

DPI Primefact

African Swine Fever (ASF) Investigation

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Animal Biosecurity, NSW DPIRD

To date, there has never been a reported case of ASF in Australia. If you suspect ASF you should immediately phone:

- **the Emergency Animal Disease Watch Hotline on 1800 675 888 – which is monitored 24 hours a day or**
- **your Local Land Services on 1300 795 299 (during business hours)**

Introduction

ASF may cause sudden deaths or unexplained illness in pigs. If illness or deaths in pigs you have attended are potentially consistent with disease due to ASF, samples should be submitted to exclude or confirm infection. This Primefact is a resource for veterinarians investigating suspect ASF cases and provides guidance on collecting and submitting samples, and links to resources about farm biosecurity, Personal Protective Equipment (PPE), personal decontamination and restraining and collecting blood from pigs. In the event of an outbreak it is possible some of the following advice will be modified. This updated information would be communicated broadly.

For further information about clinical presentation of ASF please refer to [African swine fever – A guide for veterinarians](#).

While on farm, collect as much relevant history, clinical and epidemiological information as possible. An example of an animal biosecurity field investigations questionnaire can be found on the DPIRD [Resources and publications webpage](#) under 'Forms and templates – surveillance, WHS and risk'.

If you have visited a pig farm and suspect ASF, contact NSW DPIRD on the 24-hour Emergency Animal Disease Hotline **1800 675 888** or the Local Land Services on **1300 795 299** (during business hours) before leaving the farm for advice. Do not

visit other pig farms until you have received negative laboratory results from the suspect farm.

Biosecurity

In countries where there have been outbreaks of ASF, poor biosecurity has been found to have contributed to the spread of the disease.

Farm entry and exit

It's important that visitors to farms do not spread disease. Always use good farm biosecurity practices and follow any established on-farm biosecurity protocols.

Park your car where there is less chance it can pick up infectious agents (e.g. at the farm entrance gate, or designated clean areas separate from, and external to, production areas). If your car does come in to contact with potentially infected material, then it must be decontaminated either on farm or before entering another pig farm. Remove gross organic material from the wheels, footsteps, and wheel arches of vehicles. Wash the vehicle with water containing soap or detergent (usually under pressure) followed by a registered disinfectant.

Ensure flying insects within the vehicle are killed.

Resources:

- [Farm Biosecurity videos](#)
- [Visiting a farm fact sheet](#)

Personal Protective Equipment (PPE) and personal decontamination

There are no public health implications for ASF, however, personal decontamination and PPE should be used to minimise spread of disease as you enter and exit a property.

Follow established farm biosecurity protocols relating to movements between sheds.

Resources:

- [DPIRD Personal Decontamination Guide](#)
- [AVA Suit Up video](#)
- [Taking off protective gloves](#)
- [WHO – How to handwash](#)
- [WHO - How to handrub](#)
- [Emergency animal diseases](#): A field guide for Australian Veterinarians

Personal effects should not be taken into a piggery, and equipment should be limited strictly to that required to perform the task required.

What to do about footwear and clothes:

- Footwear and overalls should ideally be provided by the piggery. If these are not made available, use disposable overalls and plastic overshoes or boots.
- All clothing and footwear worn on the site should be left on site when exiting. If this doesn't occur, remove organic matter as much as possible from clothes and scrape soles of shoes/gumboots and use a screwdriver to clean treads prior to washing with soap and water, then rinsing and followed by disinfection.
- If clothes or shoes cannot be left on farm or if cleaning on farm is not possible, place potentially contaminated clothes in a plastic bag and wash and disinfect them when back at the vet hospital. Based on a risk assessment of the likelihood of ASF, clothes may need to be soaked in disinfectant prior to laundering. Clothes should be washed in regular laundry detergent and hot water.
- Ideally, shower, including washing hair using soap at the farm if appropriate facilities are provided, otherwise make sure you shower, including washing hair before the next pig farm visit.

Disinfect shoes and other contaminated equipment (such as post-mortem or ultrasound instruments), containers or ziplock bags containing samples. Wash dirty items with soapy water and, if required, a scrubbing brush and then rinse prior to disinfection. Items such as phones used for taking photographs should be placed in a waterproof casing or a ziplock bag during the visit to allow for decontamination. Wash hands and exposed skin with soap or detergent and water for at least 20 seconds. Dry hands and then disinfect with alcohol-based sanitiser (at least 60% alcohol) by rubbing hands/skin for at least 15 seconds.

Follow any stand-down periods required by the next pig farm you plan to visit before entering or if you suspect ASF, until the laboratory results have returned negative, whichever is the longer period. In the absence of a stand down policy, if you suspect ASF, do not enter another pig farm until the laboratory results have returned negative. See 'Laboratory testing arrangements' section below for estimated laboratory turnaround times.

Disinfectants

If ASF is suspected, use a disinfectant permitted by the Australian Pesticide and Veterinary Medicines Authority (APVMA) for use against ASF virus (ASFV) in [Permit 88135](#) and applied for sufficient time to inactivate the virus (as per label). When using the APVMA permit search enter PER88135.

There are important health and safety considerations with some disinfectants. Always follow the product label including safety directions and the Safety Data Sheet issued by the supplier/manufacturer of the products. If products or prepared solutions come in to contact with the skin or eyes, immediately wash with water. Avoid inhaling disinfectant powders.

In some cases, the safety directions listed on the label are not sufficiently protective, due to the higher concentrations to be used. Additional Safety Directions are specified in the 'caution' column below and further information is provided in Permit 88135.

Note: some disinfectants are corrosive to some metals.

Table 1. Some disinfectants permitted by the APVMA for use for treatment of equipment, fabric and surfaces in ASF or CSF outbreaks

- For all situations as much organic matter as possible should be removed before cleaning with soap and water then rinsing with water. This should be followed by disinfection.

Disinfectant	Rate	Application for clothes, footwear and small equipment	Caution	Availability
Citric acid	30g product/L Final dose 3% solution (3 tbsp/L or ¼ cup/2L)	15 minutes contact time with non-porous surfaces then rinse with water. Porous surfaces apply for 30 minutes then rinse Can be used on vehicles	Corrosive. Avoid contact with eyes and skin. Wear protective eye wear	From drug wholesalers. 25kg bags available online. Citra-clean 1kg available from pool shops & supermarkets
Virkon S and Virkon Aquatic	10g/L Virkon S 20g/L Virkon aquatic Final dose: 2-3% solution	10 minutes contact time Can be used on vehicles	Can stain clothing. Do not use on human skin. Mildly corrosive on many metals.	Chemical suppliers and drug wholesalers
Sodium Hydroxide 400g/L (Caustic soda)	50ml/L Final dose 2% solution	10 minutes contact time	Do Not use in the presence of aluminium & derived alloys or on paint work. Toxic for skin and	Chemical suppliers

eyes –
wear protective
clothing,

Sodium hypochlorite 125g/L (Bleach)	40ml/L Final dose 0.5% solution	15-30 minutes contact time Clothes, footwear, small equipment	Toxic for skin and eyes – wear protective clothing, mask and gloves. Use in well ventilated areas. Do not inhale vapour Corrosive for many metals. Bleaches clothing. See warnings on label	Widely available
Calcium hypochlorite 700g/kg	7.2ml/L Final dose 0.5% solution	10-30 minutes contact time Sheds and housing	Toxic for skin and eyes – wear protective clothing, mask and gloves. Use in well ventilated areas. Corrosive for many metals. Bleaches clothing. See warnings on label	Chemical suppliers
Sodium Carbonate (Washing soda, soda ash)	100g/L Final dose 10% solution (½ cup/L)	30 minutes contact time Can be used on vehicles	Mildly caustic for eyes and skin. Avoid use on aluminium and similar alloys	Soda Ash – available from hardware stores and swimming pool chemical shops - e.g. 2.5kg bucket and 25kg bags Lectric Washing Soda- available from supermarke ts in 1 kg packets.
Sodium carbonat	40g/L Final dose	20 minutes contact time	Mildly caustic for eyes and skin. Avoid	Chemical suppliers

e Anhydrous	4% solution		use on aluminium and similar alloys	
Sodium Hydroxide 400g/L (Caustic soda)	50ml/L Final dose 2% solution	Clothes, footwear and small equipment	mask and gloves. Use in well ventilated areas. Corrosive for many metals. Bleaches clothing. See warnings on label	Chemical suppliers
F10SC Veterinary Disinfectant	10 ml per 1L (1:100)	30 minutes contact time Can be used for disinfecting hard surfaces, equipment and air spaces	Avoid contact with eyes and skin.	Widely available

Further information about disinfectants that can be used against the ASFV may be found in the [AUSVETPLAN Decontamination manual](#).

Restraining pigs and collecting samples

Challenges in sampling pigs include pig restraint and Work Health and Safety issues. Wear earmuffs to protect hearing. If possible, have an experienced pig handler present to assist.

Pig restraint and blood collection resources are available at:

- [Video and slide show of blood collection from pigs](#)
- [Bleeding and intravenous techniques in pigs](#)
- [Oropharyngeal swab collection in pigs](#)
- [Smart Pig Handling Parts 1 & 2](#)

Correct needle size and length for different sized pigs is important for successful blood collection. Suggested needle sizes for jugular blood collection are:

- For small pigs and piglets - 1" x 20G
- For growers and finishers - 1.5" x 18G
- Larger sows and boars - 1.5" x 18G

Use vacutainers and a needle holder for jugular blood collection. Note: blood samples can be collected immediately post-mortem if required.

List of what may be required when sampling live animals:

People

-Blood collector

-Pig catcher(s)/handler(s)

Materials

-Snout rope

-Mouth gag

-Ear plugs/ear muffs

-Rubbish bag

-Vacutainers – EDTA & plain

-Needles

-Needle holders

-Gloves (consider cut resistant)

-Swabs and phosphate buffered gelatin saline (PBGS) transport media

-Marker pens

-Plastic ziplock bags

-Paper to record sample details (lab submission form)

-Method to identify pigs if required

Resources for identifying clinical disease and pathological lesions

Some photographs available to aid in identifying ASF include:

- [UK Pirbright Institute images of ASF sick pigs and gross pathology](#)
- [US Center for Food Security and Public Health images of ASF sick pigs and gross pathology](#)

List of what may be required when doing a post-mortem:

-Containers to collect samples, including sterile containers

-Formalin 10%

-Saline

-Swabs and PBGS transport media

-Knives and sharpener

-Rib cutters (pruning shears)

-Scalpel handles and blades

-Forceps

-Scissors

-Marker pen

-Paper to record sample details (lab submission forms)

-Eskies and icepacks

-Needles and syringes

-Camera

Laboratory diagnostic testing for ASF

ASF cannot be diagnosed on clinical grounds alone. Collection of samples from pigs is required to confirm or exclude a clinical suspicion of ASF. Laboratory testing is also used to exclude Classical swine fever (CSF), another pig disease exotic to Australia. CSF is clinically indistinguishable from ASF.

Diagnostic tests may be used to detect virus, virus genome and/or antibodies.

Initial screening will involve testing of fresh blood and tissue samples and/or swabs by real-time PCR (qPCR). While serological tests can be conducted, they are generally more appropriate for defining the nature and extent of an outbreak, and in the proof-of-freedom phase rather than at the initial diagnosis stage. ASF virus is likely to be present in the blood and oral fluids 1-2 days prior to the appearance of clinical signs. Viral genome can be detected in oropharyngeal swabs collected from either affected live or dead pigs.

Swabs are particularly valuable if there is difficulty collecting blood, in index cases, in single animal cases and to aid differentiation from Classical swine fever. Viral nucleic acid can sometimes also be detected in oral fluids earlier than in blood.

Sampling domestic pigs

Obtain a thorough clinical history, carefully examine live affected and at-risk pigs, and conduct post-mortems on dead pigs. Collecting a range of samples is vital.

Sample clinically affected pigs, including pyrexemic pigs, and those that have recently died (including stillborn piglets and aborted fetuses).

Samples from up to 10 pigs from a farm should be sufficient to diagnose ASF or CSF. For example, this could be:

- If there are mortalities – post-mortem samples from up to 5 pigs and collect blood and swab samples from up to 5 live sick pigs.
- If there are no mortalities – collect blood and swab samples from up to 10 sick pigs. Sample some at-risk in contact pigs if there are fewer than 10 sick pigs.

If pigs are sufficiently unwell consider euthanising and collecting full range of post-mortem samples, especially if you suspect an emergency (exotic) animal disease, or a disease of unknown cause in the herd.

If live pigs that are sampled are not already permanently identified, place an ear tag (ideally) or apply temporary identification on the skin with a stock marker such as spray-on stock paint. Record where the sampled pigs are located e.g. shed number and pen number.

Live suspect pigs

Collect bloods:

- 1 x full 7-10ml EDTA tube (purple top) and
- 1 x full 7-10ml plain tube (red or speckled red/grey top) from each pig sampled. Note: blood samples can be collected immediately post-mortem if required.

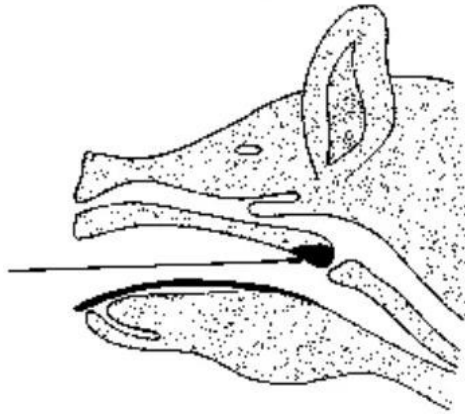
If possible and safe to do so, take oral swabs from the oropharynx otherwise from well inside the cheeks. Alternatively, collect nasal or rectal swabs. Use sterile swabs and place them in PBGS transport media.

Dead pigs

Conduct full post-mortems and collect samples from recently dead or freshly euthanased affected pigs. To minimise the risk of contamination, tissue samples should be taken as aseptically as possible. Record and photograph lesions seen. Note: individual pigs may have great variability in lesions. Even if pigs are not freshly dead, and have died within the last 24 hours, it is still worth collecting samples for testing if others are not available.

- Collect approximately 5 g (1cm x 1cm) sample of each of the following fresh tissues: spleen, lymph nodes (gastro-hepatic, mesenteric and sub-mandibular), tonsils, lung, kidney, liver and ileum into separate sterile yellow screw top containers with no additives.
- Collect a full range of tissues as above, placed into 10% neutral-buffered formalin at a 10:1 ratio of formalin: tissue. Tissues can be pooled into one container

If sufficient tissue samples as described above can't be collected, swab the pharyngeal tonsillar area and place in PBGS transport media.



Location of tonsils in pig (Image from [The Pig Site](#))

- If someone is available, an assistant can be valuable to assist labelling samples and taking photographs.

Note: sampling requirements are likely to change in the event of an outbreak and as a response progresses.

Sampling feral pigs

Feral pig mortalities that are suspected to be African swine fever should be reported to the 24-hour Emergency Animal Disease Hotline immediately on **1800 675 888** or to the Local Land Services on **1300 795 299** during business hours.

If recently dead, sample as above. Even if pigs are not freshly dead, it is still worth collecting samples for testing.

At a minimum collect tonsillar swabs and/or swabs from affected tissues into PBGS for screening to detect viral genome by qPCR.

Provide a history when submitting samples including:

- Name and contact details of person who notified about the feral pig
- Geographical location of the pig (GPS coordinates if available)
- Photos of the pig
- Whether the pig was shot or found dead
- If shot, whether the pig appeared healthy prior to death
- Clinical signs if pig(s) looked sick prior to death
- Approximate number of other feral pigs seen in the area
- Approximate number of other feral pigs sick or dead in the area
- Proximity to commercial pig farm(s), if known

Swab viral transport media

PBGS viral transport medium in 5ml screw top vials is available free of charge. Orders can be placed by sending requests to laboratory.services@dpi.nsw.gov.au using the [media request form](#).

PBGS can be stored frozen indefinitely and is an important transport medium for most virus investigations, especially for emergency animal diseases. It can be frozen and thawed many times over but should not be held at high (>25°C) temperatures. After use, PBGS containing swabs should be kept chilled but not frozen.

PBGS and swabs are also held at most Local Lands Services offices.

In an emergency situation, if no PBGS is available, place swabs in 2-3 ml of sterile saline in a sterile screw topped vial.

Labelling of samples

Carefully label each sample and cross reference them to your paperwork so they can be linked both to an animal and the premises. Record which samples are from dead, alive affected or alive at-risk non-affected pigs. Record on the paperwork which shed number and pen number the pigs sampled were in.

Vials can be labelled numerically with a key number and full details provided on a key list. Provide the key list identifying which samples are from which pigs with the submission form.

Take photos of any lesions and email them to laboratory.services@dpi.nsw.gov.au, with reference to associated sample numbers.

Packing ASF samples

Samples must be packed as a category B diagnostic specimen. [See Video 1 - Collecting, packaging and shipping samples](#) for information on packing samples.

A [specimen advice form](#) must accompany specimens submitted to the laboratory. On the form write 'ASF exclusion' in the 'Other' section under Test(s) Requested.

Allow plain blood samples to clot before transporting them over any distance. Ideally, hold clotted blood samples at room temperature until the clot has retracted, then keep chilled (but not frozen).

Samples in 10% formalin should be kept at room temperature until transported to the laboratory.

Ensure that the lids of all containers are tightly closed.

Place blood tubes, fresh samples and samples in PBGS vials into well sealed ziplock plastic bags (preferably double bagged, with smaller bags in an outer larger bag if necessary) and place fixed (10%) formalin samples into a different sealed bag.

Fresh tissue, swabs and blood samples must be sent chilled with ice packs (NOT frozen). Pack in insulated containers with sufficient ice bricks to ensure that the samples are still cold when received at the laboratory. Prevent direct contact between coolant bricks and specimens, which may otherwise become frozen. Fixed samples can be transported in the same container.

Ensure specimen submission forms are placed in their own ziplock bag on top of the samples. Do NOT include them in the same bag as the samples. Place some absorbent material (e.g. paper towel) in the esky to absorb any spillage that may occur.

Sample submission

Send samples to Elizabeth Macarthur Agricultural Institute (EMAI), Woodbridge Road, Menangle NSW 2568. Samples may be sent with Metrostate Couriers or StarTrack.

After calling the Emergency Animal Disease Hotline on **1800 675 888** to report you suspect ASF, call the EMAI Customer Service unit on **1800 675 623** 8:30am - 4:30pm Monday - Friday (excluding public holidays) to notify of intent to submit samples for ASF testing and to receive further details on submission. Urgent deliveries after hours can be arranged through the Emergency Animal Disease Hotline.

DPIRD will pay for the cost of the courier and the laboratory testing for emergency (exotic) animal disease testing.

Laboratory testing arrangements

Preliminary testing for ASF and CSF, will be conducted at EMAI. The turn-around time for initial results from EMAI will depend on when samples are submitted. They would usually be processed on the same day as being received on a weekday. Samples will be forwarded to the Australian Centre for Disease Preparedness

(ACDP) for confirmation of ASF/CSF status. The results from ACDP would usually be available within 24 - 36 hours after samples arrive at EMAI.

Additional information

For further information on ASF see:

- [African swine fever – A guide for veterinarians](#)
- The [Emergency animal diseases: A field guide for Australian veterinarians](#) Chapter 3.1 on page 58. Section 1 also includes general principles of disease investigation and Section 2 includes guides to investigation of disease syndromes, such as sudden death in pigs on page 31.
- [NSW Department of Primary Industries and Regional Development African Swine Fever information for vets webpage](#)
- Australian Government Department of Agriculture, Water and the Environment [‘Keeping African swine fever out of Australia’](#)
- [Food and Agriculture Organization of the United Nations African Swine Fever Detection and Diagnosis – A manual for veterinarians](#)
- Additional PPE videos from Biosecurity QLD and EuFMD
https://www.youtube.com/watch?v=mjA_3_RtPFM
<https://www.youtube.com/watch?v=l5lsaJZV0ds>
<https://www.youtube.com/watch?v=ljS-53r0FJk>
- NSW DPIRD [Emergency Management resources and publications](#)

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