



NATIONAL VETERINARY RESEARCH INSTITUTE

al. Partyzantow 57
24-100 Pulawy, POLAND
<http://www.piwet.pulawy.pl>

phone +48 81 889 30 00
fax +48 81 886 25 95
sekretariat@piwet.pulawy.pl

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The OPINION

of National Veterinary Research Institute in Pulawy on the product of
an *in vitro* diagnostic used in veterinary medicine:

VDx ® ASFV qPCR Kit
(Cat. No. NS-ASF-31v)

Real-time PCR (qPCR) kit used for the detection of viral DNA of African Swine Fever Virus (ASFV)
Serial number: 100T. Exp. Date: 30.09.2019.

1. Applicant

Median Diagnostics Inc.

2. The producer/manufacturer

Median Diagnostics Inc., tel. +82-33-244-0100
878, Sunhwan-daero, Dongnae-myeon, Chuncheon-si, Gangwon-do, South Korea

3. The distributor

Median Diagnostics Inc., tel. +82-33-244-0100
878, Sunhwan-daero, Dongnae-myeon, Chuncheon-si, Gangwon-do, South Korea

4. Trade name of the product

“VDx ® ASFV qPCR Kit”

5. The technical and medical name of the product

„VDX® ASFV qPCR Kit is used for the detection of viral DNA of African Swine Fever Virus (ASFV) by real-time PCR method”

6. Product description, intended purpose and restrictions on the use of the product

VDX® ASFV qPCR Kit is used for the detection of viral DNA of African Swine Fever Virus (ASFV) by real-time PCR method. This kit can measure the p72 gene of ASFV quantitatively by using TaqMan probe. The test samples appropriate to the kit: whole blood, serum and tissue homogenates from pigs (the samples should be kept as fresh as possible and frozen during storage). Required volume to the extraction of sample: 100~300 µl (recommended extraction kit: QIAmp Viral RNA Kit - Qiagen).

The product contents: 2x qPCR Master mix (A) (1 ml), 4x Oligo mix (B) (500 µl), Control DNA (100 µl), Manual (1 ea in English language). In the manual there were written also storage conditions of the ingredients (-20°C: kit is stable for 1 year), materials required (but not supplied), precautions to avoid contamination, template preparation, required equipment, preparation of the qPCR, programming the amplification, assay validation, suggested interpretation of results.

To conduct the real-time PCR experiment using VDX® ASFV qPCR Kit there is required thermal cycler capable of reading Fluorescence Dyes FAM and HEX (or VIC). Example of compatible thermal cycler: CFX96 Biorad, LC96 Roche, 7500 AB, Rotor-Gene Q Qiagen. The kit contains internal process control of amplification (detected using HEX/VIC channel).

7. Evaluation of product validation documentation

Meets the requirements.

8. Results of clinical sensitivity (sensitivity, specificity, repeatability)

The real-time PCR kit was obtained to control using:

- Positive control applied with VDX® ASFV qPCR Kit (according to the manufacturer's recommendation).
- Internal process control included in qPCR Master mix (A) (according to the manufacturer's recommendation).
- Negative control: DNase and RNase-free autoclaved D.W. (according to the manufacturer's recommendation).
- PCR Positive control: MO/060/17 1:100 (dilution).
- Panel of DNA samples delivered from EURL in Valdeolmos in Spain (ASFV gen. I, II, V, VIII, IX, X).
- Panel of DNA samples extracted from wild boars and pig tissues (ASFV positive and negative) in National Veterinary Research Institute in Puławy (Poland 2018). All of the samples used for the evaluation were previously tested with the real-time PCR UPL tests recommended by EURL in Valdeolmos in Spain and by the International Animal Health Organization (OIE). In addition, the compliance of the obtained results with the EU requirements included in the Decision of the European Union commission No. 2003/422 EC of May 26, 2003 approving the Diagnostic Manual establishing diagnostic procedures, sampling methods and criteria for evaluation of laboratory tests results to confirm ASF (Polish Journal of Laws: Dz. U. L.143 z 11.06.2003., page 47).

Sensitivity of the assay was assessed by testing ASFV positive DNA samples derived from pigs and wild boars. Specificity was assessed by examination of samples in the ASFV negative wild boars and pigs. Repeatability of the estimated results was assessed by the level of compatibility between

successive measurements under the same conditions performed by the same person. The tests were carried out in accordance with the procedure contained in the kit provided from the producer.

The DNA were extracted using QIAamp DNA Mini Kit (Qiagen). The thermocycler used in experiment was 7500 AB.

The test validity criteria have been complied.

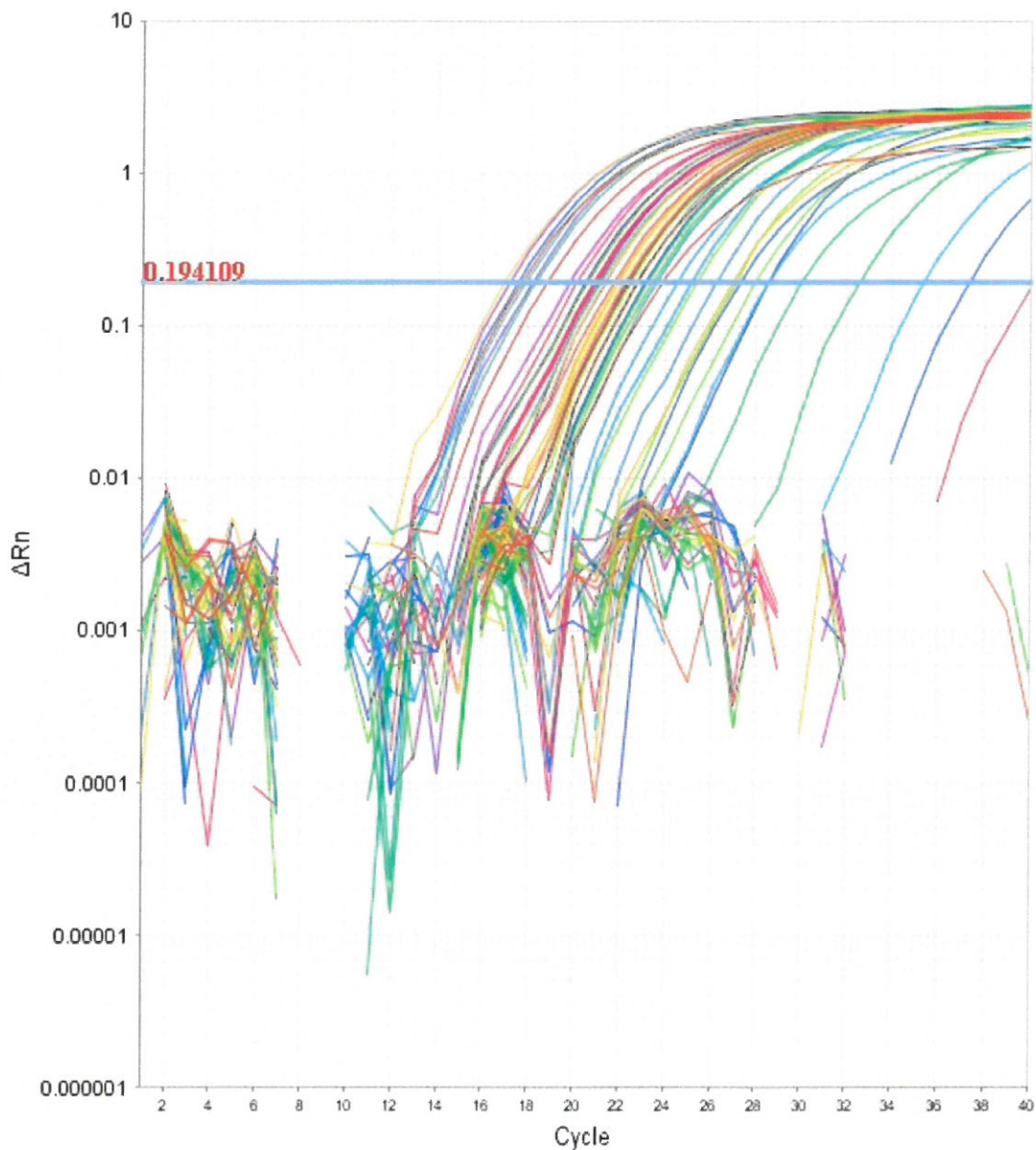


Fig. 1. Detection of ASFV DNA using VDX® ASFV qPCR Kit. Detection in FAM channel.

The results obtained with use of both panels (from EURL Valdeolmos and NVRI Puławy) were identity in 100% with intended samples status. It was concluded 100% of sensitivity, specificity and repeatability of results.

Figure 1 shows the fluorescence curves in FAM channel. Figure 2 shows the fluorescence curves in VIC channel. The Table 1 present the collected detailed results from experiment.

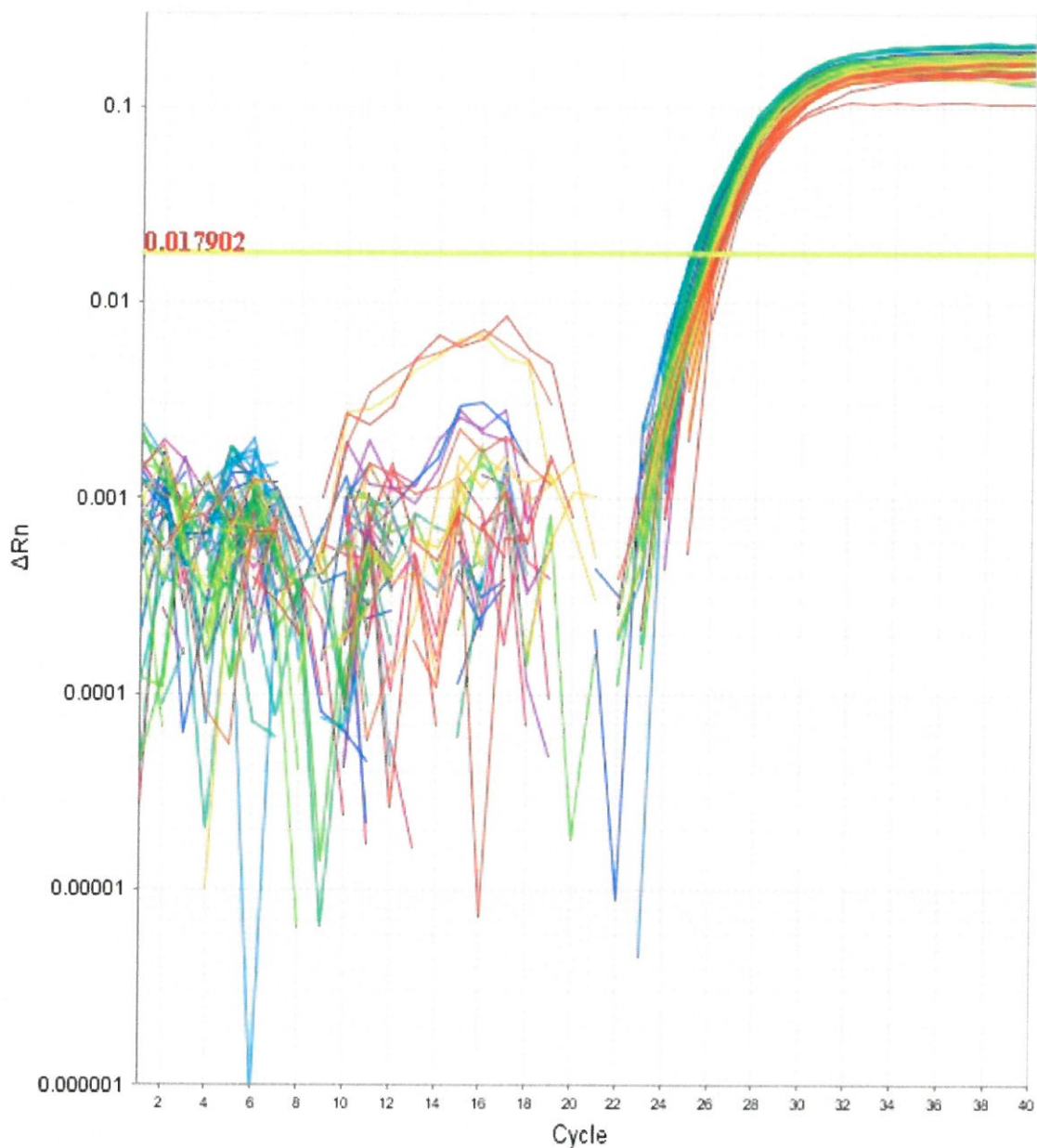


Fig. 2. Detection of IPC using VDX® ASFV qPCR Kit (internal process control). Detection in VIC channel.

In addition, thanks to internal process control (target IPC) it was possible to check the potential inhibition of amplification. It was positive results in all samples, real-time PCR process was conducted correctly (Fig. 2, Tab. 1A-B, Tab. 2).

Test No.	Sample ID	Origin	Animal	Tissue	Isolation Year	Genotype	Ct Value	Result	IPC Value	Result
1	Ang72	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen I	23,84	Positive	26,04	Positive
2	LT14/1490	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen II	26,9	Positive	25,98	Positive

3	Moz64	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen V	27,94	Positive	25,94	Positive
4	MwLil 20/1	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen VIII	29,97	Positive	26,1	Positive
5	Ken06.Bus	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen IX	28,53	Positive	26	Positive
6	Ug64	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen X	27,21	Positive	25,8	Positive
7	18/69380(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	21,02	Positive	25,61	Positive
8	18/69302(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	20,97	Positive	25,72	Positive
9	18/70239(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	22,33	Positive	26,3	Positive
10	18/70253(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	21,9	Positive	26,14	Positive
11	18/70253(2)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	25,31	Positive	25,88	Positive
12	18/70549(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	22,74	Positive	25,58	Positive
13	18/68916(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	25,07	Positive	25,84	Positive
14	18/68914(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	23,1	Positive	25,69	Positive
15	18/69318(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	21,32	Positive	25,6	Positive
16	18/69318(2)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	20,84	Positive	25,56	Positive
17	18/69315(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	22,76	Positive	26,12	Positive
18	18/69321(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	21,9	Positive	25,88	Positive
19	18/69320(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	23,38	Positive	25,49	Positive
20	18/69319(4)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	21,11	Positive	25,16	Positive
21	18/69319(3)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	23,59	Positive	25,4	Positive
22	18/55330(1)	NVRI (Poland)	Pig	spleen	2018	gen II	17,55	Positive	25,44	Positive
23	18/55330(1)	NVRI (Poland)	Pig	lung	2018	gen II	18,18	Positive	25,59	Positive
24	18/55330(1)	NVRI (Poland)	Pig	kidney	2018	gen II	22,23	Positive	25,85	Positive
25	18/64917(1)	NVRI (Poland)	Pig	lung	2018	gen II	21,69	Positive	26,12	Positive
26	18/64917(1)	NVRI (Poland)	Pig	spleen	2018	gen II	21,33	Positive	25,94	Positive
27	18/64917(1)	NVRI (Poland)	Pig	lymph node	2018	gen II	22,42	Positive	25,77	Positive
28	18/53258(1)	NVRI (Poland)	Pig	lung	2018	gen II	23,14	Positive	25,24	Positive
29	18/53258(1)	NVRI (Poland)	Pig	lymph node	2018	gen II	24,48	Positive	25,03	Positive
30	18/53258(1)	NVRI (Poland)	Pig	spleen	2018	gen II	20,5	Positive	25	Positive

31	18/50097(1)	NVRI (Poland)	Pig	spleen	2018	gen II	17,17	Positive	25,32	Positive
32	18/50097(1)	NVRI (Poland)	Pig	kidney	2018	gen II	20,92	Positive	25,73	Positive
33	18/50097(1)	NVRI (Poland)	Pig	lung	2018	gen II	17,72	Positive	26,16	Positive
34	18/50098(1)	NVRI (Poland)	Pig	spleen	2018	gen II	16,97	Positive	25,87	Positive
35	18/50098(1)	NVRI (Poland)	Pig	kidney	2018	gen II	20,59	Positive	25,71	Positive
36	18/50098(1)	NVRI (Poland)	Pig	lung	2018	gen II	18,32	Positive	25,09	Positive
37	18/50099(1)	NVRI (Poland)	Pig	spleen	2018	gen II	17,89	Positive	25,03	Positive
38	18/50099(1)	NVRI (Poland)	Pig	kidney	2018	gen II	22,34	Positive	25,36	Positive
39	18/50099(1)	NVRI (Poland)	Pig	lung	2018	gen II	19,84	Positive	25,37	Positive
40	18/50101(1)	NVRI (Poland)	Pig	blood	2018	gen II	20,13	Positive	25,45	Positive
41	18/70757(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	-	NoCt	Negative	26,15	Positive
42	18/70757(1)	NVRI (Poland)	Wild Boar	bone marrow 1:10	2018	-	NoCt	Negative	26,09	Positive
43	18/70797(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	-	NoCt	Negative	26,08	Positive
44	18/70797(1)	NVRI (Poland)	Wild Boar	bone marrow 1:10	2018	-	NoCt	Negative	25,97	Positive
45	18/70377(1)	NVRI (Poland)	Wild Boar	spleen	2018	-	NoCt	Negative	26,12	Positive
46	18/70824(1)	NVRI (Poland)	Pig	spleen	2018	-	NoCt	Negative	25,99	Positive
47	18/70824(1)	NVRI (Poland)	Pig	kidney	2018	-	NoCt	Negative	25,14	Positive
48	18/70824(1)	NVRI (Poland)	Pig	lung	2018	-	NoCt	Negative	25,96	Positive
49	18/70825(1)	NVRI (Poland)	Pig	spleen	2018	-	NoCt	Negative	26,08	Positive
50	18/70825(1)	NVRI (Poland)	Pig	kidney	2018	-	NoCt	Negative	25,93	Positive
51	18/70825(1)	NVRI (Poland)	Pig	lung	2018	-	NoCt	Negative	25,78	Positive
52	18/70826(1)	NVRI (Poland)	Pig	spleen	2018	-	NoCt	Negative	26,13	Positive
53	18/70826(1)	NVRI (Poland)	Pig	kidney	2018	-	NoCt	Negative	26,07	Positive
54	18/70826(1)	NVRI (Poland)	Pig	lung	2018	-	NoCt	Negative	26,14	Positive
55	18/70827(1)	NVRI (Poland)	Pig	spleen	2018	-	NoCt	Negative	26,14	Positive
56	18/70827(1)	NVRI (Poland)	Pig	kidney	2018	-	NoCt	Negative	25,78	Positive
57	18/70827(1)	NVRI (Poland)	Pig	tonsil	2018	-	NoCt	Negative	26,01	Positive
58	18/70828(1)	NVRI (Poland)	Pig	kidney	2018	-	NoCt	Negative	25,96	Positive

59	18/70828(1)	NVRI (Poland)	Pig	lung	2018	-	NoCt	Negative	25,83	Positive
60	18/70828(1)	NVRI (Poland)	Pig	tonsil	2018	-	NoCt	Negative	25,87	Positive
61	K+PCR	VDx control	-	-	2018	-	26,16	Positive	25,81	Positive
62	MO/060/17 1:100	ASFV DNA	-	-	2017	gen II	28,54	Positive	26,05	Positive
63	K-PCR	H2O	-	-	2018	-	NoCt	Negative	26,05	Positive

Tab. 1A. Clinical sensitivity of VDX® ASFV qPCR Kit.

Test No.	Sample ID	Origin	Animal	Tissue	Isolation Year	Genotype	Ct Value	Result
1	Ang72	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen I	23,98	Positive
2	LT14/1490	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen II	28,41	Positive
3	Moz64	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen V	29,23	Positive
4	MwLil 20/1	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen VIII	26,68	Positive
5	Ken06.Bus	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen IX	24,2	Positive
6	Ug64	CISA-INIA (Spain)	Cell culture	Cell culture	2016	gen X	29,51	Positive
7	18/69380(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	25,64	Positive
8	18/69302(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	21,34	Positive
9	18/70239(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	22,5	Positive
10	18/70253(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	22,3	Positive
11	18/70253(2)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	26,1	Positive
12	18/70549(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	23,57	Positive
13	18/68916(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	25	Positive
14	18/68914(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	23	Positive
15	18/69318(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	21,3	Positive
16	18/69318(2)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	20,8	Positive
17	18/69315(1)	NVRI (Poland)	Wild Boar	spleen	2018	gen II	22,6	Positive
18	18/69321(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	21,6	Positive
19	18/69320(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	21,7	Positive
20	18/69319(4)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	20,3	Positive

21	18/69319(3)	NVRI (Poland)	Wild Boar	bone marrow	2018	gen II	22,9	Positive
22	18/55330(1)	NVRI (Poland)	Pig	spleen	2018	gen II	17,29	Positive
23	18/55330(1)	NVRI (Poland)	Pig	lung	2018	gen II	19,46	Positive
24	18/55330(1)	NVRI (Poland)	Pig	kidney	2018	gen II	21,77	Positive
25	18/64917(1)	NVRI (Poland)	Pig	lung	2018	gen II	23,28	Positive
26	18/64917(1)	NVRI (Poland)	Pig	spleen	2018	gen II	22,5	Positive
27	18/64917(1)	NVRI (Poland)	Pig	lymph node	2018	gen II	24,02	Positive
28	18/53258(1)	NVRI (Poland)	Pig	lung	2018	gen II	26,05	Positive
29	18/53258(1)	NVRI (Poland)	Pig	lymph node	2018	gen II	28,27	Positive
30	18/53258(1)	NVRI (Poland)	Pig	spleen	2018	gen II	23,78	Positive
31	18/50097(1)	NVRI (Poland)	Pig	spleen	2018	gen II	17,5	Positive
32	18/50097(1)	NVRI (Poland)	Pig	kidney	2018	gen II	21,6	Positive
33	18/50097(1)	NVRI (Poland)	Pig	lung	2018	gen II	18,5	Positive
34	18/50098(1)	NVRI (Poland)	Pig	spleen	2018	gen II	17,2	Positive
35	18/50098(1)	NVRI (Poland)	Pig	kidney	2018	gen II	21,3	Positive
36	18/50098(1)	NVRI (Poland)	Pig	lung	2018	gen II	20,2	Positive
37	18/50099(1)	NVRI (Poland)	Pig	spleen	2018	gen II	18,99	Positive
38	18/50099(1)	NVRI (Poland)	Pig	kidney	2018	gen II	22,84	Positive
39	18/50099(1)	NVRI (Poland)	Pig	lung	2018	gen II	20,7	Positive
40	18/50101(1)	NVRI (Poland)	Pig	blood	2018	gen II	19,6	Positive
41	18/70757(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	-	NoCt	Negative
42	18/70757(1)	NVRI (Poland)	Wild Boar	bone marrow 1:10	2018	-	NoCt	Negative
43	18/70797(1)	NVRI (Poland)	Wild Boar	bone marrow	2018	-	NoCt	Negative
44	18/70797(1)	NVRI (Poland)	Wild Boar	bone marrow 1:10	2018	-	NoCt	Negative
45	18/70377(1)	NVRI (Poland)	Wild Boar	spleen	2018	-	NoCt	Negative
46	18/70824(1)	NVRI (Poland)	Pig	spleen	2018	-	NoCt	Negative
47	18/70824(1)	NVRI (Poland)	Pig	kidney	2018	-	NoCt	Negative
48	18/70824(1)	NVRI (Poland)	Pig	lung	2018	-	NoCt	Negative

49	18/70825(1)	NVRI (Poland)	Pig	spleen	2018	-	NoCt	Negative
50	18/70825(1)	NVRI (Poland)	Pig	kidney	2018	-	NoCt	Negative
51	18/70825(1)	NVRI (Poland)	Pig	lung	2018	-	NoCt	Negative
52	18/70826(1)	NVRI (Poland)	Pig	spleen	2018	-	NoCt	Negative
53	18/70826(1)	NVRI (Poland)	Pig	kidney	2018	-	NoCt	Negative
54	18/70826(1)	NVRI (Poland)	Pig	lung	2018	-	NoCt	Negative
55	18/70827(1)	NVRI (Poland)	Pig	spleen	2018	-	NoCt	Negative
56	18/70827(1)	NVRI (Poland)	Pig	kidney	2018	-	NoCt	Negative
57	18/70827(1)	NVRI (Poland)	Pig	tonsil	2018	-	NoCt	Negative
58	18/70828(1)	NVRI (Poland)	Pig	kidney	2018	-	NoCt	Negative
59	18/70828(1)	NVRI (Poland)	Pig	lung	2018	-	NoCt	Negative
60	18/70828(1)	NVRI (Poland)	Pig	tonsil	2018	-	NoCt	Negative
61	MO/060/17 1:100	ASFV DNA	-	-	2017	gen II	30	Positive
62	K-PCR	H2O	-	-	2018	-	NoCt	Negative

Tab. 1B. Clinical sensitivity using Fernandez-Pinero method (UPL-162 probe).

9. Analytical sensitivity

In order to determine the analytical sensitivity of VDX® ASFV qPCR Kit, it was conducted the measurement of limit of detection of ASFV DNA. The DNA of ASFV (gen. II) with known viral titer ($10^{6,62}$ HA050/mL) was dissolved and analysed. The last detectable dilution was 10^5 (Tab. 2).

Virus	Genotype	Virus Titer	Results			
			Dilution	Ct Value	Results	IPC Value
ASFV	II	10(6,62)HA050/mL	0	18,81	Positive	26,55
			10	22,16	Positive	26,13
			10(2)	26,96	Positive	26,15
			10(3)	32,57	Positive	26,15
			10(4)	35,35	Positive	26,21
			10(5)	37,27	Positive	26,21
			10(6)	-	Negative	26,16
			10(7)	-	Negative	25,84

Tab. 2. Analytical sensitivity of VDX® ASFV qPCR Kit.

10. General conclusion

The analysed VDX® ASFV qPCR Kit is suitable for detection of ASFV DNA equally from pig and wild boar samples. The limit of detection of ASFV DNA gen. II is 105 (the starting viral titer $10^{6,62}$ HA050/mL).

Prepares the opinion:.....

dr. Maciej Tront, mgr. Magdalena Łępiel

Head of Swine Diseases Department:.....

Wojciech
D Y R E K T O R

Director General of NVRI:.....

dr hab. Krzysztof Niemczuk
profesor nadzwyczajny