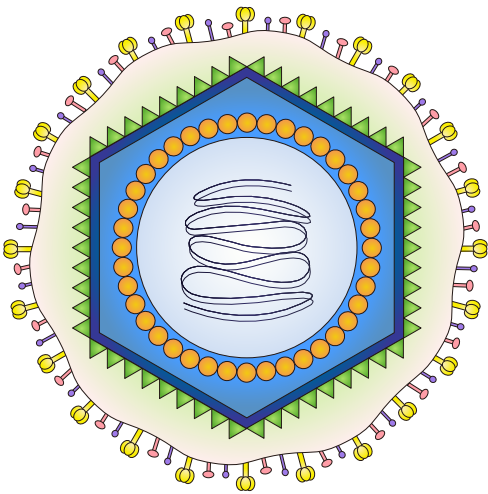MEDIAN Diagnostics Inc.

ASF Total Diagnostic System

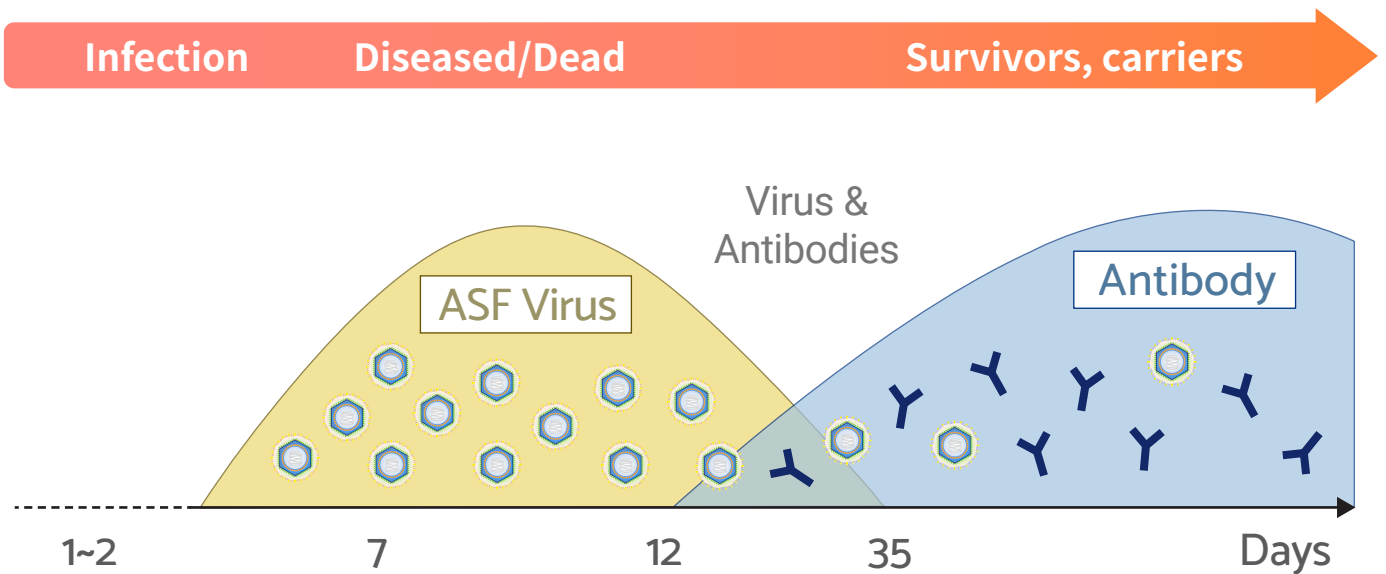For One Health

MEDIAN
Diagnostics

What is African Swine Fever (ASF)?

African Swine Fever Virus

African Swine Fever (ASF) is an infectious disease of domestic and wild pigs of all breeds and ages, caused by African Swine Fever Virus (ASFV). The clinical signs vary from peracute, acute, subacute to chronic, depending on the virulence of the virus. Acute disease is characterised by high fever, hae-morrhages in the reticuloendothelial system, and a high lethality.
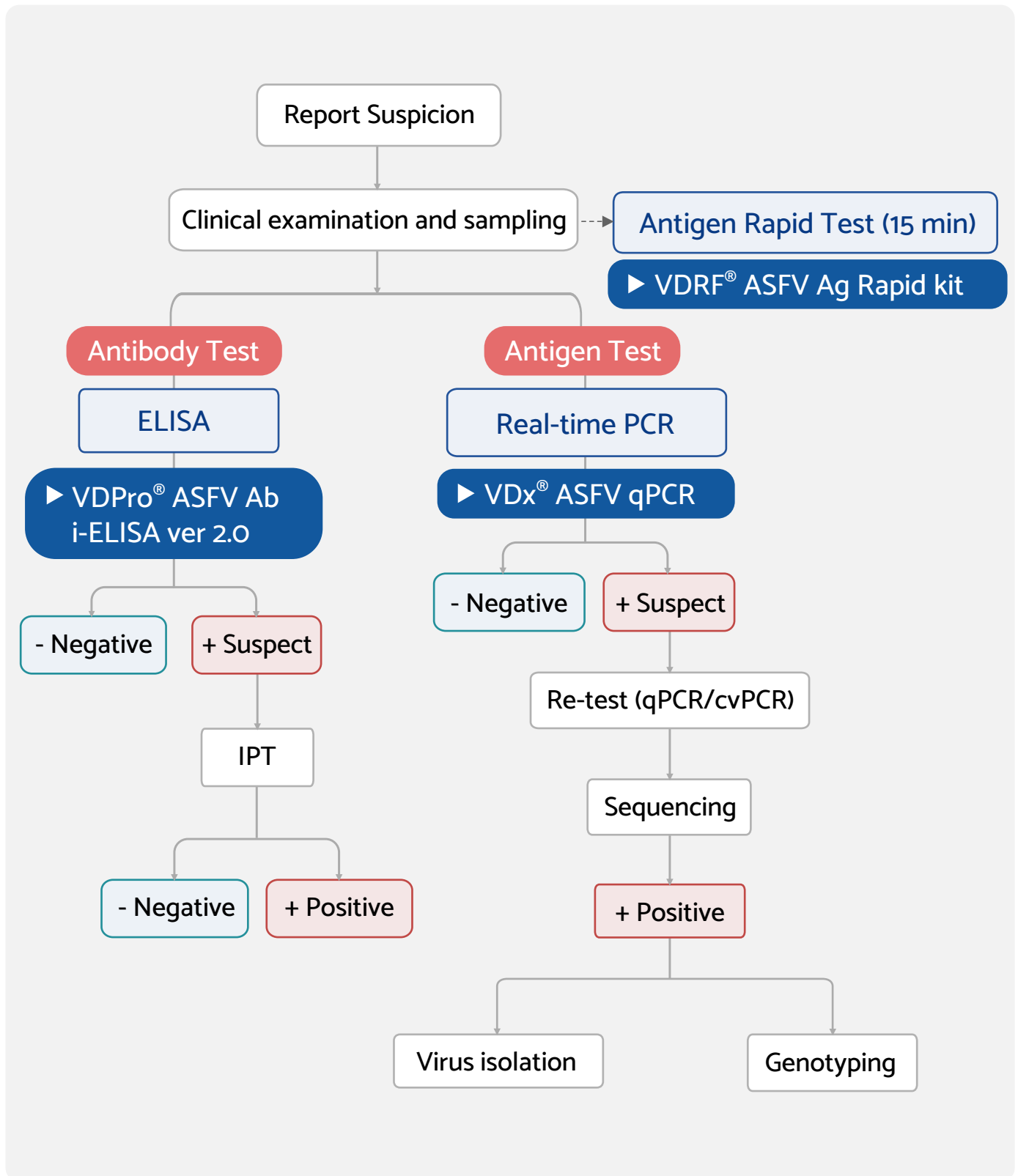
- ASF is an economic devastating disease
- Pathogen: large (175~215nm), lipoprotein-enveloped, dsDNA virus
- 23 genotypes but only genotype 1 and 2 are active out of Africa
- 150 genes encodes 150 ORF and 50 structural proteins
- Natural hosts are warthogs, bush pigs, giant forest hogs and soft ticks
- No commercial vaccine

Infection   **Diseased/Dead**   **Survivors, carriers**

ASF Virus

Virus & Antibodies

Antibody

| 1~2 | 7 | 12 | 35 | Days |

Acute Form 90~100% Lethality   **Subacute Form** 30~70% Lethality

Recovered Animals <20% Lethality

Diagnostic SOP of ASF (South Korea Case)

```
Report Suspicion
        │
Clinical examination and sampling ----> Antigen Rapid Test (15 min)
        │                                ▶ VDRF® ASFV Ag Rapid kit
   ┌────┴────────────────┐
Antibody Test         Antigen Test
   │                      │
 ELISA               Real-time PCR
   │                      │
▶ VDPro® ASFV Ab      ▶ VDx® ASFV qPCR
  i-ELISA ver 2.0         │
   │                 ┌────┴────┐
┌──┴──────┐      - Negative  + Suspect
- Negative + Suspect              │
           │                Re-test (qPCR/cvPCR)
          IPT                     │
           │                 Sequencing
     ┌─────┴─────┐                │
 - Negative   + Positive      + Positive
                           ┌──────┴──────┐
                      Virus isolation  Genotyping
```

MEDIAN Diagnostics Inc provides various solutions for the diagnosis of African Swine Fever (ASF).

ASFV Antigen Rapid kit

How to use VDRF® Rapid kit
→ https://youtu.be/C1ZrCe9mayO

VDRF® Reader Tutorial
→ https://youtu.be/6jIA3x7yrzE

☀ Intended Use

Detection of ASFV antigen

🧪 Specimens

Whole blood
from swine or wild boar

🗒 Kit Components

ASFV Ag Rapid Device

Dropper

Sample dilution buffer

VDRF® Reader (*seperate component)

VDRF® ASFV Ag Rapid kit is a lateral flow chromatographic immunoassay for detection of African swine fever virus (ASFV) antigen in blood of swine. This rapid kit specifically detects ASFV P30 antigen using blood as well as some kinds of tissue samples.

VDRF® Reader is a highly sensitive fluorescence analyzer that connects via Bluetooth to your smart phone app. After entering the sample name on the smart phone, tap or click the detect button to send a command to measure the immune complex by the antigen and antibody reaction using a light source device. The measured fluorescence signal is converted into an electric signal and sent to the smart phone and output as a reading value.
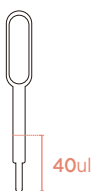
At first, Cloud Application (App) should be installed in the Smart phone according to instruction manual of VDRF® Reader because the reader is operated by Cloud App. The result of VDRF® ASFV Ag Rapid kit can be checked only by the reader.
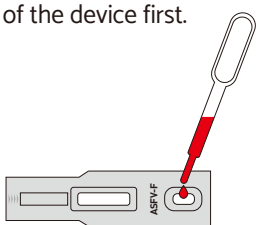
Test Procedure (using Dropper Cap)

* Fresh blood sample (collected within 3 days) should be used for the test. If the sample aged, the blood sample become sticky and may lead to non-specific reaction).
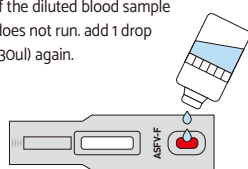
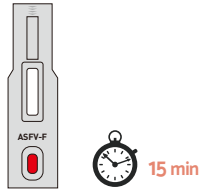* Tranfer the kit to room temperature before the test and use it after about 30 minutes.

1 Collect whole blood sample to the scale by using dropper (40ul).

40ul

2 Drop the blood (40ul) onto the sample hole of the device first.

3 After 1-3 minutes, add 2 drops (60ul) of dilution buffer.

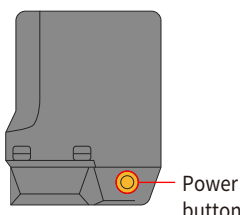If the diluted blood sample does not run. add 1 drop (30ul) again.
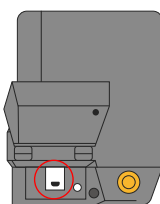
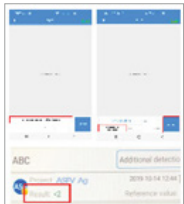**4** Read test results after 15 minutes.

15 min

Result Reading (15 min after sample dropping)

1 Turn on Bluetooth and start the cloud detection app.

2 Turn on the Reader (VDRF® Reader).

Power button

3 Open the cover, insert the device until it clicks and close the cover.
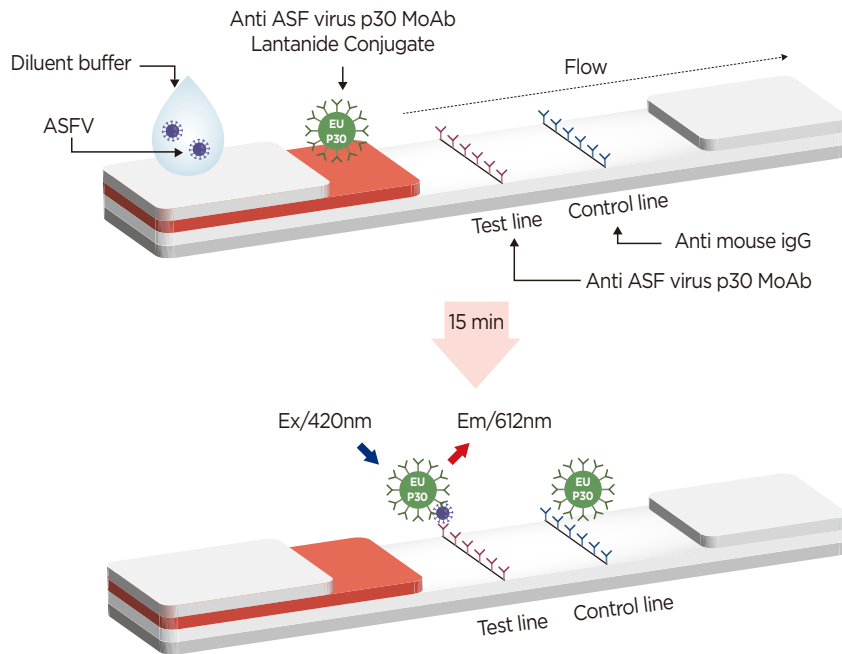
4 Recognize the kit #lot, fill in the sample name and tap detect to read the result.

ASFV Antigen Rapid kit

Principle

Performance

✓ Clinical Sensitivity : 100% on herd base(n=15), 79.4% on individual base (n=136)

Herd		VDRF® ASFV Ag Rapid kit		Sum
		Positive	Negative	
VDx® ASFV qPCR	Positive	15	0	15
	Negative	0	3*	3
	Sum	15	3	18

* 3 ASF free area

Individual		VDRF® ASFV Ag Rapid kit		Sum
		Positive	Negative	
VDx® ASFV qPCR	Positive	108	28	136
	Negative	0	37*	37
	Sum	108	65	173

* 37 ASF negative samples

✓ Clinical Specificity : 97.7% on herd base(n=44), 99.9% on individual base(n=903)

Herd		VDRF® ASFV Ag Rapid kit		Sum
		Positive	Negative	
VDx® ASFV qPCR	Positive	0	0	0
	Negative	1	43	44
	Sum	1	43	44

Individual		VDRF® ASFV Ag Rapid kit		Sum
		Positive	Negative	
VDx® ASFV qPCR	Positive	0	0	0
	Negative	1	902	903
	Sum	1	902	903

✓ Validated by NVRI* in Poland

Test	Results
Analytical Sensitivity	Genotype 1 : $10^{4.16\pm0.36}$ HAD$_{50}$/ml Genotype 2 : $10^{5.69}$ HAD$_{50}$/ml
Clinical Sensitivity	100% (n=59, Ct-value : 15.39~31.4)
Clinical Specificity	100% (n=61)

For the evaluation it was used the panel of 59 positive and 61 negative (both wild boar and domestic pig) blood and tissue samples previously tested in NVRI using qPCR kit.
* National Veterinary Research Institute, Poland

✓ Sensitivity based on Ct value of qPCR

Ct-value	VDRF® ASFV Ag Rapid kit		Sum	Sensitivity
	Positive	Negative		
< 21	100	0	100	100.0%
< 22	101	1	102	99.0%
< 24	101	3	104	97.1%

✓ Cross Reactivity : 0% (testing 8 kinds of bacteria and viruses including CSFV)

ASFV Antibody indirect-ELISA kit

Intended Use

Detection of ASF antibody

Method

Indirect ELISA

Specimen

Serum from swine or wild boar

VDPro® ASFV Ab i-ELISA ver 2.0 is designed to detect presence of anti-ASFV antibody in swine and wild boar serum. The ELISA plate is coated with recombinant ASFV antigens.

The need for ASF antibody test

ASFV antibody assay is a useful technique for assessing the status of the epidemic.
Experimental evidence with currently circulating strains indicates that some infected animals can remain asymptomatic and might even survive the infection. The increasing trend in potential survivors enhances the importance of ASF antibody test.
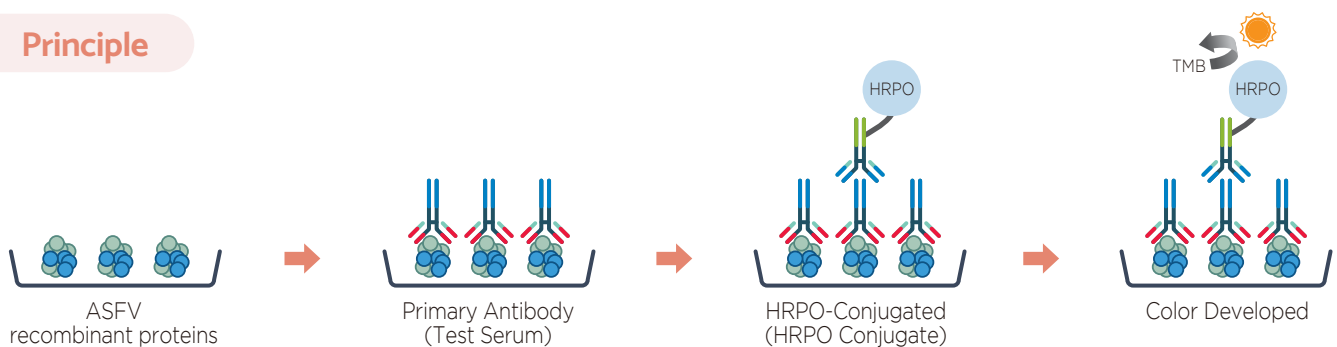
*Ref. Evolution of the ASF Infection Stage in Wild Boar Within the EU (2014-2018), Frontiers in Veterinary Science, 01 April 2020

An accurate ASF diagnosis must include the virological and serological results together with the clinical, pathological, and epidemiological findings.
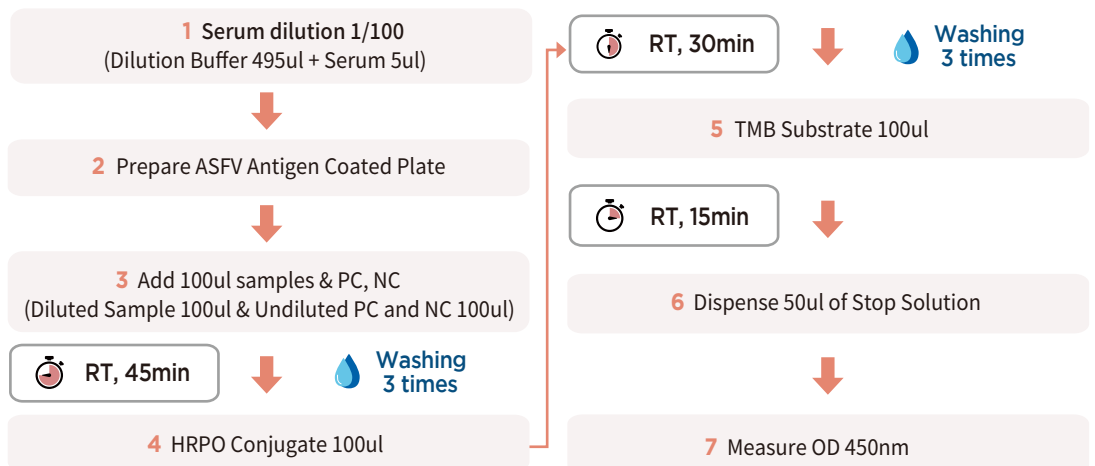
*Ref. Food and Agriculture Organization (FAO)
- Laboratory diagnosis of ASF

Food and Agriculture Organization (FAO) also recommends antibody testing for accurate diagnosis of ASF.

Principle

ASFV recombinant proteins → Primary Antibody (Test Serum) → HRPO-Conjugated (HRPO Conjugate) → Color Developed

Quick protocol

1 Serum dilution 1/100
(Dilution Buffer 495ul + Serum 5ul)

↓

2 Prepare ASFV Antigen Coated Plate

↓

3 Add 100ul samples & PC, NC
(Diluted Sample 100ul & Undiluted PC and NC 100ul)

↓

RT, 45min → Washing 3 times

4 HRPO Conjugate 100ul

RT, 30min → Washing 3 times

5 TMB Substrate 100ul

RT, 15min

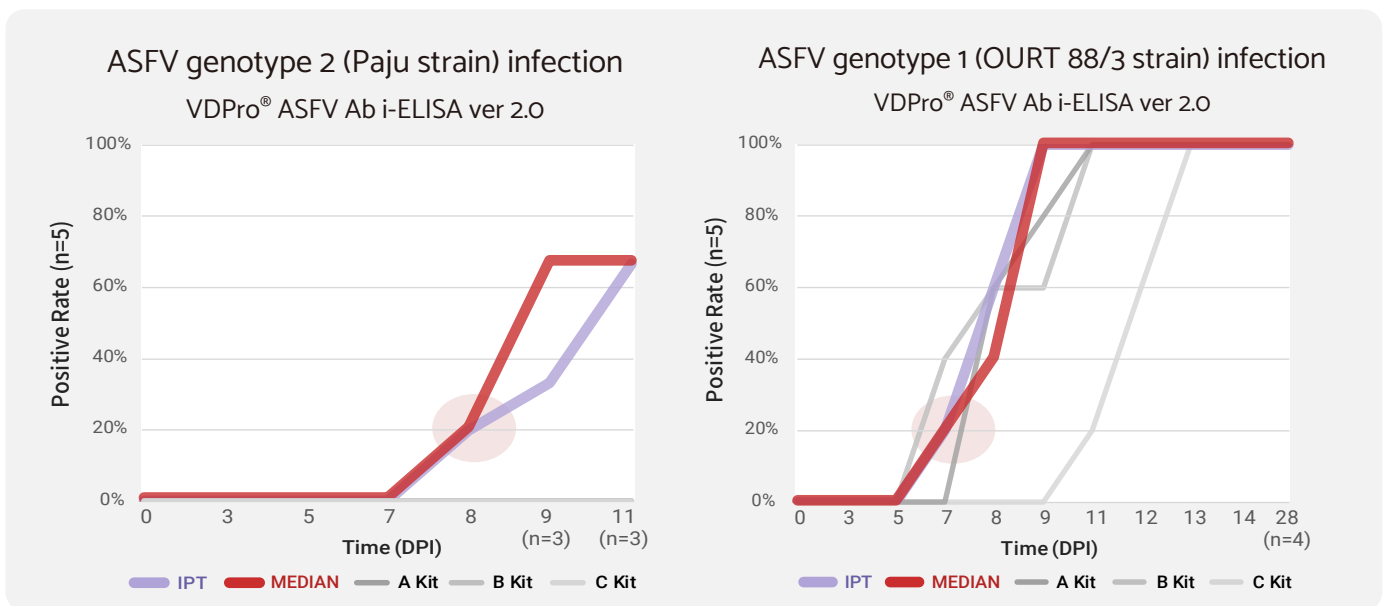**6** Dispense 50ul of Stop Solution

↓

7 Measure OD 450nm

ASFV antibody indirect-ELISA kit

✓ Excellent agreement with the IPT reference test (91.1%)

*ASFV challenge sera

Strain	Species	No. of sample*	Result	VDPro® ASFV Ab i-ELISA ver 2.0		IPT Result		Agreement rate with IPT
				No. of samples	Sensitivity	No. of samples	Sensitivity	
OURT 88/3 (Genotype 1)	Swine	49	Positive	32	65.3%	33	67.3%	98.0%
			Negative	17		16		
Paju (Genotype 2)	Swine	30	Positive	5	16.7%	9	30.0%	80.0%
			Negative	25		21		

ASFV genotype 2 (Paju strain) infection
VDPro® ASFV Ab i-ELISA ver 2.0

ASFV genotype 1 (OURT 88/3 strain) infection
VDPro® ASFV Ab i-ELISA ver 2.0

→ Genotype 2 : Detecting ASFV antibody as early as 8-9 days post infection

→ No detection of all 3 competitors' products

→ Genotype 1 : Detecting ASFV antibody as early as 7-9 days post infection

High detection ability of antibodies against genotype 2 as well as genotype 1

✓ Clinical Specificity : 99.8% (1131/1133)

Country	No. of sample*	VDPro® ASFV Ab i-ELISA ver 2.0		Country	No. of sample*	VDPro® ASFV Ab i-ELISA ver 2.0	
		No. of negative	Specificity			No. of negative	Specificity
USA	541	540	99.8%	South Korea	592	591	99.8%

* Swine sera collected from ASF non-outbreak domestic and oversea area

✓ Cross Reactivity : 0% (testing 7 kinds of bacteria and viruses including CSFV)

ASFV real-time PCR kit

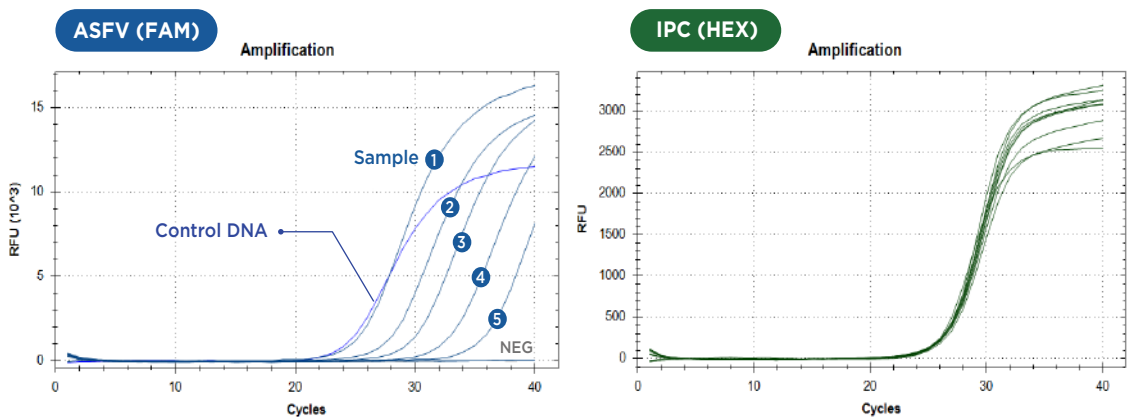**Intended Use**
Detection of ASFV

Method
Real-time PCR, qPCR

Specimen
blood, tissue, feces, livestock processed goods, and food wastes from swine or wild boar

Target Gene
ASFV p72 gene

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 TapMan probe.
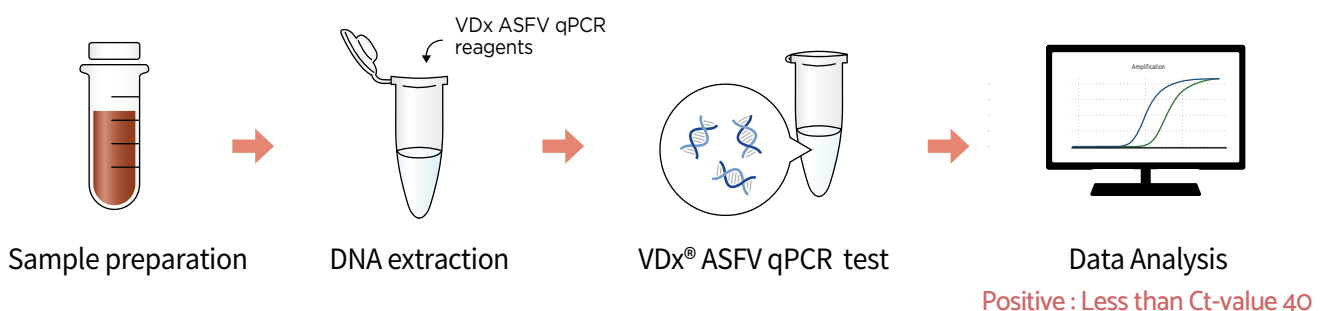
Techinal Data

ASFV (FAM)

IPC (HEX)

Performance

✓ Clinical Sensitivity : 100% (n=208)

✓ Clinical Specificity : 100% (n=550)

✓ Detection limit : 1copy/ul, $10^{1.62}$ HAD_{50} (Genotype 2), 10^0 HAD_{50} (Genotype 9)

✓ Cross Reactivity : 0% (testing 19 kinds of bacteria and viruses including CSFV)

Work Flow

Sample preparation

DNA extraction

VDx® ASFV qPCR test

Data Analysis
Positive : Less than Ct-value 40

Solution for ASF diagnostic system

Antibody screening test

VDPro® ASFV Ab i-ELISA ver 2.0

ASF diagnosis

Point of care
ASFV antigen test

VDRF® ASFV Ag Rapid kit

Confirmation test

VDx® ASFV qPCR

MEDIAN Diagnostics Inc provides a total portfolio for ASF diagnosis.

MEDIAN Diagnostics Inc provides real-time PCR kit, antigen rapid kit, and antibody ELISA kit for the diagnosis for ASF.

Our company provides diagnostic solution to monitor and diagnose diseases such as Foot-and-Mouth Disease (FMD), Classical Swine Fever(CSF), Avian Influenza (AI), as well as various common industrial diseases such as brucellosis and Japanese encephalitis. Moreover, we also provides diagnostic products to effectively diagnose infectious diseases caused by the increase of companion animals and global aging.

MEDIAN Diagnostics Inc will complete the world-class diagnostic system and provide diagnostic kit with the highest quality to the customers.

ASF Total Diagnostic System

Easy-to-Handle rapid kit at field farm condition

Fast identification of result within 15 minutes

One drop (0.04cc) of blood sample is enough for the test

Accurate result by measuring the immuno-fluorescent reaction signal

VDRF® ASFV Ag Rapid kit

Serological test for early detection of ASF in swine herd

Excellent agreement with the IPT reference test

Superior performance comparing to competitor's product

High detection ability of antibodies against genotype 2 as well as genotype 1

VDPro® ASFV Ab i-ELISA ver 2.0

Confirmatory diagnosis of ASFV

High sensitivity(100%) and specificity(100%)

High reproducibility and high repeatability

Applicable to all suspect specimens (blood, tissue, feces, livestock processed goods, and food wastes)

Most conserved p72 gene targeting for detecting all genotypes of ASFV

VDx® ASFV qPCR

Product Information

Cat. No.	Product Name	Quantity
PS-ASF-15	VDRF® Reader	1 Reader
PS-ASF-11	VDRF® ASFV Ag Rapid kit	30 Tests/kit
ES-ASF-05	VDPro® ASFV Ab i-ELISA ver 2.0	192 Tests, 480 Tests/Kit
NS-ASF-31	VDx® ASFV qPCR	96 Tests/kit