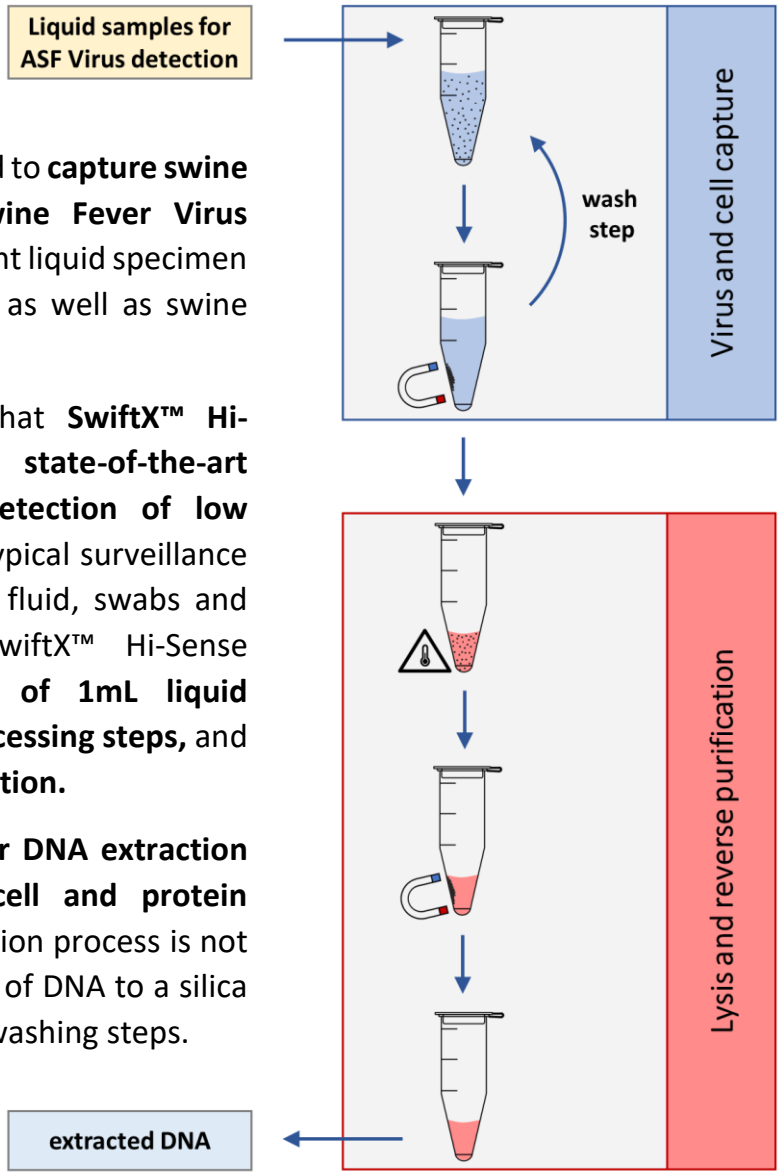
Extraction of African Swine Fever Virus (AFSV) DNA from liquid samples

Related product

This application note is related to **SwiftX™ Hi-Sense** (SXHS-25).



Summary

SwiftX™ Hi-Sense was applied to **capture swine host cells and African Swine Fever Virus (ASFV) particles** from different liquid specimen types **to extract ASFV DNA** as well as swine genomic DNA.

This study demonstrates that **SwiftX™ Hi-Sense is superior to state-of-the-art extraction methods for detection of low concentrations of ASFV** in typical surveillance samples such as swine oral fluid, swabs and environmental samples. SwiftX™ Hi-Sense allows routine **processing of 1mL liquid samples**, requires **fewer processing steps,** and provides a **faster DNA extraction.**

It is **particularly superior for DNA extraction from samples with low cell and protein content**, because the extraction process is not prone to suboptimal binding of DNA to a silica matrix and DNA loss during washing steps.

Introduction

African Swine Fever (ASF) originates from West Africa and has spread mainly to Eastern Europe and large parts of East Asia. The agent causing ASF is a highly infectious DNA virus transmitted through contaminated feed, contact to infected animals, and ticks. The virus replicates in macrophages of infected animals causing a hemorrhagic fever. Infected animals show a high mortality rate, which renders it a serious threat to livestock farms and the local food industry. In order to avoid local spreading of the disease, infected animals are isolated and culled.

ASFV detection and surveillance is important in endemic as well as non-endemic regions. Current DNA extraction and detection methods, however, do not enable a high sensitivity for environmental samples and require whole blood samples for the diagnostic of animals. A higher sensitivity for environmental samples is critical especially before repopulation of previously infected facilities. Thus, high diagnostic sensitivity from non-invasive samples such as swabs and oral fluids is needed to enhance monitoring and to lower the cost of diagnostics.

In this application note, we demonstrate and validate the enhanced sensitivity reached by the SwiftX™ Hi-Sense, which utilizes a different approach to DNA extraction by capturing cells and virus particles from liquid samples and "reverse purification" of extracted DNA.

Experimental details

The following samples from pig farms have been processed and tested:

- Swine oral fluid
- Oral and nasal swabs eluted in water or saline
- Environmental gauze soaking liquid

Extraction with the SwiftX™ Hi-Sense kit was performed essentially as described in the handbook. The following variations have been applied: for the sample types “swine oral fluid” and “oral/nasal swabs”, the wash step using Buffer EN was not performed. The DNA extraction was performed in 75µL Buffer DL spiked with 2µL IC DNA of the detection assay. The samples were incubated at 95°C for 15 minutes.

In parallel, all samples have been extracted using the MagAttract cador Pathogen Kit (QIAGEN, Germany) as a reference. The sample input volume of this kit is 0.2mL. The IC DNA was added to the sample before extraction and purification. The extracted DNA was finally eluted in a volume of 75µL to match the extraction volume of the SwiftX™ Hi-Sense kit.

Finally, 5µL of extracted DNA was used for ASFV detection using 25µL reactions of the Virotype ASFV 2.0 Assay (Indical Bioscience, Germany). This assay is a triplex real-time PCR assay that simultaneously detects ASFV DNA, swine DNA (as sampling and extraction control), as well as an IC DNA (as inhibition control).

For separation of the magnetic particles, the **magnetic rack (cat.no. MAG-12)** can be used, which is available for a competitive price from Xpedite Diagnostics.

Results

Detection of ASFV in liquid samples suspected to contain low concentrations of the virus was performed using a commercial real-time PCR kit. The sample DNA was extracted either with a silica magnetic bead-based extraction kit (standard sample input volume: 200µL) or with the SwiftX™ Hi-Sense kit (standard sample input volume: 1mL).

Figure 1 illustrates the comparison of quantitative PCR results with DNA retrieved from both extraction methods. Sample extraction using SwiftX™ Hi-Sense results in significantly lower Ct values compared to samples extraction using the silica extraction method. Interestingly, the observed Ct difference goes beyond the expectable value of 2.3 (which comes from the use of the higher sample volume): for oral fluids and swabbed samples, the median Ct difference is 3.85 and 3.40, respectively. This equals to a 10- to 14-fold concentration of ASFV DNA in SwiftX™ Hi-Sense extractions compared to the QIAGEN extraction.

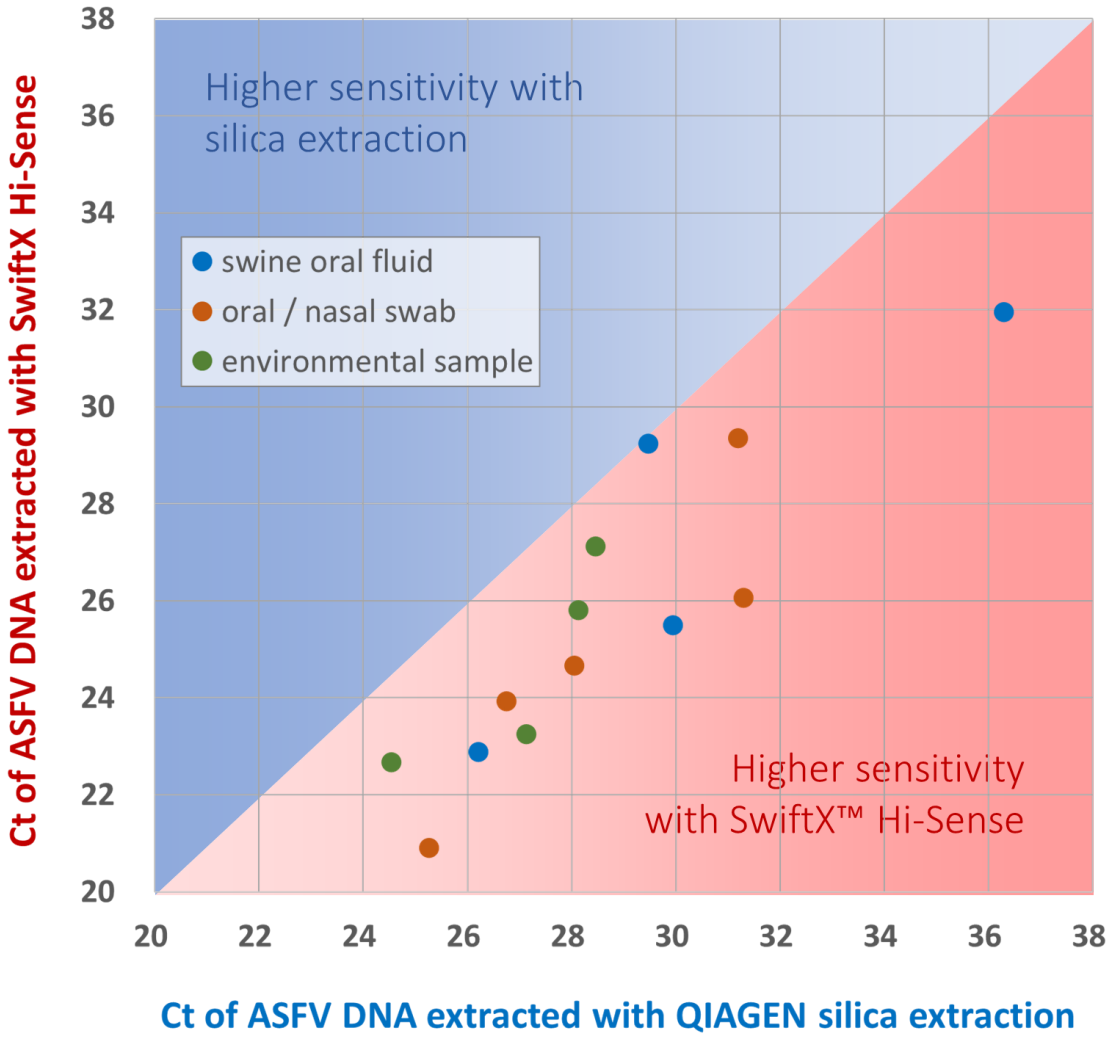


Fig. 1: Real-time PCR Ct values of ASF Virus DNA extracted with SwiftX™ Hi-Sense and a standard silica extraction method, respectively.

Tab. 1: Detailed information on Ct values for the ASFV detection channel of the real-time PCR analysis and the differences in Ct values between the two extraction methods.

Sample types	ASFV DNA		median ΔCt
	QIAGEN	SwiftX Hi-Sense	
oral fluid	29.47	29.23	3.85
	36.30	31.94	
	29.95	25.49	
	26.21	22.88	
oral / nasal swab	26.76	23.92	3.40
	31.20	29.34	
	28.06	24.66	
	31.31	26.05	
	25.27	20.90	
environmental gauze soaked liquid	27.14	23.25	2.12
	28.14	25.80	
	28.47	27.12	
	24.55	22.66	

Figure 2 summarizes the real-time PCR quantification of swine genomic DNA in the two types of extracted DNA. The genomic DNA serves as a sampling and extraction control for the ASFV assay. All extracted DNAs contained enough genomic DNA to securely indicate that the sample contained material of pig origin and that the extraction was successful. This illustrates nicely that SwiftX Hi-Sense also captures the animal cells in the sample.

Tab. 2: Detailed information on Ct values for the genomic DNA and IC DNA detection channel, respectively, of the real-time PCR analysis.

Sample types	Pig genomic DNA		Internal control DNA	
	QIAGEN	SwiftX Hi-Sense	QIAGEN	SwiftX Hi-Sense
oral fluid	31.44	32.31	21.42	20.59
	30.69	32.44	21.47	20.31
	25.77	23.04	21.51	20.29
	27.78	26.06	21.68	20.56
oral / nasal swab	21.53	19.87	21.40	20.22
	22.58	21.83	22.07	20.39
	25.93	23.89	21.21	20.52
	28.11	25.14	21.01	20.58
	28.26	23.4	21.22	20.09
environmental gauze soaked liquid	25.22	24.92	22.20	20.16
	29.30	26.74	21.13	20.73
	32.53	32.47	21.95	20.11
	25.93	24.62	21.19	20.41

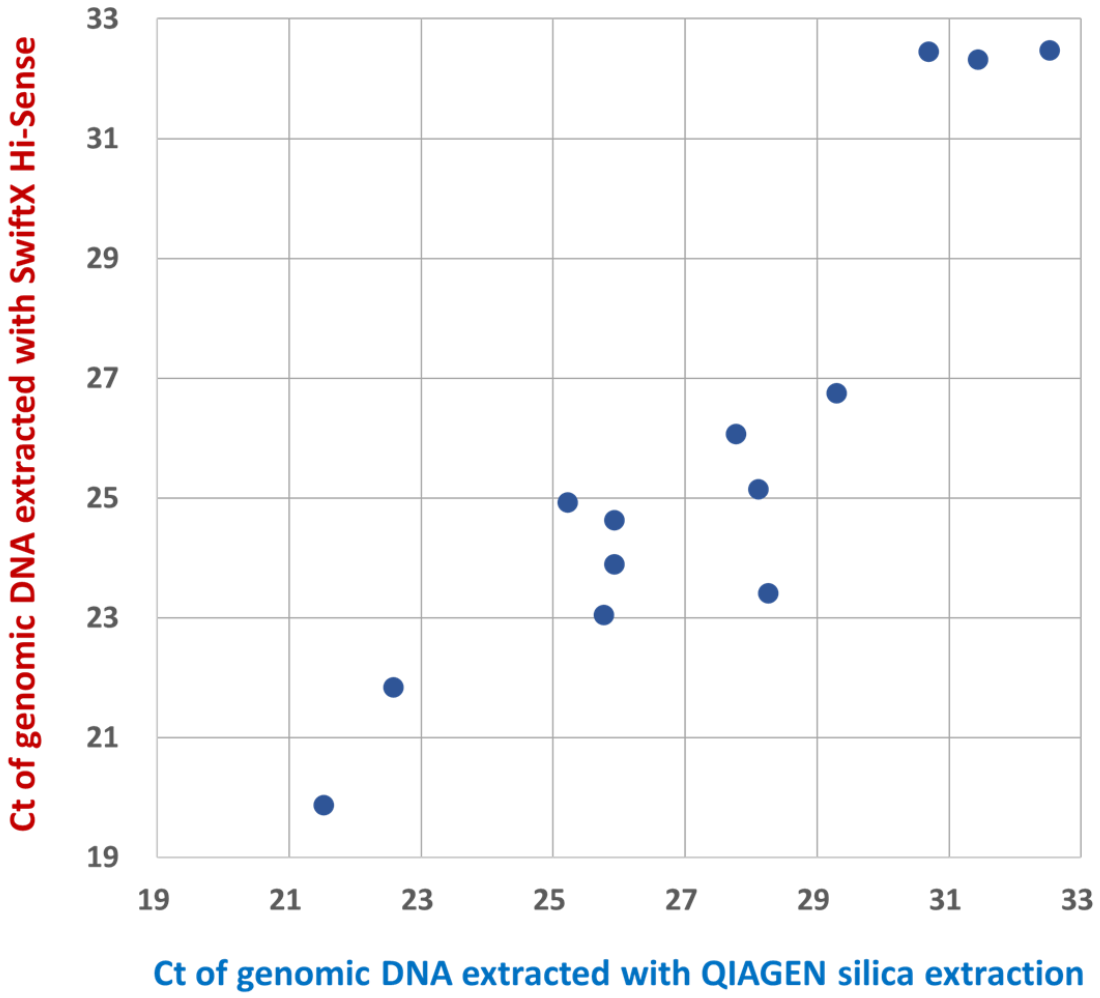


Fig. 2: Real-time PCR Ct values of pig genomic DNA extracted with SwiftX™ Hi-Sense and a standard silica extraction method, respectively.

Finally, Figure 3 shows the amplification results of the Internal Control DNA, which was added during the extraction process to provide additional feedback on the DNA extraction efficiency and on the presence of inhibitors. The consistency in recovery of internal control DNA after the extraction process is similar in both methods. However, SwiftX™ Hi-Sense recovers on average a two-fold amount of IC DNA compared to the silica method (average delta Ct = 1.1).

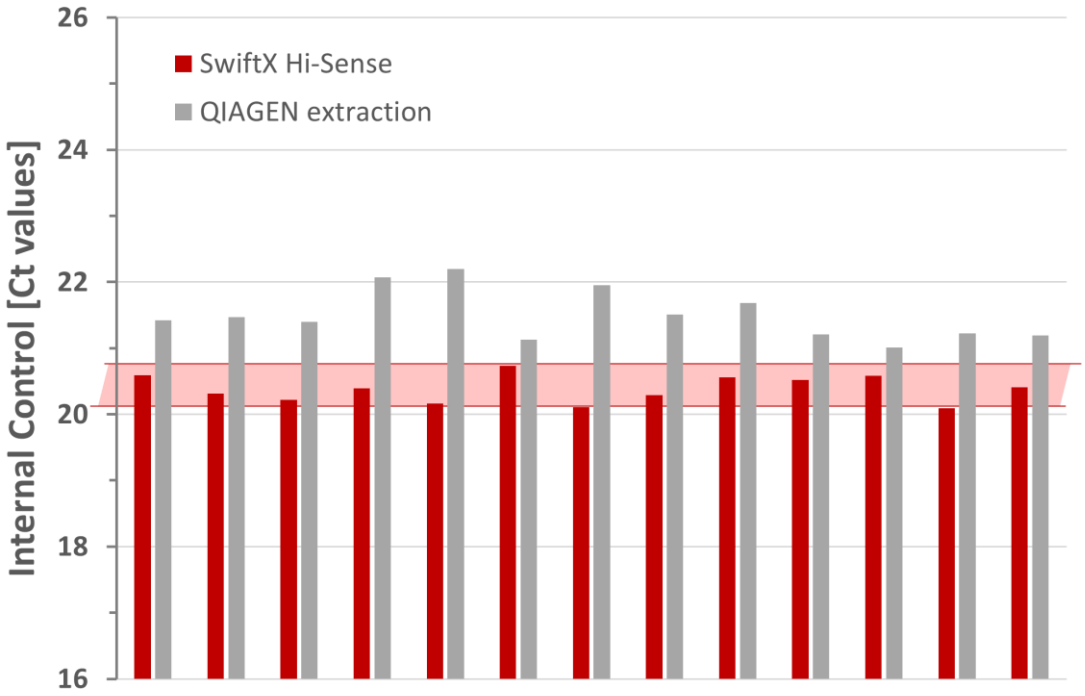


Fig. 3: Real-time PCR Ct values of IC-DNA extracted with SwiftX™ Hi-Sense and a standard silica extraction method, respectively.

Discussion

The data presented here demonstrate the high sensitivity of SwiftX™ Hi-Sense, which makes it a perfect solution for ASFV detection from low-positive samples. This is supported by the following observations:

- the magnetic bead-based capturing of ASF Virus particles from liquid samples leads to exceptionally good recovery of ASFV DNA
- the reverse purification mechanism provides efficient removal of inhibitory substances and avoids DNA loss as observable with silica-based purification methods.

SwiftX™ Hi-Sense unlocks the use of non-invasive sample types for monitoring and diagnostic of African Swine Fever in pigs as well as the use of environmental samples to confirm the negative status of cleaned facilities or trucks and other equipment and surfaces. Actually, SwiftX technology can be used to extract DNA from even larger volumes of up to 20mL liquid samples. For this, additional buffer EN is needed, which is available from Xpedite Diagnostics as a separate accessory.