

### Objective

African swine fever (ASF) is an OIE notifiable viral disease, which provokes severe economic losses and for which no vaccine is currently available. Last years in Ukraine emerged very difficult situation with regard to ASF. However ASF problem - it's not just a question of the security of Ukraine, but also the European Union as a whole.

Effective modern method of early diagnosis of ASF is a polymerase chain reaction (PCR). That's why by scientists of our institute developed, tested and registered in the established order the test kit for the diagnosis of ASF for molecular genetic techniques.

### Methods

We have analyzed the situation that appeared to Ukraine with regard to ASF.

Development of a diagnostic test kit for PCR was carried out with the recommendations of OIE. Testing of specificity and sensitivity was performed on the sample, the total DNA isolated from pigs that died from ASF and of the recombinant plasmids carrying the gene fragment of the main ASF virus capsid protein. As a negative control were used materials from healthy domestic pigs. Activity and specificity of the means of has been assessed comparing with similar imported commercial test kits.

### Results

In Ukraine, the first case of ASF was registered in 1977 year in the Odeska region, where ASF was subsequently controlled through culling of the entire pig population within a 30-kilometer radius from the outbreak source. The next case was detected in 2012 year in domestic swine in the Zaporizhia region. Control measures were taken to bring the spread of the virus to a halt, but new cases of ASF were reported in 2014 (16 cases of ASF, 12 - in wild boars and 4 - in domestic pigs), 2015 (39 cases of ASF, 5 - in wild boars and 34 - in domestic pigs) and 2016 (98 cases, 7 - in wild boars and 91 - in domestic pigs). **The situation with regard to wild and domestic pigs changed in 2015-2016** (Fig.1, 2). Sequencing of three independent areas of the genome of ASFV from Ukrainian isolates showed that the isolates were 100% homologous with the genotype II isolate that caused the outbreaks in Eastern Europe, which started with the entry of the virus in Georgia in 2007. The virus strain that caused the disease of ASF in Ukraine include to genotype II. Course of the disease of ASF at pigs in Ukraine is: the incubation period duration of 2-6 days. For II genotype of ASF, typically disease takes place in the acute form, out within 3-7 days.

**Simple diagnostic kit based on the classical PCR variant has been developed in SSCIBSM.** It is aimed on quick detection of ACFV DNA (amplification of conservative regions B646L gene (VP72) (Fig.3), protein is the main component of the viral capsid) (Cobbold and Wileman, 1998) in biological materials and environment (Fig.4). We modified the 3'-ends of the oligonucleotide primers recommended by OIE (Fig.5, 6) for this gene and this gave the possibility to increase their annealing temperature by 5 degrees and as a result to improve reaction specificity (Fig.7, 8). Sequences of the modified primers: mod-Sn 5'-ATGGATACCGAGGGAATAGCAAG-3' and mod-Asn 5'-TACCGATGAAAATGATACGCAGC-3'. DNA from recombinant plasmid pB646 is used as positive control.

Diagnostic kit is developed in two variants on test system that differ in the technique of DNA isolation. In version A, DNA isolation is performed with the use of sorbent, which has to be precipitated by centrifugation. In variant B DNA isolation is performed with the use of Ukrainian magnetite nanosorbent with saturation magnetization 37 (A·m<sup>2</sup>/kg), it can be precipitated in special magnetic holder (support, stand) without centrifugation. Such variant of DNA isolation not only minimizes the risk of contamination of the surrounding surface of the test material, but also can be used in the field.

Established that developed by us test system is specific (100%) and sensitive enough and not inferior in quality research world standards.

### Conclusions

In Ukraine, in 2012-2016 emerged a very complicated situation with regard to ASF.

Have been developed highly sensitive, highly specific and not very expensive diagnostic test kit for the diagnosis of ASF for PCR. Using this kit possible not only carry out rapid identification of the causative agent in acute forms of the disease, but also to ensure control of imported products during the quarantine of measures, necessary to detect early signs of disease.

### Outcomes

Implementation of the developed diagnostic means in practice, we hope, will contribute to improve epizootic situation with regard to ASF in Ukraine.

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### Situation with regard to the ASF in Ukraine in 2012-2016 years

Years	Domestic swine	Wild boars	Total
2012	1	0	1
2013	0	0	0
2014	4	12	16
2015	34	5	39
2016	91	7	98
Total	130	24	154

Fig.1

Fig.2



### The comparative position of modified 3'-END primers on the nucleotide sequence of the gene B646L

	mod-Sn 100.0%	Sn 100.0%
99101	AGCGCTCGCC GAAGGGGATG GATACCGAGG GAATAGCAAG GTTACCGTTC TCGTTAAACC AAAAGCGCAG CTTAATCCAG AGCGCAAGAG GGGGCTGATA TCGCGAGCGG CTTCCTTAC CTATGGCTCC CTTATCGTTC CAAGTGCAAG AGCAATTTGG TTTTCGCGTC GAATTAGTTC TCGCGTTCTC CCCCGACTAT	
99201	GTATTTAGGG GTTTGAGGTC CATTACAGCT GTAATGAACA TTACGTCTTA TGTCAGATA CGTTGCGTCC GTAATAGGAG TAATATCTTG TTTACCTGCT CATAAATCCC CAAACTCCAG GTAATGTCGA CATTACTTGT AATGCAGAAT ACAGGTCTAT GCAACGCAGG CATTATCCTC ATTATAGAAC AAATGGACGA	
99301	GTTTGGATAT TGTGAGAGTT CTCGGGAAAA TGTGTGAAAA GGAATTTCCG GTTGGTATGG CTGCACGTTT GCTGCGTATC ATTTTCATCG GTAAGAATAG CAACCTATA ACATCTCAA GAGCCCTTTT ACAACACTTT CCTTAAAGCC CAACCATACC GACGTGCAAG	

Fig.3

### Results of analysis the samples of pathological materials to source (OIE) and modified primers in PCR

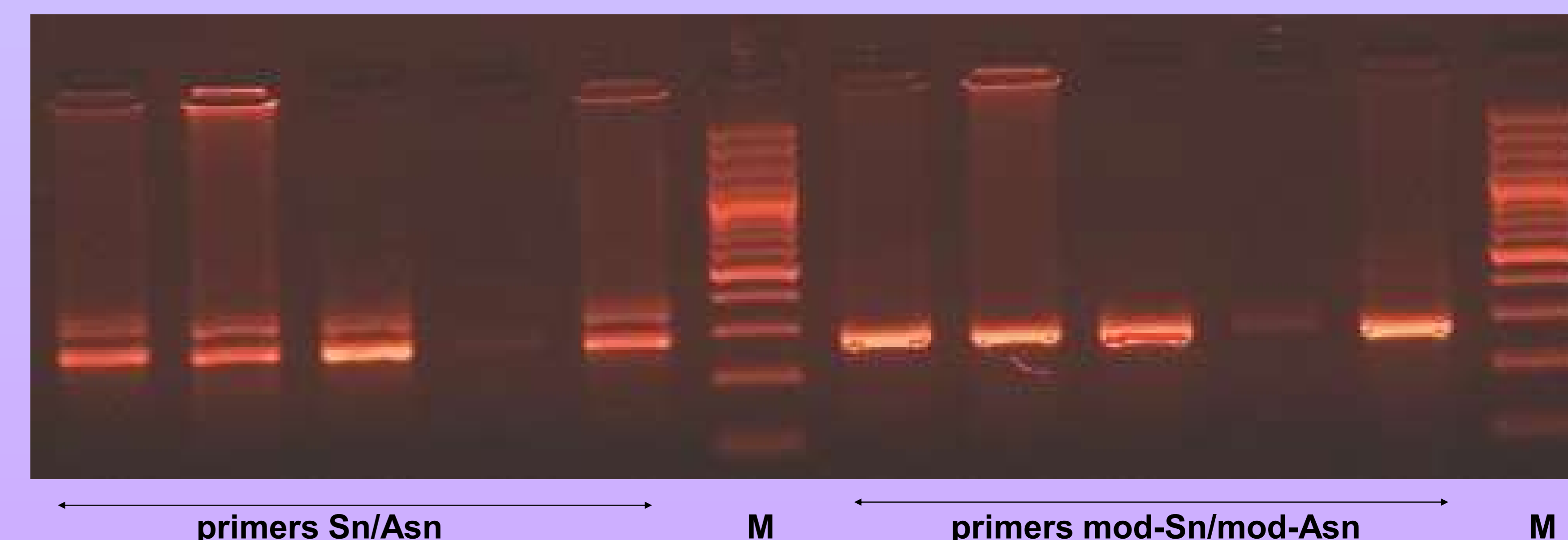


Fig.4

### Comparative characteristics of oligonucleotide primers the recommended by the OIE (Fig.5, 6) and modified (Fig.7, 8)

Oligonucleotide: Sn Length: 20		Results		Palindromes: 0 total	
[ATGGATACCGAGGGAATAGCAAG]					
Parameters: <input checked="" type="checkbox"/> DNA <input type="checkbox"/> RNA <input type="checkbox"/> Complementary Analyze Save Results Close					
dG Temperature (C)	25.0	Mol. Wt	6277.1		
Probe Conc. (pMol)	250.0	%GC	50.0		
Salt Conc. (mMol)	50.0	Therm. Tm	49.9		
% Formamide	0.0	%GC Tm	46.7		
3' End Length (bp)	7	dG	-33.5	Repeats: 0 total	
Palindromes (bp)	6	3' End dG	-13.8		
Nucl. Repeats (bp)	4	dH	-158.6		
Stem Length (bp)	3	dS	-413.5		

Fig.5

Oligonucleotide: Asn Length: 20		Results		Palindromes: 0 total	
[CTTACCGATGAAAATGATAC]					
Parameters: <input checked="" type="checkbox"/> DNA <input type="checkbox"/> RNA <input type="checkbox"/> Complementary Analyze Save Results Close					
dG Temperature (C)	25.0	Mol. Wt	6171.1		
Probe Conc. (pMol)	250.0	%GC	35.0		
Salt Conc. (mMol)	50.0	Therm. Tm	41.5		
% Formamide	0.0	%GC Tm	40.5		
3' End Length (bp)	7	dG	-28.7	Repeats: 1 total	
Palindromes (bp)	6	3' End dG	-10.7	AAAA at 12	
Nucl. Repeats (bp)	4	dH	-146.3		
Stem Length (bp)	3	dS	-388.4		

Fig.6

Oligonucleotide: mod-Sn Length: 23		Results		Palindromes: 0 total	
[ATGGATACCGAGGGAATAGCAAG]					
Parameters: <input checked="" type="checkbox"/> DNA <input type="checkbox"/> RNA <input type="checkbox"/> Complementary Analyze Save Results Close					
dG Temperature (C)	25.0	Mol. Wt	7232.7		
Probe Conc. (pMol)	250.0	%GC	47.8		
Salt Conc. (mMol)	50.0	Therm. Tm	55.3		
% Formamide	0.0	%GC Tm	50.2		
3' End Length (bp)	7	dG	-39.0	Repeats: 0 total	
Palindromes (bp)	6	3' End dG	-12.7		
Nucl. Repeats (bp)	4	dH	-181.3		
Stem Length (bp)	3	dS	-471.2		

Fig.7

Oligonucleotide: mod-Asn Length: 23		Results		Palindromes: 0 total	
[TACCGATGAAAATGATACGCAGC]					
Parameters: <input checked="" type="checkbox"/> DNA <input type="checkbox"/> RNA <input type="checkbox"/> Complementary Analyze Save Results Close					
dG Temperature (C)	25.0	Mol. Wt	7127.7		
Probe Conc. (pMol)	250.0	%GC	43.5		
Salt Conc. (mMol)	50.0	Therm. Tm	55.8		
% Formamide	0.0	%GC Tm	48.4		
3' End Length (bp)	7	dG	-38.6	Repeats: 1 total	
Palindromes (bp)	6	3' End dG	-15.7	AAAA at 10	
Nucl. Repeats (bp)	4	dH	-177.1		
Stem Length (bp)	3	dS	-458.5		

Fig.8