AFRICAN SWINE FEVER FIELD MANUAL

Steps in swine handling and sample collection for African swine fever outbreak investigation
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ACKNOWLEDGEMENTS

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PURPOSES AND SCOPE

This is a simple, easy guide for:

- Handling and restraining pigs for sample collection for ASF outbreak investigation; field sample collection from pigs and sample handling including biosafety and biosecurity measures.

- Intended for field staff who are performing sample collection and handling procedures.

- Developed based on Asian context, but may apply to other regions/continents.

- Sample handling currently follows protocols for testing of unknown pathogens.

Other resources or references may provide more details or comprehensive guidelines. This manual will be updated soon as new development or information is available.
SAMPLING DESIGN AND PREFERRED SAMPLES

MINIMUM NUMBER OF SAMPLES
At least three pigs showing clinical signs per epidemiological unit should be sampled and tested during a reported African swine fever (ASF) outbreak. A minimum of two tubes of blood samples should be extracted for each pig in case of tube breakage or any sample is compromised.

FREQUENCY
• Pigs on suspect farms should be sampled at the time of the outbreak investigation. If found to be negative, the herd should be monitored via testing every week to every few weeks during the period of risk mitigation.

• After decontamination, barns should remain fallow without any animals for at least 40 days.

• After the minimum 40-day fallow period, sentinel pigs (ten percent of the usual herd capacity) can be introduced and should be tested every week or until they start showing clinical signs over 6 weeks, as testing will detect infection before the appearance of clinical signs.

• Animals should be tested upon introduction into a herd.

TYPES OF SAMPLES
Whole blood collected in tubes coated with ethylenediaminetetraacetic acid (EDTA) anticoagulant is the best sample type due to the viral load in blood and because virus appears in blood first as compared to other sample types.

SELECTION OF FARMS AND ANIMALS
Selection of farms and animals for ASF testing should be based on risk, which is determined by increased mortality or clinical signs consistent with ASF, proximity to known ASF-positive farms or villages or pens in the farm, and contact-tracings of animals, people, and fomites with ASF-positive or suspect farms. A suspect ASF case may be reported to the authorities by a farmer prompting an outbreak investigation, or the authorities will send an outbreak investigation team to a farm-based on the levels of risk.

The following provide examples of criteria of farms for consideration, and more may be considered:
• swine farms neighbouring ASF-infected farms;
• swine farms with history of unusual mortality or pigs with clinical signs consistent with ASF;
• swine farms that have had contact with ASF infected farms via movement of animals, people, or fomites such as vehicles;
• swine farms with low biosecurity such as backyard farms;
• free range swine, or where swine have access to wild boar habitat; and
• swine farms whose farmers engage in wild boar hunting.
BEFORE GOING TO FARMS/ COLLECTING SITES

It is suggested to have briefing sessions with all staff to ensure understanding of roles and responsibilities (interviewer of the farmer, animal restrainer, sample collector, sample and sample runner). Briefings should discuss techniques for interviews and sample collection and handling as well as practices to ensure a thorough epidemiological investigation, good biosecurity (minimize likelihood of introducing pathogens into a farm or spreading pathogens from a farm to other farms) and safety for staff, farmers, and animals.

To minimize likelihood of inadvertent spread of the virus via the sampling team, a field operator team should only sample one farm or community at a time. If more than one farm or community is to be sampled, then it is recommended that multiple teams operate. All field operators should put on the complete set of personal protective equipment (masks, boots, overalls, natural or latex gloves, goggles etc.) before entering the farm.

SETTING UP SUPPLIES AND PPE

Set up of sampling and personal protective equipment (PPE) kits should be done before arriving at the farm to minimize the time needed to be onsite and ensure that only necessary supplies are brought onto the farm. Any unused supplies that cannot be disinfected will have to remain on farm or be properly discarded. To prevent unnecessary waste, the supply kits should match the expected number of animals that need to be sampled on farm and the PPE should match the expected number of personnel on the outbreak investigation team plus some extra in case of loss.

OVERVIEW OF OUTBREAK INVESTIGATION

A brief overview of the steps in an outbreak investigation is described here to provide the context of how and when to use the supplies and PPE for animal handling and sampling.
At this stage, a provisional quarantine should already be in place, free-ranging animals should be confined, and the entrance and exit of people and vehicles should be kept at a minimum.

The farm owner and/or farm manager will likely be present upon your arrival at the farm. It is important that a designated member of the outbreak investigation team speak with the farmer about the process and what the outbreak investigation team will be doing as well as the follow-up throughout the process.

1. Park the vehicle at the entrance to the farm.
2. Take latitude and longitude coordinates of the farm using the GPS device.
3. Remove watches and jewelry.
4. Place mobile phone in zip-lock bag.
5. Lay a plastic sheet down in the neutral zone to unload supplies.
6. Prepare a foot bath of boot disinfectant and place it in the dirty zone with a scrub brush, biohazard waste bag, and regular waste bag.
7. Put on disposable suit or coveralls.
8. Put on plastic booties and then rubber boots.
9. Put on overshoes or boot covers. Secure with tape to make a tight seal, if necessary.

10. Put on face mask.

11. Put on two pairs of disposable gloves. Seal gloves over the sleeves with tape if necessary.

12. Mark the clean, neutral and dirty zones with tape or other markers like sticks and stones.

13. Enter farm premises with sampling supplies.

14. Begin the interview with the farmer using the data collection form on a plastic clipboard and pen. Identify the suspect ASF-affected pigs, pens and barns.

15. Establish disinfection points with foot baths, buckets of water with disinfectant, and scrub brushes for people and vehicles at entrances and exits of the pig barns.

16. Set up a sample station outside the affected pen or barn to ensure smooth workflow, reduce stress of the animal from prolonged restraint and ensure good quality samples. The photos below show examples of how equipment are set up on site.

**TIPS**

- It is good to have an empty tube storage racks or boxes to transfer those blood tubes that are already filled.
- Use plastic sheet or tablecloth to cover a table for easy cleaning and disinfection.
- In case a farm does not have one, prepare a footbath with disinfectant and place it in front of an entrance into animal areas. Ensure that it is clean and properly disposed after use.
The restrainer should be put on the upper snout.

**RESTRAINING**

17. Sorting panels can be used to isolate and immobilize the pigs selected for sampling.

18. Use an iron snare, nylon rope or nylon stocking on the snout of the pig to immobilize it. The snare position should be behind the canine teeth. The neck must be stretched well upwards. The position of pigs should be as shown in the photos.

19. Damp a cotton round with ethanol or isopropyl alcohol. Unpack and connect a syringe and needle or vacutainer with needle.

20. Locate the jugular groove. The venipuncture point is the deepest point of the jugular groove, where the alcohol swab is placed in the picture.

21. While holding the syringe or vacutainer and needle, wipe the skin of the sampling site on the pig clean to avoid infection of the venipuncture site.
USING SYRINGES

22. If a syringe is used:

22.1. Uncap the needle and slowly insert the needle into the venipuncture point. Insert the needle at a 45-degree angle into the vein and apply gentle pressure on the plunger.

22.2. Slightly pull the syringe to create negative pressure inside as the needle goes in.

22.3. Once the needle goes into the vein (blood seen coming into the syringe), slowly withdraw 8–9 ml of blood.

22.4. Hold a cotton round damp with ethanol or isopropyl alcohol to the venipuncture site while withdrawing the needle to prevent bleeding.

22.5. Stick the needle through the cap of an EDTA purple-top tube. The negative pressure inside the tube will draw the blood out of the syringe and into the tube. Fill one tube with 4–5 ml of blood, and then fill a second EDTA tube with the remaining 4–5 ml of blood.
USING VACUTAINERS

23. If a vacutainer is used:

23.1. Slowly insert the vacutainer needle (connected with the vacutainer holder) into the venipuncture point until blood is seen at the tip of the holder (suggesting that the needle has gone into the vein). Hold the holder firmly.

23.2. Connect an EDTA purple-top tube to the other end of the vacutainer needle (inside the holder). The negative pressure inside the tube will draw the blood out of the vein and into the tube.

23.3. After the first EDTA tube is filled, remove the tube while keeping the needle and vacutainer in place.

23.4. Connect the second EDTA tube. Blood is seen entering the second EDTA tube.

23.5. Hold a cotton round damp with ethanol or isopropyl alcohol to the venipuncture site while withdrawing the needle to prevent bleeding.

TIPS

- Right-handed operators will usually find it easiest to use the animal’s right jugular vein, and vice versa.

- Consistent positioning of the pig will assist the person collecting the blood samples. Working in pairs can increase efficiency of sampling (i.e. good handler with a good bleeder working together).

- Operators should be aware when the vacutainer needle goes out of the vein during blood collection. Air might have entered the vacutainer tube and this should be considered as waste and replaced with a new one.

- If the operators are unsuccessful in getting sample after a few tries, it is suggested to skip the animal and move on to a new one as the animal may become very stressed (owners too!).

- The operators should be extremely cautious when handling needles after sample collection to avoid injuring himself or herself and preventing potential contaminations.

After taking a blood sample, put it into the appropriate tube.
SAMPLE HANDLING IN THE FIELD

Blood

1. Take the needle out of the EDTA tube (still attached to the syringe). Recap the needle and dispose of the needle in a sharps container.

2. Recap the syringe.

3. Dispose of the syringe/Vacutainer holder in a biohazard waste bag. (Vacutainer holders can be used about five times or until contaminated with blood).

4. Gently invert the filled EDTA tube to mix the blood with the EDTA coated on the inside surface of the tube.

5. Place a label on the tube.

6. Use a fine-tipped permanent marker to label the tube with the animal identification (ID), farm ID and date.

SAMPLE TRANSPORTATION TO LABORATORIES

This will depend on how long samples have to be kept in field conditions before they reach laboratories. Generally, samples should reach laboratories as soon as possible. Understandingly, in some circumstances, samples may have to be kept in the field for days before they are delivered to laboratories due to logistical challenges. This section will attempt to describe how each type of sample should be kept and transported depending on the duration before they reach a laboratory.

Blood

If the samples arrive at the laboratory within 24 hours:

- Keep the filled blood tubes in coolers with ice pack (at 4 °C) until they reach the laboratory.
- Upon arrival in the laboratory, prepare serum aliquots (1-2 ml) and store at -80 °C until further processing.
- Store the samples in the dry shipper or liquid nitrogen container, if possible, if it will take more than 24 hours to reach the laboratory.

Any remaining EDTA blood samples are not foreseen to be used after transfer. However, if the remaining blood can be used for other tests (slides, capillaries, hemoparasite detection, etc.), it must be stored for a maximum of six hours at 4 °C. All samples must then be safely discarded (refer to “Safety and Biosecurity” section).
SAFETY AND BIOSECURITY

The instructions under this section are NOT optional. Operators must ensure safety of themselves, other personnel working with them, as well as animals they handle. They also must ensure that the best biosecurity measures feasible for the situation are followed and practised.

SAFETY OF OPERATORS

• Operators must wear proper PPE when handling animals and samples.

• In case an operator is injured during the sample collection (for example when restraining the animal or pricked by a needle while preparing the different samples), medical assistance should be immediately sought. The case should be immediately reported to the direct line manager of the operator and the ECTAD Team Leader of the FAO country office.

• Operators must ensure that they are not carrying pathogens into and outside of livestock units, whereby proper cleaning and disinfection of themselves must be done before entering and exiting the units.

• Safety and welfare of animals we work with should be one of the priority concerns. Animals must be handled humanely and care should be given to not injure them unnecessarily. It is also a good practice to contact animal owners following the operation (a few days after) to inquire if all animals we handled are safe.

BIOSECURITY AND WASTE MANAGEMENT

• No waste generated from this operation, hazardous or non-hazardous, should be left at the livestock unit. All waste should be collected and taken out of the farm for proper disposal.

• All sharp objects, such as needles, pins and scalpels (if used) must be disposed of in the sharp container.

• All waste generated from this operation should be assumed as hazardous and must be disposed of, destroyed and incinerated accordingly.

SAMPLE COLLECTION

• Equipment that will be reused, such as plastic tablecloths, writing pads, folding tables, footbaths, coolers and animal restrainers, must be properly cleaned and disinfected between moving to the next farm.

• It is preferable to use disposable PPE. However, if some PPE are supposed to be reused (such as overall and boots), they must be properly cleaned and disinfected.

Be mindful and follow biosecurity protocols of the livestock unit you are visiting.
SUPPLIES AND PERSONAL PROTECTIVE EQUIPMENT

SUPPLIES

1 Mobile phone with location services (i.e. smart phone) or GPS device.

2 Data collection form, clipboard (plastic) and tube labels.
1. Scissors and sealing tape to seal PPE around wrists and ankles, and to seal garbage bags.

2. Pens to fill out data collection forms and permanent markers (fine point for tube labels and thick point for bag labels).

3. Vacutainer needle holders.
6 Vacutainer needles (21 gauge 1.50 inches).

7 Needles (21 gauge 0.50 inch).

8 10 ml syringes.
9 Vacutainer EDTA tubes.

10 Cooler with ice packs.

11 Tube storage racks or boxes.
Pig restraint devices: iron snares or nylon rope/stocking.

Sorting panels which can be used to direct individual swine into pens for restraints.

First aid kit (for both human and animals).
15 Cotton swabs for disinfecting the skin of sample site.

16 Ethanol or isopropyl alcohol for disinfectant of skin at sample site.

17 Disposal bags for both non-biological and biological waste.
18 Sharps container.

19 Ziplock bags for phones.

20 Buckets.
21 Scrubbing brushes.

22 Detergent and sponges for equipment (i.e. liquid soap).

23 Disinfectant for boots.
24 Hand sanitizer.

25 Disinfectant wipes.

26 Plastic sheet or tablecloth.
Paper towel and tissues.

Water (enough to dilute disinfectants).
PERSONAL PROTECTIVE EQUIPMENT (PPE)

29 Rubber boots.

30 Overshoes or boot covers.

31 Disposable coverall.
32 Waterproof suit or coveralls (alternative to disposable suit).

33 Disposable mask.

34 Examination gloves.