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Development and Validation of a Quantitative Synthetic Molecular Standard for African Swine Fever Virus

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Background and Introduction

African swine fever virus (ASFV) is a double-stranded DNA virus within the *Asfarviridae* family. ASFV infects both wild and domestic pigs, resulting in the highly contagious and deadly disease African swine fever.[1,2] The virus can be transmitted either directly from sick to healthy pigs or indirectly through contact with contaminated products or the bite of infected *Ornithodoros* ticks, which are natural hosts and reservoirs of the virus. The mortality rate is close to 100% for infected domestic pigs and clinical symptoms include sudden death, fever, reddening of skin, vomiting, diarrhea, abortion in pregnant sows, and malaise. There is currently no treatment or vaccine for ASFV, and prevention and control include culling of infected individuals and disinfection of the infected zone and surrounding area.

Diagnosis of infected individuals is necessary for infection management and preventing further spread of the virus. Disease presentation of ASFV is similar to classical swine fever and bacterial septicemia; therefore, laboratory testing is required for accurate diagnosis. While a culture-based approach can be used for detection, viral growth and purification can be difficult, time-consuming, and costly. PCR-based methods provide a sensitive and rapid alternative approach; however, these molecular-based methods are dependent on the availability of high-quality reference materials. To address this need, ATCC has designed and developed a quantitative synthetic molecular standard for ASFV. We used a proprietary strategy that incorporated key target regions from the genome as well as conserved regions used for viral detection and identification in various published assays.

The synthetic standard was validated through next-generation sequencing, and precise DNA copy number (copies/µL) was quantified using droplet digital™ PCR (Bio-Rad). The standard was then tested using an independent, publicly available qPCR assay, which verified the efficacy of the design against a relevant assay.[3] Ten-fold dilutions were used to create a standard curve, with DNA concentrations ranging from approximately 10 to 10^6 copies/µL. The ASFV standard had an R^2 value of 0.997, indicating a high degree of linearity. This standard displayed high efficiency and amplification, with a slope of M = -3.342. Overall, these data demonstrate the applicability of the ASFV synthetic molecular standard as a control in the development and validation of molecular-based detection and quantification assays.

ATCC Synthetic Molecular Standards

BSL-1	Quantitative	Stabilized

ATCC Catalog Number	Product Description
ATCC® VR-3283SD™	Quantitative Synthetic African swine fever virus DNA

Applications

- Generation of a standard curve for quantitative PCR
- Positive control for qPCR assays
- Assay verification and validation studies
- Monitor assay-to-assay and lot-to-lot variation
- Molecular diagnostics assay development

Materials and Methods

Quantitative Synthetic DNA
Using a proprietary method, we designed a synthetic DNA construct for African swine fever virus (ATCC VR-3283SD). This standard is comprised of fragments from the following genomic regions: B646L, A489, 505-2R, C717R, B962L, B119L, G1340L, and D1133L. Following construction, the standard was authenticated via next-generation sequencing and then quantified via droplet digital PCR.

qPCR Assay
The qPCR assay was performed using the CFX96™ Real-Time PCR Detection System (Bio-Rad) according to the manufacturer's instructions with slight modifications.

Droplet digital PCR Assay
Droplet digital PCR assays were performed according to the manufacturer's instructions using the QX200™ droplet reader and QuantaSoft™ software 1.7.4.0917 (Bio-Rad) for droplet generation and data analysis.

Results

The designed synthetic is a compatible control for a commonly used assay for detection of African swine fever virus

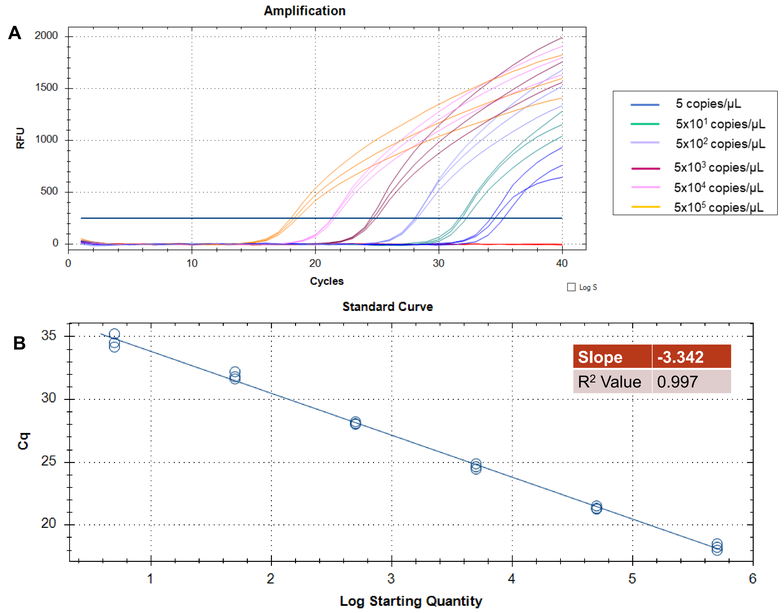

Figure 1: qPCR assay to verify the functionality of the synthetic molecular standards. (A) An amplification plot and (B) standard curve were generated with the African swine fever virus DNA standard. The qPCR assay was performed as previously described by King et al.[3] Cycling conditions were 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 sec and 58°C for 1 min. Standard curves were generated using serial 10-fold dilutions that ranged from 5 copies/µL to 5×10^5 copies/µL. The DNA standard was tested in triplicate.

Results (continued)

Absolute quantification of synthetic African swine fever virus DNA via droplet digital PCR

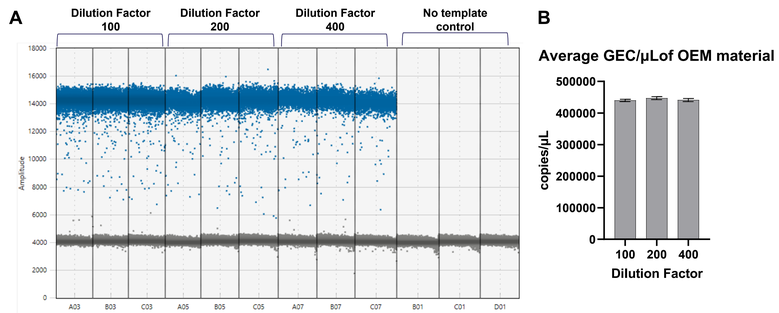

Figure 2: Absolute quantification of synthetic African swine fever virus DNA via droplet digital PCR. (A) One-dimensional (1-D) amplitude scatter plots of positive and negative digital PCR droplet reactions for three dilutions (dilution factors 100, 200, and 400) in triplicate were quantified by ddPCR by using a proprietary assay for quantitation of synthetic materials manufactured by ATCC. Droplets were analyzed in the QX200 droplet reader. Data were analyzed with QuantaSoft software (Bio-Rad). (B) Average calculated genome equivalent copy (GEC) numbers/µL per dilution of stock material.

Conclusions

- Our proof-of-concept data demonstrates that the ATCC quantitative synthetic molecular standard for African Swine Fever virus can be used as a control for assay development, verification, and validation.
- The standard was manufactured under ISO 13485 guidelines and can be used to determine the microbial load of unknown African swine fever virus samples through the generation of a standard curve.
- The standard is compatible with numerous published assays and exhibited minimal variability, as evident from the slope and R^2 values of the tested assay.
- This standard provides a well-characterized control for viral detection and quantification.

Order the ASFV molecular standard

References

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2. Turlewicz-Podbielska H, et al. African Swine Fever Virus as a Difficult Opponent in the Fight for a Vaccine-Current Data. Viruses 13(7):1212, 2021. PubMed: 34201761
3. King DP, et al. Development of a TaqMan PCR assay with internal amplification control for the detection of African swine fever virus. J Virol Methods 107(1):53-61, 2003. PubMed: 12445938

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