NEW RT-PCR FOR CLASSICAL SWINE FEVER VIRUS DETECTION IN SWINE AND WILD BOAR, ALLOWING FOR PARALLEL TESTING WITH ID GENE ASF qPCRs

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INTRODUCTION

Classical swine fever (CSF) is a contagious viral disease of pigs, including wild boar. The causativevirus is a member of the genus Pestivirus of the family Flaviviridae, and is closely related to the viruses of bovine viral diarrhoea and border disease. There is only one serotype of CSF virus (CSFV). Reliable and accurate diagnostics are the key for rapid implementation of control measures to detect and prevent the spread of CSFV.
Innovative Diagnostics has developed a rapid and specific RT-qPCR for detection of CSFV RNA, the ID GENE™ Classical Swine Fever Virus duplex (product code: IDCSF).

CONCLUSION

The **ID Gene™ Classical swine fever virus Duplex** kit offers:

- → **Rapid and specific detection** of RNA from CSFV (target in the non 5' UTR region)
- → **Excellent performance**: high inclusivity on all 21 CSFV strains; LDPCR = 4 copies / PCR
- → **Optimal test reliability** thanks to swine cells specific endogenous internal control included in the kit
- → **Can be performed in one cycler run with** ID Gene™ African swine ever qPCR kits, allowing, from the same extracts, for a parallel testing CSFV and ASFV, which share similar clinical patterns

KIT PRINCIPLE

The **ID Gene™ Classical Swine Fever Duplex** kit is a ready-to-use RT-qPCR kit assay detecting simultaneously CSFV and an endogenous internal positive control.

CHARACTERISTICS	
Targets	CSFV and endogenous control
Matrices	Blood, serum, swabs, Organ and tissues Individual samples or pools up to 10 or 20
Duration	60 min (rapid protocol)
Species	Domestic pig, wild boar, warthogs
Format	Ready-to-use liquid format

SAMPLE RESULT	CSFV SIGNAL	ENDOGENOUS CONTROL SIGNAL	INTERPRETATION
Positive	+	+/-	Valid; Animal detected as positive for CSFV No signal for endogenous because of competition
Negative	-	+	Valid; Animal detected as negative for CSFV
Degraded sample or partial PCR inhibition	-	-	Not Valid; Analytical process successful, no signal for endogenous control due to poor sample quality A problem occurred during sample distribution or extraction processes and/or RT-qPCR reaction was inhibited. Dilute the extracted DNA 10 times in nuclease-free water or re-extract the sample or consider it uninterpretable.

ID Gene™ Classical Swine Fever Virus duplex

RESULTS

INCLUSIVITY

Analysis of 32 reference CSFV strains provided by the Friedrich-Loeffler-Institut (FLI, Germany) and the French National Reference Laboratory (ANSES Ploufragan, France).

CSFV STRAIN	SUB-GENOTYPE	ORIGIN	IDCSFV RESULTS
Baker A	1.2	FLI, Germany	Detected
Guatemala HC	1.3		Detected
VRI 4167	1.3		Detected
39 Margarita	1.4		Detected
PR VP32/10	1.4		Detected
907/1	2.1		Detected
A-2	2.1		Detected
Parma 98	2.2		Detected
Bergen	2,.2		Detected
100/06	2.3		Detected
Kanagawa	3.4		Detected
C strain	1.1		Detected
Stamm	1.1		Detected
Koslov1128	1.2		Detected
SchweizII	2.1		Detected
Pader	2.1		Detected
Israel	2.1		Detected
Bergen	2.2		Detected
D4886/82/Ro	2.2		Detected
Uelzen	2.3		Detected
Spante	2.3		Detected
Congenital Tremor	3.1		Detected
Kanagawa	3.4		Detected
Alfort	1.1	ANSES, France	Detected
Brescia	1.2		Detected
Diepholz	2.3		Detected
Roesrath	2.3		Detected
Visbeck	3.1		Detected
Moselle1	2.3		Detected
Bas rhin	2.3		Detected
Peru I8	1.1		Detected
Paderborn	2.1		Detected
Litauen	2.1		Detected
Eystrup	1.1		Detected

The ID Gene CSF qPCR kit successfully detected all isolates tested

EXCLUSIVITY

The following 69 selected isolates were tested:

SAMPLE NUMBER	STRAINS	IDCSFV RESULTS
1-4	Influenza (H1N1, H5N2, H7N1, H9N2)	Not detected
5-11	African Swine fever	Not detected
12-13	PRRSV (EU and NA types)	Not detected
14-24	BVDV (1a, 1c, 1d, 1e, 1g, 1h, 1l, 2a, 2b, 3, 4)	Not detected
25-28	BHV (1,2,3,4)	Not detected
29-69	40 other viruses, bacteria and parasites from different species	Not detected

The ID Gene CSF qPCR kit did not show any cross-reactions with the 69 other pathogens tested

OVERALL PERFORMANCE

PCR CHARACTERISTICS	
Efficiency / R^2	94,09 % / 0,99
Limit of detection	4 copies/PCR with rapid amplification program
Robustness	Unaffected by all parameters tested (temperature and volume of sample)
METHOD CHARACTERISTICS (WITH THE ID GENE™ MAG FAST EXTRACTION KIT)	
Experimental limit of detection (with rapid amplification program)	3,2 x10^3 copies/ml for swine blood, serum and spleen samples

DIAGNOSTIC SENSITIVITY AND SPECIFICITY

Diagnostic sensitivity and specificity was evaluated on 66 field samples provided by ANSES Ploufragan (France), Landeslabor Schleswig-Holstein Neumünster (Germany) and the FLI (Germany) previously characterized by other methods.

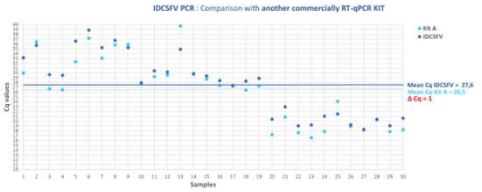
SAMPLE TYPE	NUMBER OF SAMPLES TESTED	DIAGNOSTIC SENSITIVITY (Se)	DIAGNOSTIC SPECIFICITY (Sp)
		CSFV TARGET	
Organs or tissues	11	4/4	7/7
Whole bloods and serums	22	10/10	12/12
Swabs	23	N/A*	23/23
Cellular culture supernatants	10	7/7	3/3
TOTAL	**66**	**21/21 100%**	**45/45 100%**

* N/A : Non analyzed

The test correctly identified all positive and negative samples tested

COMPARISON WITH ANOTHER COMMERCIALLY AVAILABLE RT-qPCR KIT (KIT A)

The performances of the ID Gene CSF test were compared to another commercially available RT-qPCR kit (Kit A) on 21 RNAs provided by the FLI (Hannover, Germany) and 9 RNAs from EILA panel (Ring test trial 2018) composed of field samples spiked with different CSFV strains provided by ANSES (Ploufragan – France). All samples were tested with the Kit A and the ID Gene assay.



→ **The ID Gene CSF qPCR kit assay shows excellent status correlation with the other commercial kit tested.**

(1) OIE Terrestrial Manual, Classical Swine Fever (infection with classical swine fever virus), chapter 3.8.3, 2019.