Diagnosis of ASF

When large numbers of pigs of all ages die and the clinical signs and post mortem lesions look like those of ASF, that is the first disease that should be suspected. Confirmation is by demonstration of the virus in samples sent to a laboratory.

Laboratory tests need to be carried out to confirm the diagnosis of ASF, because the drastic control measures are expensive and cause hardship to owners and government alike. These measures should not be put in place unnecessarily. The tests that exist are used to detect the virus itself by growing it, evidence that the virus was present (virus antigen, genetic material) or the reaction of the animal to the virus (antibodies in blood serum). In acute outbreaks of ASF, it may not be possible to detect antibodies, as the pigs die before they have time to produce them. The standard tests therefore involve detection of the virus. Tests to detect antibodies are useful for identifying pigs that have survived infection and for carrying out surveys to determine whether the disease is endemic in a country or area.

Detection of the virus in cell culture
ASF virus grows best in pig macrophages derived from bone marrow or lung washing. With many strains of ASF virus, the presence of virus in cell cultures can be demonstrated by adding red blood cells to the culture. These are attracted to the surface of infected cells, to which they cling and form “rosettes”, a phenomenon known as haemadsorption. The virus may be injected into pigs to demonstrate that it is capable of infecting pigs and causing disease. Some strains of virus do not cause red blood cells to adsorb to the surface of cells that they have infected, but dead cells in the culture will become obvious after a few days.

The advantage of culturing the virus is that it can then be characterized to determine the strain.

Detection of virus antigens by immunofluorescence
Impression smears of lymph nodes and spleen on glass slides are treated with antibodies labelled with a dye that will fluoresce when examined under a special microscope (Figure 17). Positive and negative controls
are used to ensure that the slides are interpreted correctly. This test can be carried out fairly rapidly and is used in most African laboratories that have the capacity to diagnose ASF.

Detection of virus antigens by polymerase chain reaction (PCR)
This test requires specialized facilities. It is used most frequently in reference laboratories to obtain a rapid diagnosis, as isolation in cell culture and demonstration of the virus by adsorption of red blood cells or cell damage (cytolysis) usually takes several days. Results are obtainable within 24 hours, and rely less on personal interpretation than immunofluorescence. The test can be carried out on a variety of tissues, but for practical purposes lymph nodes and spleen on ice or in glycerosaline are the samples of choice.

Detection of viral antigen by immunoperoxidase staining
Viral antigen may be detected in cells in histopathological preparations from formalin-fixed material by the immunoperoxidase staining technique. This method, which usually takes 5-7 days, is slower than PCR or immunofluorescence, but is useful if the only tissues available have been preserved in formalin. It is useful as a research tool to determine the distribution of viral antigen.

**FIGURE 18**

**Immunofluorescent - positive reaction**
Positive fluorescent antibody (FA) test: the ASF antigen is indicated by bright green fluorescence when examined with a special fluorescence microscope.
Detection of antibodies against ASF virus
The enzyme-linked immunosorbent assay (ELISA) is the test most commonly used to detect antibodies to ASF in serum. Other tests that are sometimes used are indirect immunofluorescence and immunoblotting. Antibodies may not be detected in pigs that have died of acute ASF. The test is used to detect animals that have survived infection with ASF and in surveys to determine whether the disease might be endemic in an area.

When submitting samples to a reference laboratory in another country, notify the laboratory so that they can send you an import permit, notify customs and collect the samples promptly. They will need to know the flight number, waybill number and date and time of arrival. A reliable courier service can be used.

All samples should be accompanied by the following information:
• name, address, telephone/fax numbers of sender;
• telephone/fax numbers for official reporting of results, if different from above;
• name, address and contact numbers of owner;
• date of sampling and submission;
• type and number of samples (including whether on ice, preserved, etc.);
• disease suspected and tests required.

And, most important, the samples should be accompanied by a detailed history of the outbreak that includes:
• age, sex and identification (if any) of each pig sampled;
• number and ages of animals dead;
• number and ages of animals sick;
• herd size;
• any recent movement of animals into or out of herd;
• date of first death;
• date when signs of disease were first observed;
• what signs of disease were observed;
• post mortem findings;
• treatment if any;
• what the animals are fed.

When unusually high numbers of deaths occur, a pig should be presented to the nearest laboratory or field station/official for post mortem examination and sampling. Farmers can also take samples of spleen and lymph nodes and present them, as quickly as possible, to
It is better not to freeze samples, as freezing at -20°C may inactivate the virus. Samples should be kept refrigerated for as long as possible. However, evidence of the presence of the virus (DNA) can still be detected by special laboratory techniques (PCR) and if there is a long delay in transmission it would be better to freeze the samples at an unsuitable temperature than allow them to become badly autolysed.

Because they will contain the highest concentrations of virus, lymph nodes, spleen and tonsils are the organs of choice to submit to a laboratory capable of performing the tests. They should be submitted on ice as soon as possible. If ice is not available, the specimens can be preserved in 50 percent glycerosaline, which prevents bacterial activity. If neither ice nor glycerosaline are available or if it is very unlikely that samples on ice will reach the laboratory chilled, samples in 10 percent buffered formalin should also be submitted. This will enable a diagnosis to be made by histopathological examination and the immunoperoxidase method, available at some international reference laboratories.
Handling blood sample prior to dispatch. Stand overnight at about 4° C (not frozen). Centrifuge and decant serum OR remove clot with bent paperclip. Note that if the tube is stored upside down, the clot will adhere to the stopper and can be pulled out carefully.

FIGURE 19

Blood sample collection
Collecting a blood sample from the jugular vein.
laboratories that have the capacity to perform histopathological examination.

Sample as many animals as possible, as this increases the chance of a diagnosis. Samples should be taken from animals that have died within 12 hours or that have been slaughtered. ASF virus is resistant to autolysis but in practice it is easier to culture virus from fresh specimens.

**DETECTION OF ANTIBODY**

Blood is collected in tubes without an anti-coagulant (red cap) and submitted to the laboratory as soon as possible after collection, on ice. Allow the samples to stand for a few hours at room temperature before refrigerating. Do not freeze the blood, as this results in the red blood cells breaking up and staining the serum. If refrigeration is not possible, remove the clot by centrifugation or by one of the methods shown in the diagram.
Control of ASF

There is no vaccine.

**TO PREVENT ASF:** Pig farmers and field personnel should be aware of ASF, able to recognize ASF and know what to do if they suspect ASF.

- Pigs should be kept in well-constructed pig sties under hygienic conditions with controlled entry to the piggery.
- Movement of pigs inside the country and especially across international borders should be controlled.
- Pigs should not be fed swill that might contain remains of pigs. To ensure safety, swill should be boiled for 30 minutes and cooled before feeding.

**DURING AN OUTBREAK:**

- infected and suspected infected farms must be placed in quarantine;
- no movement of pigs or any products of pig origin should be allowed;

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**FIGURE 20**

*Well constructed pig sties*

*Well constructed pig sties that are designed to keep the herd animals in and any stray animals out; they are also comfortable for the pigs and easy to keep clean.*
• all infected and in-contact pigs must be slaughtered;
• carcasses must be burnt or buried deeply on site;
• vehicles should be disinfected on entering and leaving farms;
• personnel should ensure that shoes, clothes and equipment are disinfected between farms.

No entry sign at gate
A “No Entry” sign at the gate to a pig farm indicates that sanitary measures are in place and that visitors may only enter the farm with the permission of the owner or manager.
A footbath filled with disinfectant at the entry to a pig farm, to ensure that people do not enter with contaminated material on their shoes.
Dead pigs

Pigs shot during a control operation after ASF broke out in a small piggery in the ASF control area in South Africa

Pig carcasses being disposed of during the stamping out exercise in Tema, Ghana; note the deep burial and later covering with a layer of lime
Recognizing African swine fever

FIGURE 24

Disinfecting a pigsty and a vehicle

A picture showing disinfection of a pigsty during the stamping out exercise in Accra, Ghana.

Disinfection of a vehicle after an exercise of stamping out in Accra, Ghana.