±0.2 UpH) (1gr of the tissue per 10ml of sterile PBS 1X)
b. Mechanical homogeneization [TissueLyser II (Qiagen) or similar]
   → Mix 50 mg of the tissue in 950 µl of sterile PBS 1x, pH 7.2(±0.2 UpH) with 1% of antibiotic (gentamicin sulphate) and on steel ball.
   →Place the tubes into the TissueLyser II (or similar) sample adapters, during 4 minutes at 30 Hz.
c. Centrifuge to clarify at 1,100g (3,000 r.p.m.) for 10 minutes.
d. Recover the supernatant and filter with MINISART filters 0.45µm.
e. Treat the resultant fluids with 0.1 % of antibiotic (*gentamicin sulphate*) during 1h at 4±3ºC. The **treated homogenate tissue** labelled with the ID registration number can be used immediately for ASF virus and genome detection or *store at <-70ºC until further use.* In PCR tests it is recommended to process at 1/10 dilution of the supernatant in parallel with the undiluted material.

Soft tick's samples: are ground in porcelain grinders with 1.5 ml cold phosphate buffered saline (PBS1x) supplemented with 0.1 % of antibiotic (*gentamicin sulphate*). Suspensions are clarified by centrifugation at 5,000 g during 5 min and the supernatants can be used immediately for ASF virus and genome detection or *store at <-70ºC until further use.*

5.3. SECURITY MEASURES

- Read the protocol previously.
- Avoid any reagent and sample contamination.
- Do not use the components after expiration dates.
- Do not eat, smoke or drink while the manipulation of reagents.
- Do not pipette by mouth.
- Use a new tip for each sample.
- The samples should be carefully labeled, and store at the appropriate conditions for further application on ASF diagnostic tests.