Detection of novel ASF variant via triplex Real-time PCR assay

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Triplex real-time PCR assay

This assay can be used to detect and identify viral nucleic acid (DNA) of ASF virus variants containing genome deletions in the *MGF360/505* and *EP402R* (CD2v) genes.

- The assay cannot be used to determine genotype. Additional conventional PCR testing and sequencing for p72 genotyping is required using specific primers.
- No cross-reactions with other porcine viruses have been detected.

PCR Protocol

Samples

- Tissue (spleen, lymph nodes, liver, tonsils and lung *et al*)
- Whole Blood in anti-coagulant
- Blood swabs or dried blood spots on filter paper
- Serum
- Oral-nasal swabs, oral fluids from rope chew collections, deep throat swabs

PCR Protocol

- **DNA Extraction**
  - There are several commercially available nucleic extraction kits that are suitable for the extraction and purification of ASF viral DNA.
  - Manually
  - automatically

Primer and Probe Mix for detection of ASFV variant

Primer	Sequence (5’ to 3’)	Volume per reaction (µl)	Final Conc. (nM)
CD2v_F (20 µM)	AGAAGAACAATGTCAGCATGATGAC	0.5	400
CD2v_R (20 µM)	CGACTGTAAGGCTTAGGAAGTAATGG	0.5	400
CD2v_P_VIC (10 µM)	VIC-CCACTTCCATACATGAACCATCTCCCAGA-BHQ1	1	400
MGF-14L _F (20 µM)	AGAAGACGGGGTTCGGATACAG	0.5	400
MGF-14L_R (20 µM)	GCAAATCCTGAATATGGGCTTATACG	0.5	400
MGF-14L_P_Cy5 (10 µM)	CY5-CTCCCAGTTCCGCACACAGCCGC-BHQ2	0.8	320
p72_F (20 µM)	AAGTTTCGGTACGCATTCTTTGT	0.4	320
p72_R (20 µM)	TATGACTGGGACAACCAAACACC	0.4	320
p72_P_FAM (10 µM)	FAM-ACAAGCGTGTAAACGGCGCCCTC-BHQ1	0.4	160

Designed and verified by Li Lin

Preparation of mastermix

Reaction Component	Volume (µl)
Nuclease-Free Water	2.5
2X RT-PCR Buffer	12.5
ASF CD2v Primer Probe Mix	2
ASF MGF360-505R Primer Probe Mix	1.8
ASF p72 Primer Probe Mix	1.2
Total volume mastermix	20.0/reaction tube or well
Addition of template	Volume (µl)
Sample DNA	5.0 µl
NC	5.0 µl
PC	5.0 µl

Total volume of reaction mixture 25µl

Program of the Real-time PCR

Setting	Temperature (°C)	Time	Cycle
Pre-incubation	50	2 min	1 cycle
Pre-denaturation	95	5 min	
Denaturation	95	15 sec	45 cycle
Annealing and elongation	60	60 sec	

Fluorescence is recorded in the FAM, VIC and Cy5 channels during annealing.

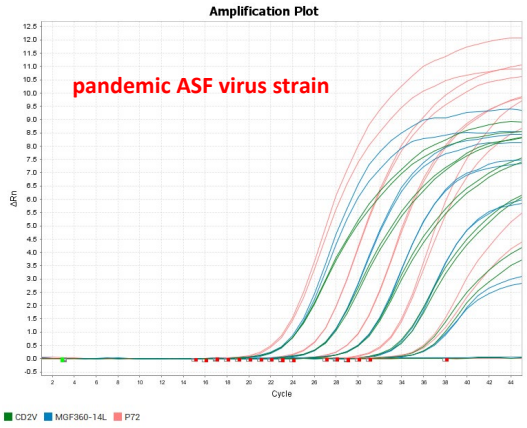
Result Analysis

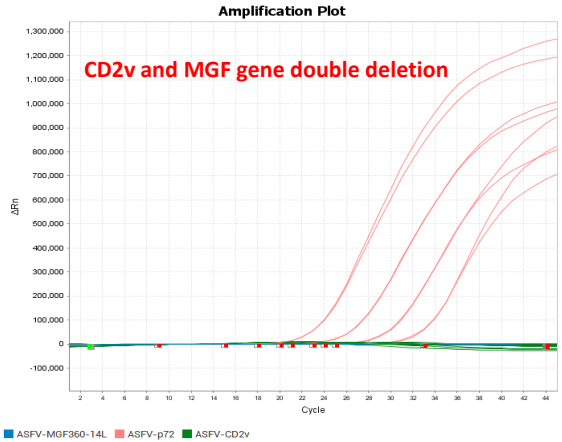
- **Positive result:** Cts < 35 for CD2v/MGF-14L/p72 **AND** the appearance of specific amplification curves for each assay
- **Negative result:** Ct ≥ 40 or no value
- **Indeterminate result:** Ct between >35 and 40, if indeterminate result is obtained, repeat the test to confirm.
- Where the p72 assay is positive AND one or both the MGF and CD2v assays are negative, this indicates that a **variant ASF virus** has been detected.
- Where all assays are positive, this indicates that the **parental or pandemic ASF virus strain** has been detected.
- Conventional PCR and sequencing for the variant strains.

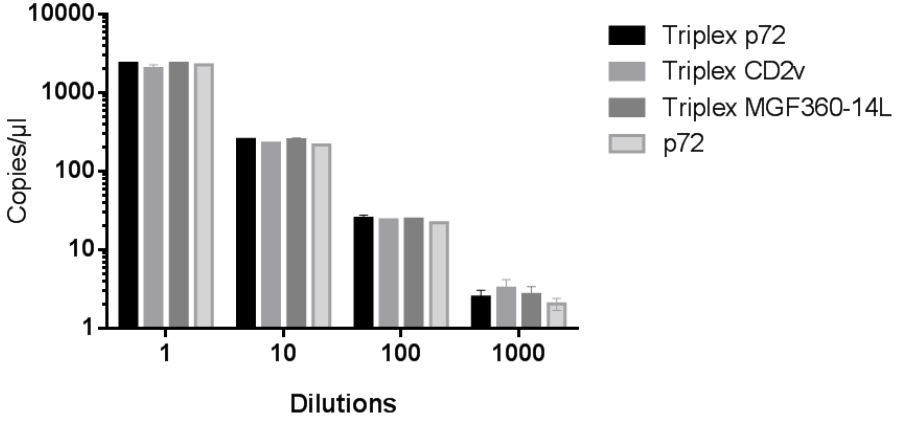
Interpretation of ASF virus triplex PCR results

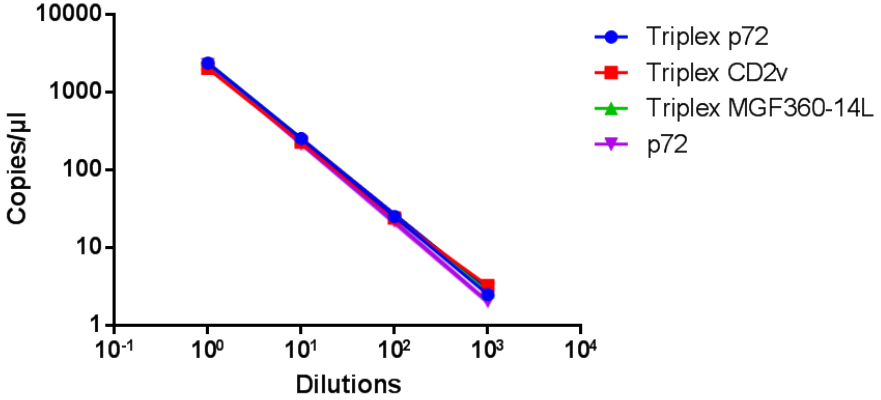
Interpretation	Test Results		
	P72-FAM	CD2v-VIC	MGF-Cy5
ASFV pandemic strain positive	+	+	+
ASFV CD2v gene deletion strain positive	+	-	+
ASFV MGF gene deletion strain positive	+	+	-
ASFV CD2v and MGF gene double deletion strain positive	+	-	-
ASFV negative	-	-	-

Method Validation










Thank you!