



## Techniques and sampling collection for ASF diagnosis

To be effective, **proper samples combined with a proper selection of diagnostic methods**, is of major importance in order to make an early, rapid and reliable diagnosis.

At the following table are summarized the target samples for ASF virus and antibody detection, the ASF diagnostic tests and their recommended use.

DETECTION	TECHIQUE	TARGET SAMPLES	RECOMMENDED USE
Nucleic acid detection	PCR tests (i.h. conventional and real time PCR tests and commercial tests)	<b>Organs:</b> spleen, lymph nodes, liver, tonsil, heart, lung, kidney, bone marrow (wild boar). <b>Anticoagulated blood*</b> <b>Ticks</b>	Early detection: suspicion, outbreak investigation, surveillance. Individual and herd testing. Movements from restricted zones
	Virus detection	Virus isolation and identification by haemadsorption (HAD) test (i.h)	Confirmation of primary outbreak.
Antigen detection	Direct Immunofluorescence (DIF) (i.h)	<b>Organs:</b> spleen, lymph nodes and tonsil.	Individual and herd testing (in case of clinical signs), early detection. <i>It is recommended its use in parallel with antibody detection tests.</i>
	Antigen ELISA commercial kit <small>(INgezim PPA DAS, Double Ab Sandwich.</small>	<b>Organs:</b> spleen and lymph nodes. <b>Plasma</b> from anticoagulated blood	Surveillance Herd testing (in case of clinical signs).
	Lateral flow device (LFD) commercial kit <small>(INgezim ASF CROM Ag)</small>	Anticoagulated <b>blood*</b>	Herd testing (in case of clinical signs). <i>It is recommended its use in parallel with LFD antibody detection tests.</i>
Antibody detection	ELISA (i.h ELISA tests and commercial methods)	<b>Sera</b>	Individual and herd testing when deemed appropriate. Surveillance
	Immunoblot (IB) (i.h)	<b>Sera</b>	Confirmatory test Individual and herd testing when deemed appropriate.
	Indirect Immunoperoxidase test (IPT) (i.h)	<b>Sera</b> <b>Plasma</b> from anticoagulated blood <b>Exudates from tissues</b> <b>Corporal fluids</b> (pericardial, intraarticular, thoracic, etc)	Confirmatory test Individual and herd testing when deemed appropriate. Surveillance; epidemiological studies (time of the infection)
	Immunofluorescence Antibody (IFAT) test (i.h)	<b>Sera</b> <b>Plasma</b> from anticoagulated blood <b>Exudates from tissues</b> <b>Corporal fluids</b> (pericardial, intraarticular, thoracic, etc)	Confirmatory test Individual and herd testing when deemed appropriate. Surveillance; epidemiological studies (time of the infection)
	Lateral flow device (LFD) commercial kit <small>(INgezim ASF CROM Ag)</small>	<b>Sera</b>	Herd testing <i>It is recommended its use in parallel with LFD Antigen detection tests.</i>



*\*Heparin (green stoppers) should be avoided as it affects the performance of both PCR (false-negative results) and haemadsorbing (HAD) test (false-positive results).  
i.h. (in house methods).*

#### **Alternative methods for sampling:**

- **Blood samples collected with cotton swabs from pigs or wild boar** can be used for virus DNA and antibody detection by PCR and ELISA, respectively (Petrov et al., 2014; Pikalo et al., 2020; Sauter-Louis et al., 2020), and using the pen side tests like lateral flow devices (LFD) for ASFV antibody and antigen detection (Carlson et al., 2018).
- **Dry swabs of organs and bone marrow** in case of absence of trained staff for ASFV genome detection (Beltrán-Alcrudo et al., 2017; Carlson et al., 2017; OIE, 2019).
- **Dried blood spot sampling (DBS)** on filter papers (for both DNA and antibody detection) or FTA card (for nucleic acids detection only) (Beltrán-Alcrudo et al., 2017; Majumdar et al., 2011; Randriamparany et al., 2016).