EU Reference Laboratory for ASF
Animal Health Research Centre
(CISA), INIA
Ctra Algete-El Casar s/n
28130, Valdeolmos, Spain

ASF diagnosis workflow in case of ASF suspicion

In **case of an ASF suspicion**, the **PCR is by far the most sensitive method for the detection of the agent and the method of choice for first-line laboratory diagnosis** at the EURL and NRL reference laboratories. It is a basic diagnostic tool for surveillance in the EU, considering the long-term viremia, the high viral load in the infected animals suffering acute or subacute clinical courses. It is quick and can be used for individual as well as pooled samples (as it has been deeply studied and validated at the EURL). A variety of PCR tests, including both conventional and real time (rtPCR), as well as commercial kits have been developed and validated to detect a wide range of ASF isolates belonging to different known virus genotypes, non-HAD strains, and diverse virulence. Nevertheless, although rare, **to avoid any false positive PCR results**, (e.g., due to lab contamination or other factors) several procedures are implemented. Thus a **primary outbreak (or wild boar case) of ASF should be confirmed by virus isolation of ASFV and the identification by the HAD assay**, by the EURL and/or the NRLs, and **by genetic typing** at the laboratories. However, this might not always be possible due to technical limitations, absence or appropriated facilities or the reduced sensitivity, particularly in samples obtained from altered carcasses or hunted wild boar, or in weak positive PCR samples.

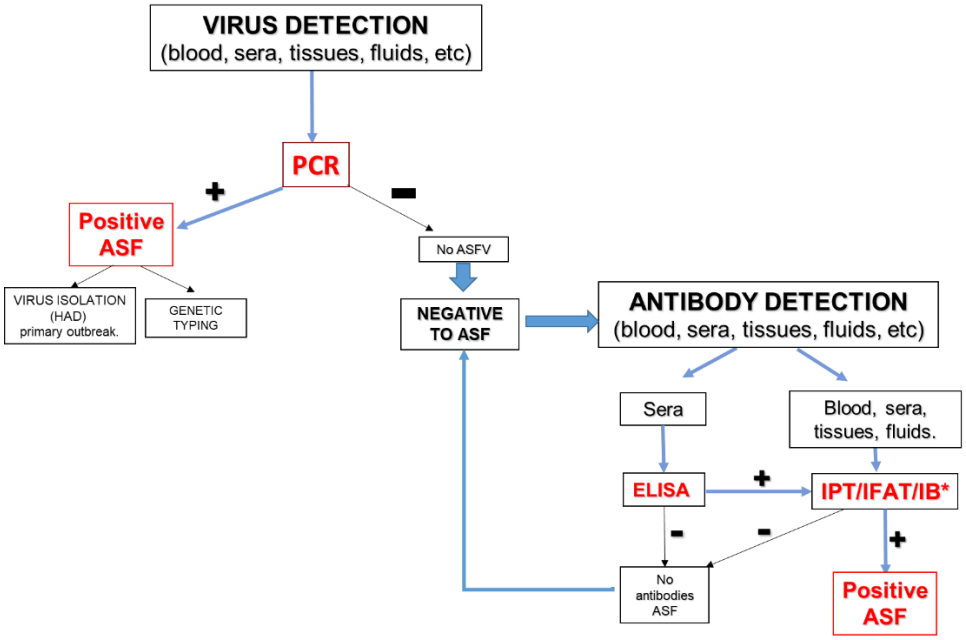
When virus isolation or the HAD identification is not possible, It is stablished that **PCR results must be confirmed by pathology examination or at least two distinct virus or antibody detection tests on the same-suspected pi**g.

In the case of **wild boar samples**, if virus isolation is not possible, **a primary case of ASF must be confirmed by at least two virus or antibody detection tests have given a positive result** (**EU 2003/422/EC**).

Whenever **the suspicion is raised that ASFV is circulating in a swine population**, **a negative PCR result cannot excluded the presence of ASF**. Since animals usually develop antibodies within the second week after infection, they can test positive for both ASF virus (ASFV) and antibodies simultaneously for at least two months. Samples from animals surviving this period are usually positive for ASFV-specific antibodies, but negative for ASFV and its genome. Therefore, if the PCR gave a negative result but there is a suspicion that ASFV is circulating, **serological assays should also be used for the diagnosis**. The current EU (2003/422/EC) and OIE recommendations for ASFV antibody detection involve the use of an ELISA for antibody screening, backed up by Immunoblotting (IB), Indirect Immunofluorescence test (IFAT) or the Indirect immunoperoxidase tests (IPT) as confirmatory tests (OIE 2019). The **ELISA remains the most useful method for large-scale serological studies in serum samples**: it is fast, easy to perform and economical. However, only serum can be analysed, which restricts its application range, especially in case of passive surveillance of wild boar when animals are usually found dead. In addition, hemolysed serum samples could arose either false positive or negative results depending of the ELISA format employed. Therefore, **positive ELISA results should always be confirmed by additional methods such as IPT, IFAT or IB tests**, as recommended by the OIE (OIE 2019). The IB is a rapid and sensitive assay but, similarly to that described above, only serum

samples can be tested. On the contrary, IPT or IFAT can be easily used for analysing **all type of porcine samples**, including exudates from tissue, whole blood, fluids and even bone marrow. **The antibody detection by IPT in exudates tissue samples is a common successful method when wild boar are analysed.**

Figure→ ASF diagnosis workflow in case of ASF suspicion.



**IB (for serum samples)*