GF-TADs
GLOBAL FRAMEWORK FOR THE PROGRESSIVE CONTROL OF TRANSBOUNDARY ANIMAL DISEASES

Standing Group of Experts on African swine fever in the Baltic and Eastern Europe region

under the GF-TADs umbrella

Eight meeting (SGE ASF8) - Chisinau, Moldova, 20-21 September 2017

Laboratory diagnostics and capability

Dr Alexey Igolkin

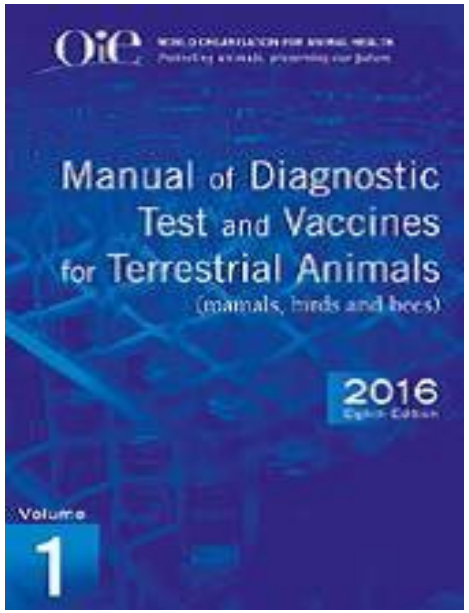
List of topics :

- Collection of samples
- Transport of samples
- Laboratory diagnostic of ASF
- Ring trial carried out recently

The OIE manual for the diagnosis of ASF sets out uniform diagnostic procedures, sampling methods and criteria for evaluating the results of laboratory examinations:

- How to recognize ASF and what are the principles of differential diagnosis;
- The main criteria to be taken into account for the recognition of the household as suspicious [with regard to ASF];
- Procedures for inspection and sampling, as well as their transportation;
- Virological examinations and evaluation of results;
- Serological surveys and evaluation of results;
- Safety requirements for laboratories.

Decision of the Commission 2003/422 / EC of May 26, 2003, approved the guidelines for the diagnosis of African swine fever



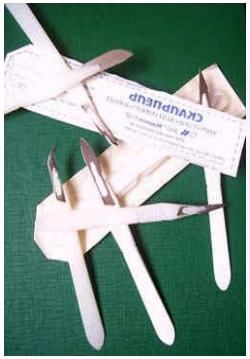
Collection of samples *is the starting point for all laboratory studies of animal diseases is sampling*

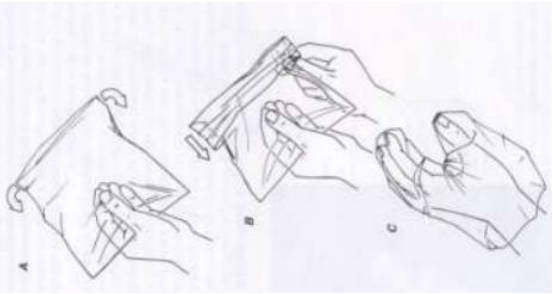
Principals of sampling

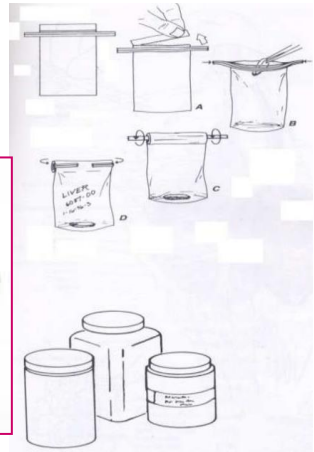
- When sampling for each disease takes into account its own specificity
- Sampling for research is conducted to diagnose the disease, confirm the status of a healthy animal in order to monitor it, determine the timing of vaccination
- The quantity and quality of the samples must be consistent to obtain reliable results
- Samples should be taken as safely as possible for the animal and the selection process must be carried out in compliance with all safety standards for the operator
- Aseptic technologies that exclude contamination should be used for selection
- Sampling is carried out by trained personnel
- Samples should be placed in the laboratory the shortest possible time after the selection

Tools, containers for the selection of samples of pathological material

Sampling for pathological material

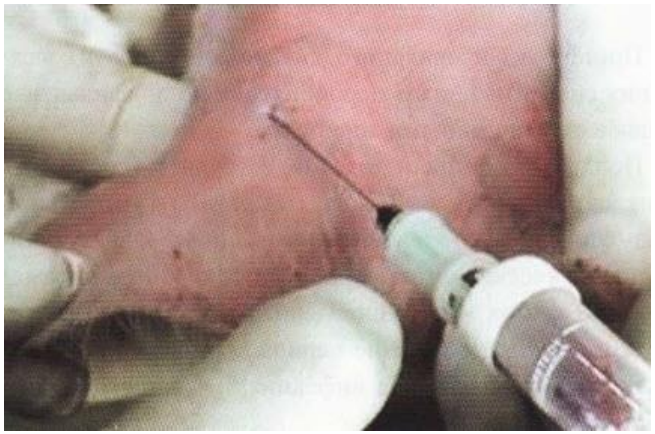




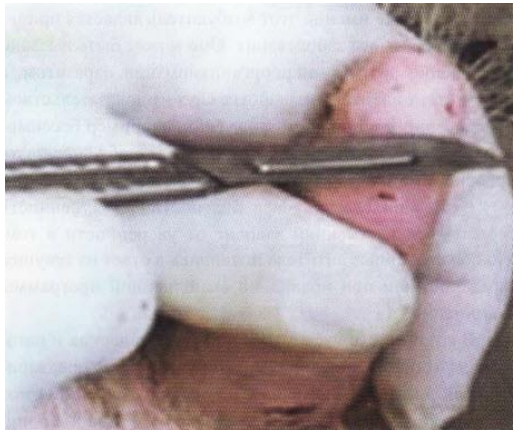


Blood sampling

- It is necessary to add an anticoagulant (Trilon B, EDTA)
- It is forbidden to use heparin as an anticoagulant
- Freezing whole blood samples is unacceptable!



Taking blood from the ear vein

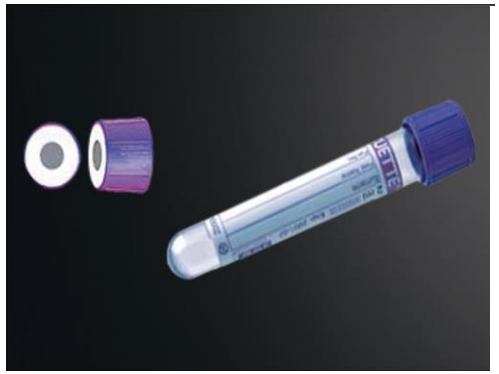


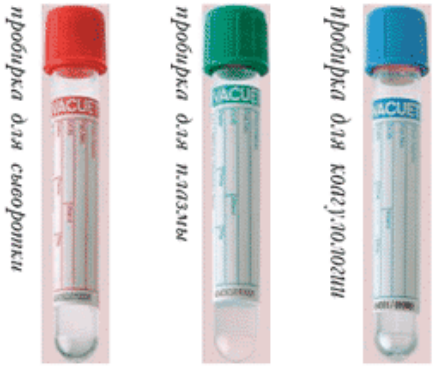
Taking blood from the vessels of the tail

Serum selection for serological studies

Serums are delivered to the laboratory for a week in a non-frozen form. If the delivery time of samples to the laboratory is more than a week, then they must be frozen

Hemolysis serum is not suitable for the research!

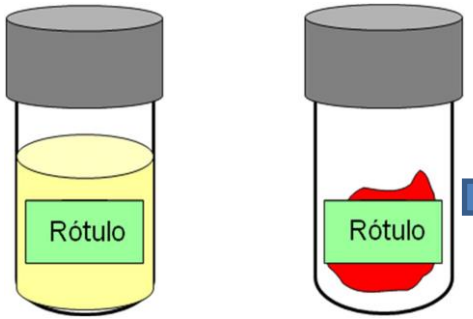




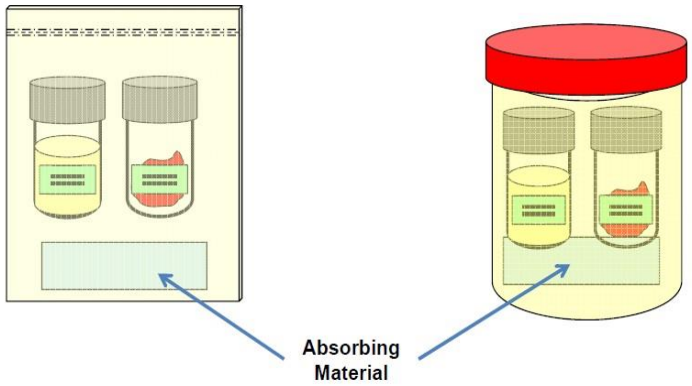
The noncontact method of selection of saliva in a wild boar

Packing and transportation of samples

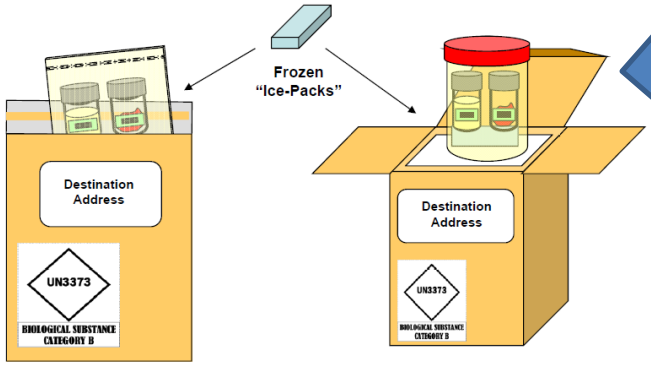
Step 1:



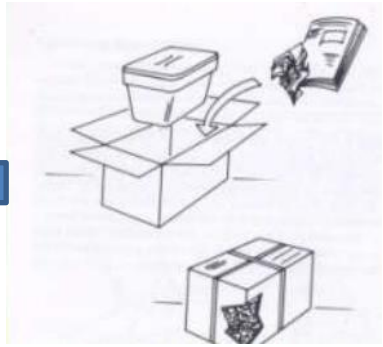
Step 2:



Step 3:



Step 4:



Lab

Supporting documentation
(domestic pigs)

- Name of the farm
- Phone numbers, postal address, E-mail
- Inventory of samples (by end-to-end numbering)
- List of necessary studies
- Date of sampling
- Epizootic information (livestock of animals, the date of origin and description of clinical signs of the disease, the number of diseased and dead animals)
- Information about vaccination, the date of the last vaccination, the series of vaccine used

Supporting documentation
(wild boar)

- Name of the hunter
- Hunter's address and phone number
- Type of sex-age belonging of an animal
- Type of study, name of the disease
- Type of send sample
- Number of samples
- Place of boar shooting or boar corpse detection
- Coordinates of the sending person, organization

Notes

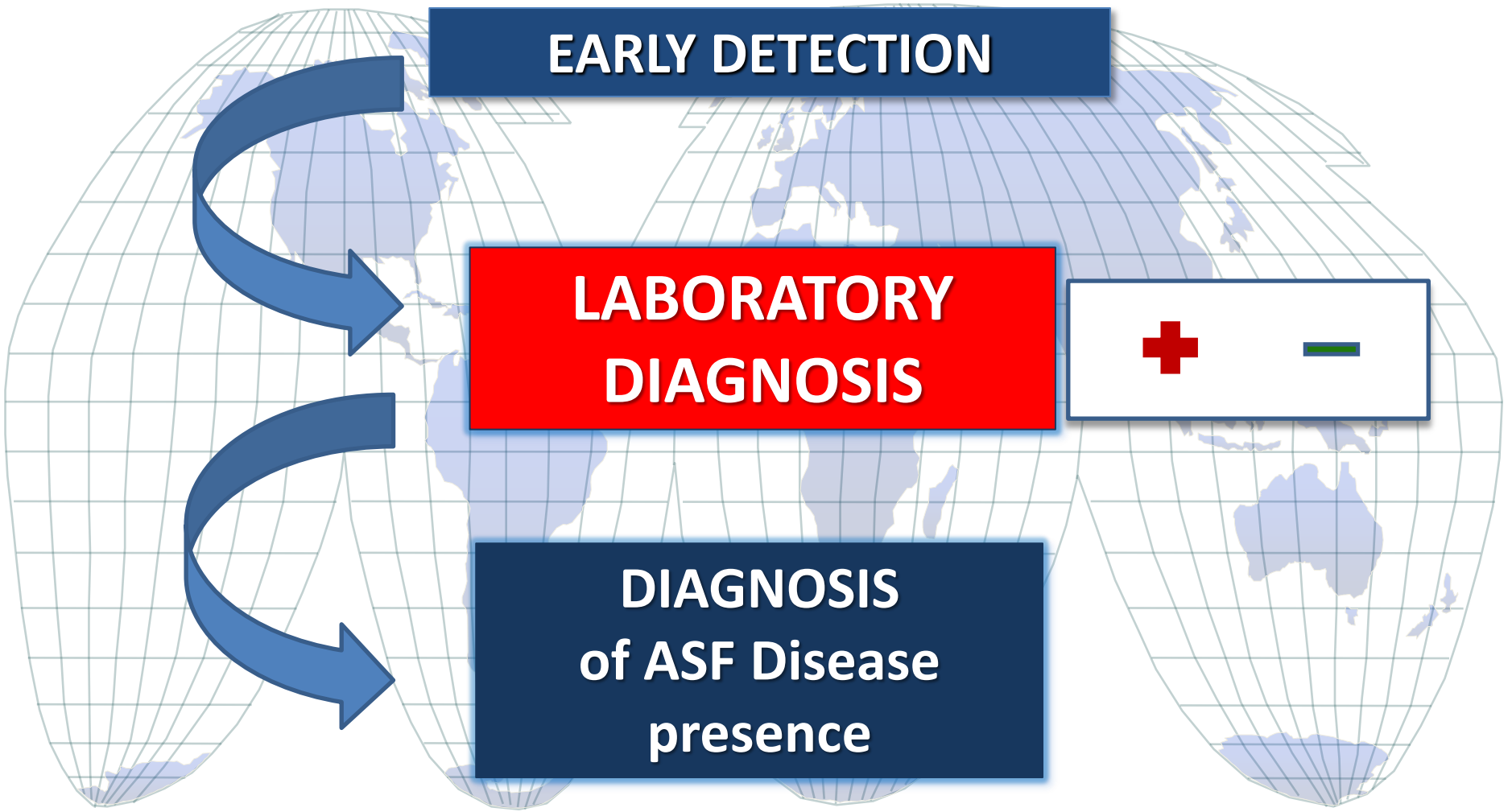
- It is required to avoid sending, diagnostic samples packed with "dry ice". There are many restrictions for using dry ice in a passenger airplane or in closed transport (trucks);
- When dispatching liquids by air, containers designed for an internal pressure of 95 kPa should be used.

Infringements at packing of a material

After collection and packaging of samples

- Disinfection of the sampling site
- One-time clothing is destroyed by burning
- Working clothes for multiple research are packed in 2 polyethylene bags before transport to the place where it can be decontaminated

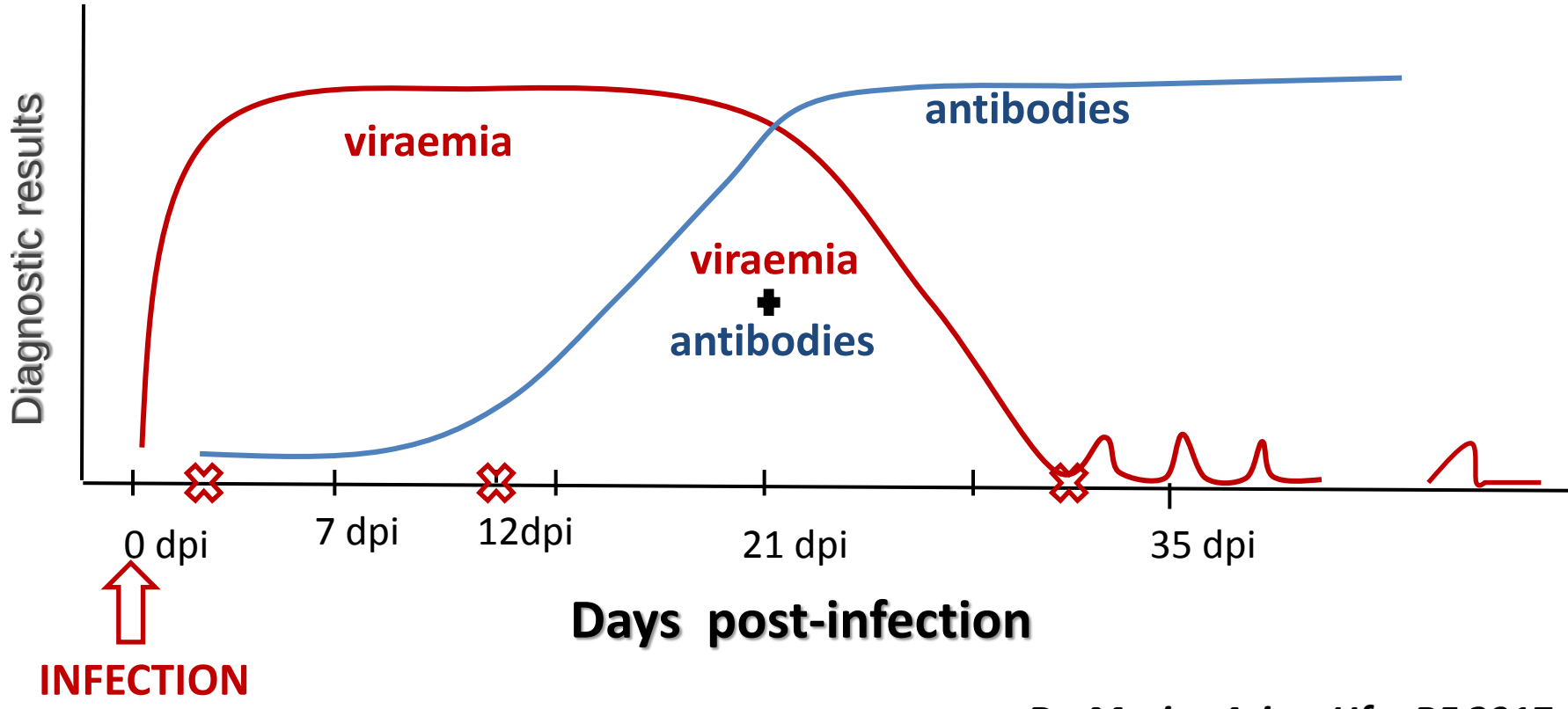
Samples of pathological material for study in a sealed container sent by courier as soon as possible, not exceeding 24 hours from the time of selection


EARLY DETECTION
LABORATORY DIAGNOSIS
+
–
DIAGNOSIS of ASF Disease presence

Dynamic of infection / Diagnostic interpretation

DISEASE- DEATHS

RECOVERING SURVIVORS



Dr. Marisa Arias, Ufa, RF 2017

AVAILABLE DIAGNOSTIC TESTS

VIRUS DETECTION TECHNIQUES

AVAILABLE TESTS		TYPE, In house/ Commercial	Recommended Use	REFERENCE
Virus Isolation		*VI /Haemadsorption (HAD) test (i.h.)	Confirmation of primary outbreak.	Malmquist and Hay, 1960
Antigen detection		*Direct Immuno fluorescence (FAT) (i.h.)	Individual testing	Bool et al., 1969
		ELISAIngezim-K2, Double AbSandwich/ Commercial	Surveillance Herd testing	INGENASA
		ELISA (i.h.) Penside test	Not in use Surveillance, individual testing	Pastor et al.1990; Hutchings and Ferris, 2006; Sastre et al, 2016 ;
PCR	**Conventional**	*Conventional (i.h.)	Surveillance Individual and Herd testing	*Aguero et al. 2003.
		Multiplex ASF-CSF (i.h.)	Co-circulation ASF and CSF	Aguero et al. 2004.
	Real Time	Taqman Probe (i.h.)	Surveillance Individual and herd testing	*King et al., 2003; *Zsack et al. 2005; Tignon et al. 2011
		UPL Probe (i.h.)	Surveillance Individual and herd testing	Fernandez-Pinero et al. 2013
		MGB Probe (i.h.)	Not in use	McKillen et al., 2010
		TETRACORE dried down (Commercial)	Surveillance Individual and herd testing	TETRACORE
		Multiplex ASF-CSF (i.h.)	Co-circulation ASF and CSF	Haines et al.2013
Isothermal Tests		Invader Assay	Not in use	Hjertner et al., 2005
		LAMP assay	Not in use	James et al., 2010

*Included in the OIE Terrestrial Manual for Diagnostic Test and Vaccines, 2012.

AVAILABLE DIAGNOSTIC TESTS

ANTIBODY DETECTION TECHNIQUES

AVAILABLE TESTS	TYPE, In house/ Commercial	Recommended Use	REFERENCE
ELISA Tests	*OIE Indirect ELISA (i.h.)	Surveillance Herd testing	Sánchez-Vizcaíno et al.1982; Pastor et al., 1990.
	Recombinant proteins (rp)-ELISA (i.h.)	Surveillance Herd testing	Gallardo et al. 2006,2009, Pérez-Filgueira et al,, 2006
	ELISA Ingezim-K3, Bloking/Commercial,	Surveillance Herd testing	INGENASA
	ELISA ID-VET Indirect/Commercial	Surveillance Herd testing	Not available
	ELISA-Svanova Indirect/Commercial	Surveillance Herd testing	Not available
Pen side Tests	Ingezim PPA-CROM Commercial	Surveillance Individual Testing	INGENASA
	Dot Blot (i.h.)	Surveillance Individual Testing	Pastor et al. 1992
Confirmatory Antibody tests	*Immunoblot (IB) Test (i.h.)	Confirmatory Herd testing	Pastor et al. 1989
	*Immunofluorescence Antibody (IFA) test (i.h.)	Confirmatory Herd testing	Pan et al., 1974
	Indirect Immunoperoxidase test (IPT)	Confirmatory Herd testing	Gallardo et al.2013

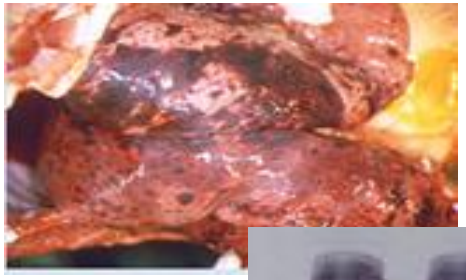
Included in the OIE Terrestrial Manual for Diagnostic Test and Vaccines, 2012.

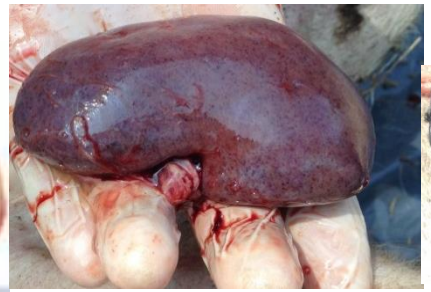
Sensitivity of diagnostic methods for ASF

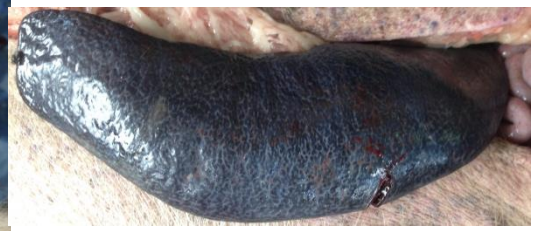
- The bioassay allows to reveal 0,1-1 HAD
- Hemadsorption reaction - 1 HAD
- Reaction of direct immunofluorescence 10-100 HAD
- PCR - 10-100 HAD
- ELISA - 100-1000 HAD
- Immunochromatography - 1000-10000 HAD

Material used for the diagnosis of ASF

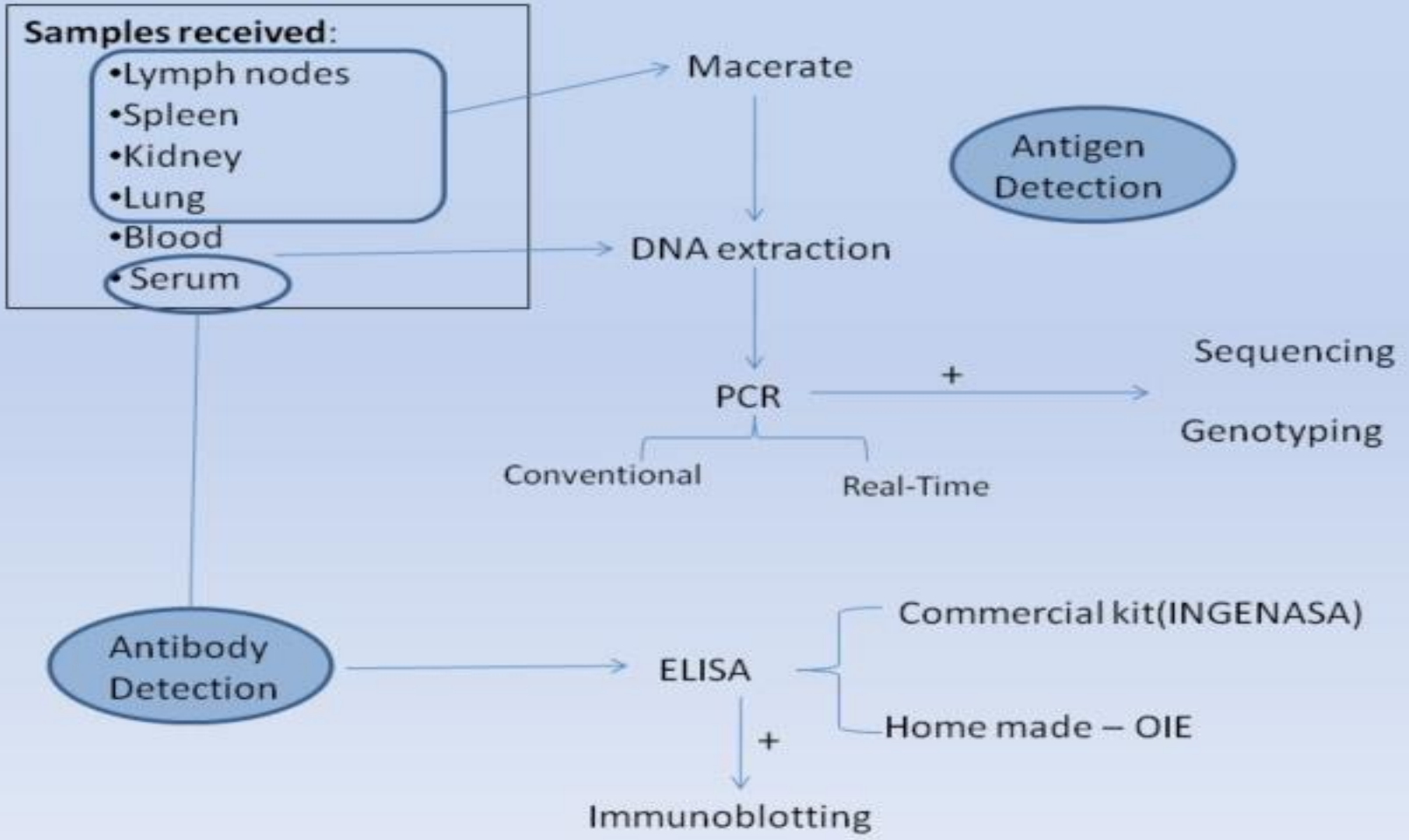
- Pieces of organs: spleen, kidney, lungs, lymph nodes
- Tubular bone
- Blood with anticoagulant
- Swabs
- Blood serum







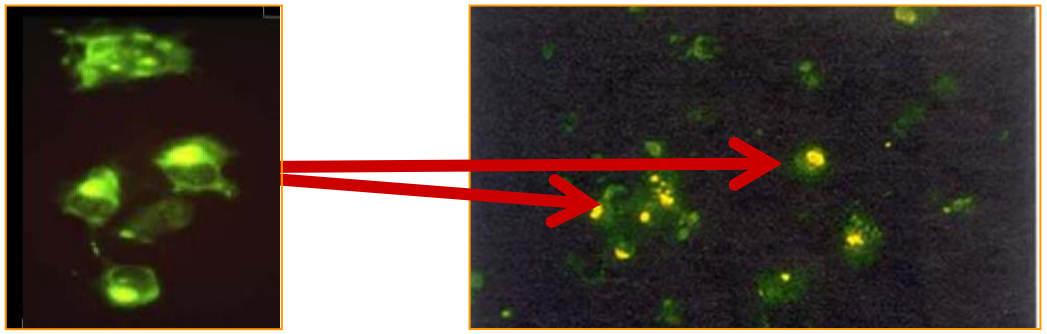
LABORATORY PROTOCOL MOST FREQUENTLY USED



Laboratory diagnostics

1. Isolation and identification of the virus:

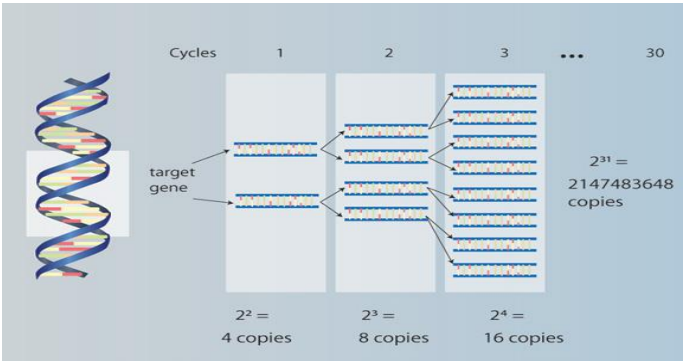
- inoculation of the primary culture of porcine monocytes / macrophages or bone marrow cells (most virus isolates cause haemadsorption);
- inoculation of pigs not vaccinated and vaccinated against CSF (specific bioassay);
- antigen determination by the method of direct immunofluorescence ins mears-prints (Ag detection).



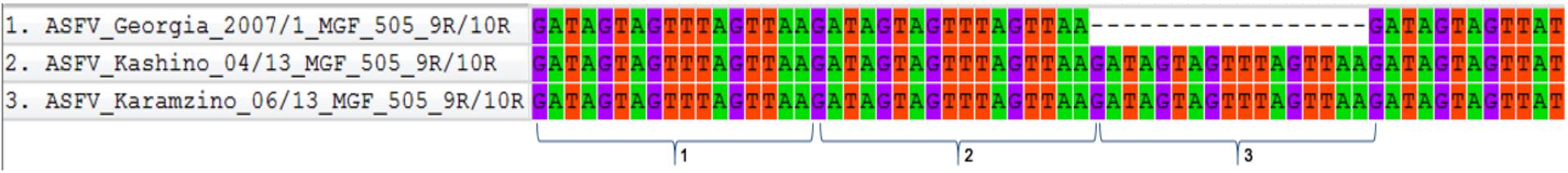
2. Molecular genetic methods for diagnosis of ASF

The method of polymerase chain reaction (PCR):

- Conventional;
- PCR in real time;



The Sequencing



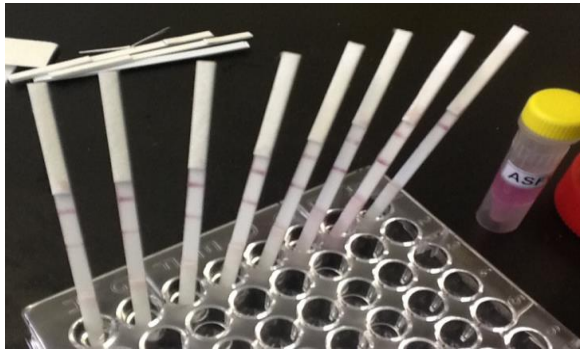
3. Serological tests :

- is detection of group-specific (common) antibodies:

- ELISA;
- Indirect Immunoperoxidase test (IPT);
- Immunoblotting;

- typing of isolates in delayed haemadsorption.

4. Immunochromatographic test system for ASF express diagnosis



Recording and interpretation of the results

«C» - control zone

«T» - test zone

«S» - applied sample

Positive result

Negative result

- Monoclonal antibody-based immunochromatographic test system to vp72 of ASF virus
- Time = 5-10 min
- Used in the field

"Gold standard" in diagnostics of ASF:

- ✓ gemadsorption reaction
- ✓ direct immunofluorescence method
- ✓ Indirect Immunoperoxidase test (IPT)
- ✓ PCR

Differential diagnostics:

- CSF, Ayesky disease, swine erysipelas, pasterellosis and ets.

ANALYSIS OF THE RESULTS OBTAINED BY FEDERAL CENTRE FOR ANIMAL HEALTH (FGBI "ARRIAH") *(laboratory designation code 45).*

REPORT

RESULTS OBTAINED IN THE

XIV INTERLABORATORY COMPARISON TEST FOR

AFRICAN SWINE FEVER 2017 (ASF-ILCT 2017) BY

NATIONAL REFERENCE LABORATORIES (NRLs)

ORGANIZED BY THE

European Union Reference Laboratory for ASF

Centro de Investigación en Sanidad Animal (CISA-INIA)

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GOBIERNO DE ESPAÑA | MINISTERIO DE ECONOMÍA, INDUSTRIA Y COMPETITIVIDAD | INIA Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria

	AB DETECTION		VIRUS DETECTION		ASF diagnostic conclusion	
	EURL	*ID 45*	*EURL*	*ID 45*	*EURL*	*ID 45*
S1	NEG	neg	NEG	neg	NEGATIVE	negative
S2	NEG	neg	NEG	neg	NEGATIVE	negative
S3	POS	pos	WEAK	pos	POSITIVE	positive
S4	POS	pos	WEAK	neg	POSITIVE	positive
S5	POS	pos	POS	pos	POSITIVE	positive
S6	NEG	neg	POS	pos	POSITIVE	positive
S7	NEG	neg	POS	pos	POSITIVE	positive
S8	WEAK	pos	POS	pos	POSITIVE	positive
S9	POS	pos	NEG	neg	POSITIVE	positive
S10	POS	pos	POS	pos	POSITIVE	positive
S11	NEG	neg	NEG	neg	NEGATIVE	negative
T1			NEG	neg	NEGATIVE	negative
T2			POS	pos	POSITIVE	positive
T3			POS	pos	POSITIVE	positive
T4			POS	pos	POSITIVE	positive
T5			NEG	neg	NEGATIVE	negative
T6			POS	pos	POSITIVE	positive
T7			POS	pos	POSITIVE	positive

In Valdeolmos 31th March 2017,

Approval

Dr. Virginia Pelayo
Laboratory scientist at ASF-URL

Dr. Carmina Gallardo
Researcher, Laboratory Coordinator
EU reference laboratory for ASF
CISA-INIA

Conclusions:

- Early detection and qualitative laboratory diagnostics are the basis of timely measures taken to eliminate the outbreaks of ASF
- Training of personnel in the principles and methods of setting up laboratory studies is the key to the success of establishing an accurate diagnosis for ASF
- Participation in qualification tests helps to verify the competence of the laboratory in conducting research

Thank you for attention !

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