



**NATIONAL CENTRE FOR ANIMAL HEALTH,
NATIONAL VETERINARY LABORATORY,
MICROBIOLOGY: VIROLOGY**



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SOP No.	Title	Version No.	Total Pages
NCAH/LAB/MOLE 13	SOP for Sample collection for ASF diagnosis	1	7

Issue Date	Effective Date	Review Period
01-3-2021	01-3-2021	

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Distribution:			
Location	Molecular section, NCAH		
REVISION SUMMARY			
Version No	Effective Date	Reason for Change	Details of the change
01	01-03-2021	Original release	

Document Type: Standard Operating Procedure		Issue No:
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A. Purpose:

The purpose of this document is to describe the procedure for sample collection, storage and shipment of samples to the National Veterinary laboratory for the detection of ASFv.

B. Scope:

This procedure can be applied in any kind of sample collection from porcine for detection of viral disease by PCR and histopathology.

C. Equipment and Materials:

- EDTA vacutainers for whole blood for PCR
- Sterile HP container
- Sterile 50ml plastic tubes
- Sterile syringe and needles
- Cold/Ice packs for transport
- 10% buffered formalin- histopathological samples only
- Cool Box
- Post mortem set
- Viral transport medium
- Biohazard bags
- Personal protective equipment
- Sodium/calcium hypochlorite (Bleach)- 0.03%-0.5% active chlorine
-

D. Procedure for sample collection and storage:

1. Molecular

- Sample collection - Where ASF is suspected, the following samples should be sent to the laboratory:
 - ✓ Blood - shall be collected from ear vein or cranial venacava (non-surgical) in EDTA anticoagulant tube.
 - ✓ Tissues- spleen, lymph nodes, lung, tonsil kidney and bone marrow.

The fresh tissues should be kept as cold as possible, without freezing, and should be shipped to nearest laboratory at the earliest. After the samples arrive at the laboratory, they should be stored at -70°C if processing is going to be delayed.

2. Histopathology

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Blocks of tissue not more than 0.5 cm thick and 1–2 cm long are cut and placed in neutral buffered 4–10% formalin, which should be at least ten times the volume of the tissue sample. Store and pack formalin-fixed tissues separately from fresh tissues, blood, and smears. Care should be taken to ensure that formalin-fixed tissues are not frozen.

E. Shipment of samples:

1. Sample Information

Information and case history should always accompany the samples to the laboratory and should be placed in a plastic envelope on the outside of the shipping container. The sample submission form (See table 1) should be filled and submitted to the receiving laboratory along with the samples.

2. Sample packaging

The recommended procedure for packing samples are as follows:

- Put the samples in a primary container with screw caps and wrap with paraffin film or adhesive tape individually to prevent leakage of fluid. The wrapping of primary containers should be carried out in clean surroundings. Put the primary container into a watertight, spill proof secondary container with absorbent cotton wool sufficient to absorb the entire contents of the primary container (in cases of leakage).
- Place the secondary container in an outer container. This should be a polystyrene foam box covered with a hard box or other appropriate containers (E.g. cool box).
- It is recommended that a freezer box/ice packs is put outside the secondary packaging to ensure that all materials are kept cool and not frozen during shipment. These packs should be pre-frozen at – 20 degrees centigrade before packaging.

3. Transportation of specimens

The specimens should be forwarded to the laboratory by the fastest method available. If they can reach the laboratory within 48 hours, samples should be sent refrigerated.

Table 1: Sample Submission form:

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Sl. No.	Sample collector Details		Farm Details									Animal Details							Sample Details						
	Agency	Designation (Ph. No)	Owner name	Farm type	Farm location (GPS)	Farm size	Domesticated/wild animal	Village	Gewog	District	Contact details	Species	Age	Sex	Breed	Health (Sick/dead/Normal)	status	Clinical History	Treatment details	Sample ID	Collection date	Type of sample	Pooled sample	Transport media / preservatives	Test requested

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