Validation of the ASFV African swine fever virus (ASF) qPCR Diagnostic Kit TARGETEX BIOSCIENCES. VALIDATION REPORT

PERFORMED BY THE

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Content

1. OBJECTIVE.

The study conducted at CISA-INIA/CSIC as the European Union Reference Laboratory (EURL) for African swine fever (EURL-ASF) aimed to assess the performance of the ASF Virus qPCR Diagnostic Kit developed by TARGETEX (named in this study TARGETEX ASFV qPCR kit), using **field porcine samples**. The multiplex TARGETEX ASFV qPCR kit is designed for the detection of African Swine Fever (ASF) virus DNA by quantitative PCR. In addition to the ASF virus-specific FAM™-labelled probe, the assay is capable of detecting endogenous controls (e.g., swine, boar or warthog DNA, probe labelled with HEX™) as well as exogenous controls (probe labelled with Cy5™).

2. PROCEDURE

1.1. Field samples included in the validation study

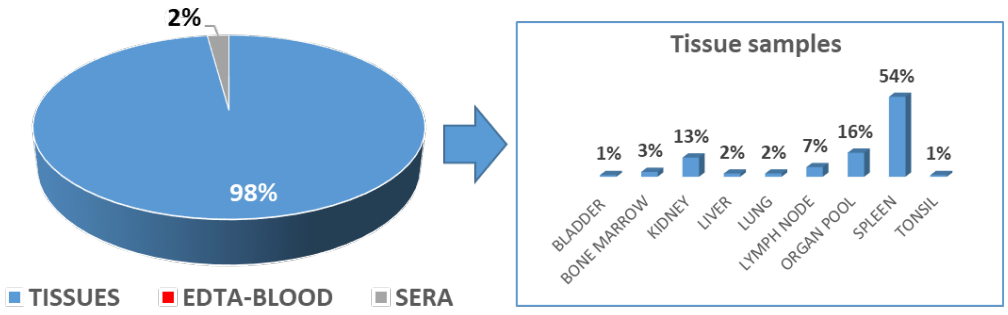
The validation study encompassed **two hundred and thirty six (236) samples** from different sources as showed in Table 1.

Table 1→ *Detailed description of field samples included in the validation study*

HOST	Affected ASF European countries	Free ASF European countries	TOTAL
Domestic pig	94	33	127
Wild boar	109	0	109
TOTAL	**203**	**33**	**236**

Specifically, for **domestic pigs in European countries affected by genotype II ASFV**, a total of 94 field samples were tested, obtained from 65 animals involved in outbreaks spanning 2021 to 2025. These samples included 92 (98%) tissue samples and 2 (2%) serum samples. Among the tissues tested, the distribution was as follows: 50 spleen samples (54%), 15 organ-pool samples (16%), 12 kidney samples (13%), 6 lymph-node samples (7%), 3 bone-marrow samples (3%), 2 lung samples (2%), 2 liver samples (2%), 1 bladder sample (1%), and 1 tonsil sample (1%) (Figure 1).

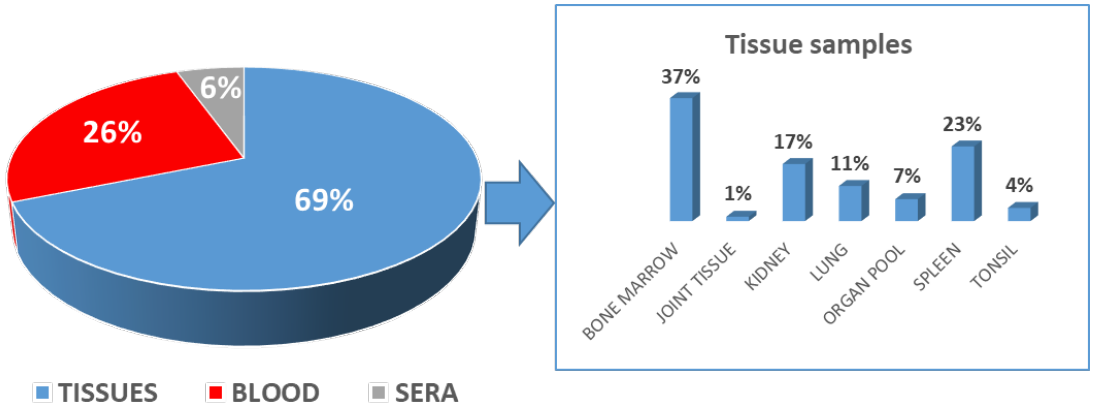
Figure 1 → *Description of the domestic pig samples tested for validation purpose.*



Additionally, a set **of 33 blood samples from domestic pigs in Spain** served as **negative samples** for diagnostic specificity assessment.

From **wild boar**, a total of **109 field samples collected from European countries affected by genotype II ASFV** between 2020 and 2025 were included in the study. The sample types **comprised 75 tissue samples (69%), 28 blood samples (26%), and 6 sera (6%)**. Among the tissue samples, bone marrow was the most frequent (n = 28; 37%), followed by spleen (n = 17; 23%), kidney (n = 13; 17%), lungs (n = 8; 11%), pool of organs (n = 5; 7%), tonsil (n = 3; 4%), and joint tissue (n = 1; 1%) (Figure 2).

Figure 2 → *Description of the wild boar samples tested for validation purpose.*



1.2. TEST PROCEDURES.

1.2.1. DNA extraction procedure.

DNA was extracted from organ homogenates and EDTA-blood using the **High Pure PCR Template Preparation kit** (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany: Ref. 11796828001 (ROCHE)] according the EURL standard operating procedure (SOP) [SOP-ASF-DNA-EXTRACTION-1_REV52021.pdf]. Briefly, 10% (w/v) clarified homogenized tissue suspensions were prepared in phosphate-buffered saline (PBS) following the EURL-SOP [SOP/CISA/ASF/SAMPLES/1]. Supernatants were filter with MINISART filters 0.45µm and then treated with 0.1 % of gentamicin sulphate 50mg/ml (BioWhittaker) during 1h at 4±3ºC prior to use for virus detection; a 10% (w/v) clarified homogenized tissue were prepared in phosphate-buffered saline (PBS). The DNA was extracted from 200 µl of each tissue homogenate or blood samples using the High Pure PCR Template Preparation Kit. DNAs were stored <-10 until use. Extracted DNAs were stored at ≤ –10 °C until use.

1.2.2. WOAH-Real time PCR procedure (UPL method) developed by Fernandez et al., 2013 (WOAH 2021)

The amplification of the ASFV amplification was performed in 96-well plate AriaMX equipment (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA) using the **WOAH-real time PCR** **(Taqman probe**

ASFV-VP72P1) developed by Fernandez *et al.*, 2013 and included in the WOAH Manual as reference test (WOAH 2021) [SOP/CISA/ASF/PCR/3].

The following controls were included in both extraction and amplification steps:

Reference	Component	Description
EURL-E+ Batch 40	**Positive control for the extraction (EURL)**	1:10.000 dilution of the ASFV reference strain E75 (genotype I) diluted in a negative sera.
NCE (E-)	**Negative control for the extraction**	Distilled water
EURL-R+ batch 38	**Positive control for the amplification (EURL)**	ASFV positive DNA extracted for the EURL
NAC (R-)	**Negative control for the amplification**	Distilled water

Assay validation: results were considered as validated if met the following criteria:

Control	Expected result	Acceptability criteria
EURL-E+ batch 40	Detected in FAM channel	Ct value within the range of 32±4.
NCE (E-)	Non detected	Ct ≥40.
EURL-R+ batch 38	Detected in FAM channel	Ct value within the range of 32±4.
NAC (R-)	Non detected	Ct ≥40.

Interpretation of the results:

- → Samples giving a **Ct value ≤ 35** are considered as **POSITIVE SAMPLES**.
- → Samples giving a **Ct value Ct ≥40** are considered as **NEGATIVE SAMPLES**.
- → Samples giving **35 ≤ Ct value < 40** are considered as **WEAK SAMPLES** if a sigmoidal plot is observed. In this case and to confirm the results, the extracted DNA from the weak sample must be tested by duplicated in a second PCR run. Sample will be considered as positive in case of the Ct value <40 in, at least, one duplicate.
- → Samples showing a Ct value >38 were considered as negative if the amplification plot had a linear shape.

1.2.3. ASF qPCR Diagnostic Kit TARGETEX BIOSCIENCES.

The amplification of the ASFV DNA was performed in 96-well plate AriaMX equipment (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA) using the TARGETEX ASFV qPCR kit according to the protocol described by the manufacturers. The kit includes both **positive and negative controls, each producing an expected Ct value of approximately 28 during amplification for ASFV and endogenous target**. Additionally, it contains an **exogenous control designed to monitor the efficiency and reliability of the DNA extraction process**. The exogenous extraction control was directly added at a 10^5-fold dilution to the extracted DNA sample (Post-extraction addition) to verify the PCR reaction itself.

Reagent	Description	Volume (µL)
Reaction Mix (100 reactions)	Master Mix includes primers and probes	1500
Positive ASF Control (PC)	ASF-specific target DNA	50
Negative ASF Control (NC)	Endogenous target DNA	50
Pos. Extraction Control (EC)	Exogenous target DNA	50

The thermal cycle used is described below:

	STEP	TEMPERATURE (°C)	DURATION
1	INITIAL DENATURATION	95	3 min.
2	DENATURATION	95	5 sec
3	ANNEAL/EXTENSION	60	30 sec

Repeat steps #2-3 for 40 cycles. The fluorescence is read at the end of the extension phase.

Assay validation: results were considered as validated if met criteria established in the following table.

Control Type	Target	Channel	Expected Ct Value
Positive ASF Control (PC)	Verifies ASFV-specific amplification	FAM	≤ 30
Negative ASF Control (NC)	Verifies endogenous target amplification	HEX	≤ 30
Pos. Extraction Control (EC)	Monitors extraction and PCR efficiency	Cy5	25–35*
No Template Control (NTC)	Excludes reagent contamination	–	no amplification

* Expected Ct value varies depending on the DNA extraction method, sample type, etc.

Interpretation of the results:

- **Positive (FAM +):** ASFV genome detected, regardless of control status. If IC (HEX) or EC (Cy5) fail, repeat extraction/qPCR is recommended to verify sample quality or EC concentration.
- **Negative (FAM – / IC + / EC +):** ASFV not detected.

- **Negative with IC or EC failure:** Result inconclusive; repeat extraction/qPCR required.
- **Invalid (all –):** No amplification of target or controls; repeat full extraction and qPCR workflow.

Result	ASF Virus (FAM)	Endogenous control (HEX)	Exogenous control (Cy5)	Conclusion
Positive	+	+	+	ASFV detected.
Positive (IC failure)	+	–	+	ASFV detected; but endogenous control failed – insufficient sample quality, repeat extraction/qPCR protocol.
Positive (EC failure)	+	+	–	ASFV detected; but exogenous control failed – repeat extraction with higher EC concentration.
Positive (IC and EC failure)	+	–	–	ASFV detected but both endogenous and exogenous control failed – repeat extraction with higher EC concentration.
Negative	–	+	+	ASFV not detected.
Negative (IC failure)	–	–	+	Endogenous control failed – insufficient sample quality, repeat extraction/qPCR protocol.
Negative (EC failure)	–	+	–	Exogenous control failed – repeat extraction with higher EC concentration.
Invalid	–	–	–	No amplification of target or controls – repeat full extraction and qPCR workflow.

1.3. DATA ANALYSIS.

The **concordance between the TARGETEX ASFV qPCR kit and the WOAH real-time PCR (gold standard)** was assessed by calculating the overall percentage agreement using two-by-two contingency tables. In addition, the Kappa coefficient (κ) statistic was used to evaluate the level of agreement beyond that expected by chance. According to the interpretation criteria, κ values of 0.81–1.00 indicate almost perfect agreement, 0.61–0.80 substantial agreement, 0.41–0.60 good agreement, 0.21–0.40 moderate agreement, 0.01–0.20 slight agreement, and 0.00 no agreement.

1.4. INTER and INTRA-ASSAY REPRODUCIBILITY

The **inter-assay reproducibility** was initially estimated using the ASF positive control included in the kit and the EURL-ASF reference positive control. Both controls were run in **six independent PCR assays** to monitor assay-to-assay variation. The mean Ct values for each positive control were calculated and subsequently used to determine the overall mean, standard deviation (SD), and coefficient of variation (CV%). The average CV% was reported as the inter-assay CV.

Additionally, **21 field samples randomly selected** were analysed in duplicate in two independent PCR runs performed on two different thermocyclers: the 96-well AriaMX (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA) and the 96-well MX3005P (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA). The CV% was calculated following the same procedure described above, and the mean CV% obtained across samples was reported as the inter-assay CV for field material.

The **intra-assay reproducibility** was also assessed using the CVs obtained from the **21 field samples tested in duplicate on the two thermocyclers mentioned above**. The mean Ct values were calculated for each sample and used to determine the SD and CV% (CV% = SD of mean Ct ÷ mean Ct × 100). The average of the individual CV% values was reported as the intra-assay CV.

3. RESULTS

2.1. Validation criteria and interpretation of the results.

All reactions were validated according to the criteria described in the Methodology section, since all controls met the required values.

Out of the 236 samples initially tested, **22 samples were deemed invalid (9.3%).** Specifically, 4 samples showed negative Ct values in all three channels, while the remaining invalid cases were due to negative values obtained in the FAM channel (used for ASFV detection) and the Cy5 channel (used to monitor extraction and PCR efficiency). All invalid cases corresponded to blood samples. Following the manufacturer's instructions, these DNA samples underwent a second extraction and PCR run, tested at 1:5 dilution. As a result, **all previously invalid samples yielded valid results when tested at a 1:5 dilution** (Table 2).

Table 2→ *Comparative results of WOAH real-time PCR and TARGETEX ASFV qPCR kit in samples initially classified as non-validated*

Nº	ID SAMPLE	TYPE	HOST[1]	WOAH real-time PCR		TARGETEX ASFV qPCR kit 1st run				TARGETEX ASFV qPCR kit 2nd run			
				Ct	RES	FAM	HEX	CY5	RES	FAM	HEX	CY5	RES
1	1	SPLEEN	EWB	29.69	POS	40	12.36	40	NV	23.12	16.09	18.44	POS
2	3	LUNG	EWB	23.53	POS	40	15.75	40	NV	22.89	19.45	18.51	POS
3	7	SPLEEN	EWB	22.84	POS	40	14.8	40	NV	20.65	19.13	18.52	POS
4	20	KIDNEY	EWB	32.55	POS	40	27.22	40	NV	33.55	29.73	18.33	POS
5	30	BONE MARROW	EWB	23.31	POS	40	17.99	40	NV	22.36	19.83	18.3	POS
6	31	BONE MARROW	EWB	25.71	POS	40	40	40	NV	23.96	23.66	18.9	POS
7	37	KIDNEY	DP	35.42	WEAK[2]	40	12.77	40	NV	36.95	14.79	18.63	POS
8	39	JOINT TISSUE	EWB	36.28	WEAK	40	18.61	40	NV	40	18.86	18.37	FN
9	46	SPLEEN	DP	22.39	POS	40	16.29	40	NV	20.87	17.17	18.67	POS
10	68	SPLEEN	DP	23.86	POS	40	15.84	40	NV	19.12	17.81	18.16	POS
11	74	LYMPH NODE	DP	26.7	POS	40	13.13	40	NV	25.01	14.54	18.12	POS
12	103	BLOOD	EWB	29.33	POS	40	24.38	40	NV	24.18	23.33	19.34	POS
13	124	SPLEEN	DP	29.61	POS	40	40	40	NV	19.54	18.27	19.65	POS
14	127	SPLEEN	DP	22.81	POS	40	14.78	40	NV	17.82	17.31	18.51	POS
15	145	SPLEEN	DP	20.3	POS	40	16.59	40	NV	20.54	19.1	19.17	POS
16	149	SPLEEN	DP	20.22	POS	40	13.23	40	NV	20.3	17.42	18.78	POS
17	166	SERUM	EWB	23.47	POS	40	16.73	40	NV	23.22	20.59	18.82	POS
18	178	SERUM	EWB	37.18	WEAK	40	17.18	40	NV	16.59	16.8	18.38	POS
19	181	SPLEEN	EWB	27	POS	40	13.76	40	NV	28.94	18.01	18.62	POS
20	199	ORGANS (POOL)	DP	25.51	POS	40	16.78	40	NV	18.13	18.43	18.24	POS
21	8Sal	BLOOD	DP	40	NEG	40	40	40	NV	40	19.66	19.16	NEG
22	10Sal	BLOOD	DP	40	NEG	40	40	40	NV	40	22.67	18.35	NEG

[1] *EWB = European wild boar; DP = domestic pig*

[2] *WEAK Ct ≥35; NV = not validated; FN = false negative*

It should also be noted that the exogenous control (EC) was added at a 10^{-4} dilution after the extraction step, instead of the 10^{-5} dilution recommended by the manufacturer. Despite this modification, all samples were successfully detected, and all controls fulfilled the required validation criteria.

2.2. Determination of the diagnostic sensitivity of the TARGETEX ASFV qPCR kit Detection Kit testing field samples.

Diagnostic sensitivity was assessed using 236 field samples collected from domestic pigs (n = 127) and wild boars (n = 109), in order to evaluate the ability of the assay to detect ASFV genome under real clinical conditions. According to the WOAH reference real-time PCR (WOAH real-time PCR)**, 203 samples were classified as positive and 33 as negative**.

The **TARGETEX ASFV qPCR kit correctly detected 200 of the 203 positives**, resulting in only three false negatives and a **diagnostic sensitivity of 98.5%** (95% CI: 95.8–99.5). All 33 negative samples were correctly identified, yielding a **diagnostic specificity of 100%** (95% CI: 89.6–100)**.** The three false-negative samples showed high Ct values with the WOAH real-time PCR: two had Ct values >36, and one sample had a Ct of 33.31. Among them, only one sample—collected from a hunted wild boar—exhibited a high antibody titer measured using the WOAH indirect immunoperoxidase test (IPT), whereas the remaining two samples were antibody-negative, indicating early infection (Table 3).

Table 3→ *Comparative results of WOAH real-time PCR and TARGETEX ASFV qPCR kit in false negative (FN) samples*

HOST[1]	SAMPLE TYPE	IPT titer	WOAH real- time PCR		TARGETEX ASFV qPCR kit			
			Ct	RES	FAM	HEX	CY5	RES
EWB	JOINT TISSUE	NEG	36.28	WEAK	40	18.61	40	FN
DP	TONSIL	NEG	38.3	WEAK	40	19.45	27.57	FN
EWB	BLOOD	1:163,840	33.31	POSITIVE	40	16.15	24.61	FN

2.3. INTER-ASSAY REPRODUCIBILITY

The inter-assay reproducibility was evaluated using the ASF positive control included in the kit (PC) and the EURL-ASF reference positive control (R+). Both controls were analysed in six independent PCR runs to assess assay-to-assay variability. The **inter-assay coefficient of variation (CV) obtained for the TARGETEX ASFV qPCR kit positive control (PC) was 2%,** while the **CV for the EURL reference control (R+) was 3%.** These values are considered acceptable for routine diagnostic testing (Table 4).

Table 4→ *Coefficient of Variation (CV) per positive controls between six different PCR runs.*

	DATE	R+ EURL	PC
PLATE 1	02/10/2025	31.11	29.19
PLATE 2	02/10/2025	31.51	29.98
PLATE 3	06/10/2025	32.87	29.12
PLATE 4	09/10/2025	30.88	29.91
PLATE 5	23/10/2025	30.90	30.4
PLATE 6	23/10/2025	29.95	30.8
	Average	**31.205**	**29.911**
	SD	0.96	0.674
	CV	**3%**	**2%**

CV = coefficient of variability; SD = standard deviation

In addition the inter-assay reproducibility was assessed with CVs obtained testing the 21 field samples randomly selected. These samples **were tested by duplicate in two different runs prepared at different times by different technical staff and using randomly two real time PCR equipment.** The results obtained are showed in Table 5.

Table 5→ *Coefficient of Variation (CV) per sample between two different PCR runs in the analysis of the 21 field- samples*

ID SAMPLE	PCR RUN (Eq1)		PCR RUN (Eq2)		MEAN	SD	%CV
	C_T1	RESULT	C_T2	RESULT			
1	23.92	**POSITIVE**	24.08	**POSITIVE**	**24**	0,11	0,47%
2	23.03	**POSITIVE**	23.26	**POSITIVE**	**23.14**	0,16	0,70%
3	20.31	**POSITIVE**	20	**POSITIVE**	**20.15**	0,21	1,09%
4	34.19	**POSITIVE**	29.53	**POSITIVE**	**31.86**	3,29	10,34%
5	23.14	**POSITIVE**	23.27	**POSITIVE**	**23.20**	0,09	0,40%
6	24.62	**POSITIVE**	24.35	**POSITIVE**	**24.48**	0,19	0,78%
7	37.02	**POSITIVE**	36.38	**POSITIVE**	**36.7**	0,45	1,23%
8	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
9	21.41	**POSITIVE**	21.48	**POSITIVE**	**21.44**	0,04	0,23%
10	17.63	**POSITIVE**	17.68	**POSITIVE**	**17.65**	0,03	0,20%
11	19.99	**POSITIVE**	20.03	**POSITIVE**	**20.01**	0,02	0,14%
12	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
13	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
14	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
15	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
16	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
17	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
18	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
19	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
20	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
21	40	NEGATIVE	40	NEGATIVE	40	0	0,00%
AVERAGE %CV							**0.77%**

CV = coefficient of variability; SD = standard deviation .
Eq1 = 96-well AriaMX (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA)
Eq2 = 96-well MX3005P (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA).

The average of the individual CVs reported as the inter-assay CV was 0.77%. This value, being below 5%, demonstrates very good stability and inter-assay reproducibility. Only one sample showed a CV slightly above 10%, considered an isolated finding without impact on the overall robustness of the assay.

2.4. INTRA-ASSAY REPRODUCIBILITY

The **intra-assay reproducibility** was evaluated by determining the CVs obtained from testing the 21 field samples in duplicate. The results obtained for each sample on each thermocycler included in the intra-assay study are presented in Table 6. This assessment ensures the reliability and consistency of the testing methodology by examining the variation observed when the same samples are tested repeatedly within the same assay run.

Table 6→ *Coefficient of Variation (CV) in the reference panel of samples tested by duplicate in the same equipment*

ID SAMPLE	PCR RUN Eq1					PCR RUN Eq2				
	C_T1	C_T2	MEAN	SD	%CV	C_T1	C_T2	MEAN	SD	%CV
1	24	23.83	23.92	0.12	0.5%	24.43	23.74	24.085	0.4879	2.0%
2	22.83	23.22	23.03	0.276	1.2%	23.2	23.32	23.26	0.0849	0.4%
3	19.9	20.72	20.31	0.58	2.9%	20.09	19.91	20	0.1273	0.6%
4	34.33	34.05	34.19	0.198	0.6%	25.23	33.84	29.535	6.0882	20.6%
5	23.42	22.85	23.14	0.403	1.7%	23.54	23.01	23.275	0.3748	1.6%
6	24.99	24.25	24.62	0.523	2.1%	24.44	24.27	24.355	0.1202	0.5%
7	36	38.04	37.02	1.442	3.9%	35.52	37.25	36.385	1.2233	3.4%
8	40	40	40.00	0	0.0%	40	40	40	0	0.0%
9	21.44	21.37	21.41	0.049	0.2%	21.43	21.53	21.48	0.0707	0.3%
10	17.6	17.65	17.63	0.035	0.2%	17.74	17.62	17.68	0.0849	0.5%
11	20.39	19.59	19.99	0.566	2.8%	20.51	19.55	20.03	0.6788	3.4%
12	40	40	40.00	0	0.0%	40	40	40	0	0.0%
13	40	40	40.00	0	0.0%	40	40	40	0	0.0%
14	40	40	40.00	0	0.0%	40	40	40	0	0.0%
15	40	40	40.00	0	0.0%	40	40	40	0	0.0%
16	40	40	40.00	0	0.0%	40	40	40	0	0.0%
17	40	40	40.00	0	0.0%	40	40	40	0	0.0%
18	40	40	40.00	0	0.0%	40	40	40	0	0.0%
19	40	40	40.00	0	0.0%	40	40	40	0	0.0%
20	40	40	40.00	0	0.0%	40	40	40	0	0.0%
21	40	40	40.00	0	0.0%	40	40	40	0	0.0%
AVERAGE CV%					**0.77%**					**1.59%**

CV = coefficient of variability; SD = standard deviation .

Eq1 = 96-well AriaMX (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA)

Eq2 = 96-well MX3005P (Stratagene, Agilent Technologies Inc., Santa Clara, CA, USA).

The average intra-assay CV ranged between 0.77% and 1.59%, confirming the high reproducibility of the results.

2.5. CONCLUSIONS.

The validation study performed at the EURL-ASF (CISA-INIA/CSIC) demonstrated that the TARGETEX ASFV qPCR Diagnostic Kit is a reliable and robust tool for the detection of ASFV genome in porcine field samples.

1. **Diagnostic performance.** The TARGETEX kit showed a **diagnostic sensitivity of 98.5%** and a **diagnostic specificity of 100%** when compared with the WOAH reference real-time PCR. The few discrepancies observed corresponded exclusively to samples with **high Ct values (>35)** and low viral loads, typically associated with chronic or late-stage infections, and therefore expected within the natural variability of ASFV detection.

2. **Analytical robustness in field matrices.** The kit successfully detected ASFV DNA in a wide diversity of field sample types originating from domestic pigs and wild boar, including tissues, blood, and sera, demonstrating **broad applicability across clinically relevant matrices**.

3. **Reproducibility and precision.** Inter-assay variability remained low, with **CV values between 2% and 3%** for positive controls, and an **average inter-assay CV of 0.77%** for field samples, indicating strong stability across runs, operators, and equipment. Intra-assay reproducibility was also high, with CV values ranging from **0.77% to 1.59%**, confirming the precision of repeated measurements within the same assay. Only one sample exhibited a higher CV (>10%), interpreted as an isolated occurrence without impact on the overall assay performance.

4. **Validation of controls and assay integrity.** All required controls (endogenous and exogenous) performed within the expected acceptance criteria. The exogenous control was tested at a higher concentration than recommended (10^{-4} instead of 10^{-5} dilution) without affecting assay validation, demonstrating **good tolerance and operational robustness**.

The validation demonstrated high concordance with the WOAH reference real-time PCR and showed excellent inter- and intra-assay reproducibility. The assay's controls behaved consistently, and variability remained within acceptable limits, confirming that the TARGETEX ASFV qPCR Detection Kit is robust, precise, and suitable for routine ASFV diagnostic testing under field conditions.

Report performed in Valdeolmos (Madrid) at 11th November 2025

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Approval

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