Rapid Detection of Tropical Arboviruses using SwiftX™ Virus

Related product

This application note is related to **SwiftX™ Virus** (Cat. No. SXV-50, SXV-50-IVD)

Summary

Combating tropical diseases requires measures beyond vector mitigation and infection control. Rapid and effective diagnostic approaches support disease surveillance, improved patient care, as well as outbreak preparedness efforts. Advanced point-of-care (POC) multiplex systems need to be complemented by robust extraction protocols capable of processing different sample types, ensuring the purity and detectability of viral nucleic acids. This report summarizes the performance of the SwiftX™ Virus protocol, for rapid extraction of blood-borne arboviral RNA from plasma and serum samples performed in approximately 6 minutes. Validation efforts demonstrate the kit's robustness, rapid and simple method of nucleic acid extraction, revealing approximately 99% diagnostic accuracy across six tropical, arthropod-borne viruses relative to established protocols. These results highlight promising opportunities for SwiftX™ Virus to be used for POC efforts, implemented in the field to support disease surveillance, preparedness, and deploying mitigation measures.

Introduction

Arboviruses, or arthropod-borne viruses are RNA viruses which are responsible for almost 700,000 deaths each year. They are transmitted by arthropod vectors such as mosquitoes, ticks, sandflies, or midges, to vertebrates through a bite [1]. Anthropological activities cause endemic regions to expand, elevating the spread of arboviral infections which are becoming a significant threat to the global population.

Examples include Dengue, Chikungunya, Crimean Congo hemorrhagic fever, etc. which have caused many fatalities globally. The arboviral families such as *Flaviviridae*, *Togaviridae Bunyavirales*, etc. exhibit overlapping symptoms, and produce similar antigens, making it difficult for conventional serological methods to detect the correct infectious agent due to cross-reactivity [2]. Left undetected, patients could also develop life-threatening conditions like hemorrhagic fevers, neurological disorders, and organ dysfunction [3]. Therefore, to aid early detection and differentiation of viral serotypes, efficient, cost-effective, and field-deployable testing methods are needed for implementation at the point-of-care.

Molecular diagnostics, mainly the RT-PCR method, is considered a gold standard for arbovirus detection during early infection cycles as they render better specificity towards the different serotypes as opposed to traditional serological and rapid antigen tests [4]. Moreover, advancements in POC systems have provided us with portable thermocyclers, isothermal amplification technologies, and next generation sequencing, reducing the need for a laboratory setup [5].

Sample preparation is critical for these methods as they use different sample types including serum, whole blood, urine and cerebrospinal fluid, whose composition is highly variable and hence pose a challenge towards designing POC-applicable nucleic acid (NA) extraction protocols [6,7]. Commercial kits employ DNA/RNA binding techniques in silica column-based or magnetic bead-based protocols, which use a variety of consumables and equipment that complicate their application in the field.

Therefore, through this application note, we demonstrate the performance of SwiftX™ Virus, a rapid, robust, and user-friendly NA extraction protocol that employs a reverse purification technique, to combine sample purification and nucleic acid isolation in under 15 minutes.

Experimental Methods

The experimental workflow assessed two sample matrices – plasma and serum. The sample belonged to two categories: pre-diagnosed, frozen clinical samples and control samples spiked in with live viral cultures, respectively. All spike-in control samples were serially diluted ten-fold, in the respective matrices, to determine the lowest range of detectable viral titers in the eluate. Triplicates of CHIKV, YFV, and USUV spike-in samples were extracted as opposed to just one for DENV. The details of the viruses evaluated for the different sample categories are listed in Table 1. Experiments were conducted at the following validation sites:

(i) Prof. Luisa Barzon's Lab, Department of Molecular Medicine, University of Padova, Italy

(i) Prof. Nazif Elaldi's Lab, Department of Infectious Disease and Clinical Microbiology, Sivas Cumhuriyet University, Türkiye.

Arbovirus	Sample Category (Clinical/Spiked-In control)	Sample Matrix (Plasma/ Serum)	Extraction Protocol compared to SXV (Reference Protocols)	Validation Site
Dengue Virus (**DENV**) serotypes 1-3	Clinical + Spiked-in controls (only DENV1)	Plasma & Serum	MagNAV	Italy
West Nile Virus **(WNV)** serotypes 1 & 2	Clinical	Plasma & Serum	MagNAV	Italy
Chikungunya Virus (**CHIKV**)	Spiked-in Control	Plasma & Serum	MagNAV	Italy
Yellow Fever Virus (**YFV**)	Spiked-in Control	Plasma & Serum	MagNAV	Italy
Usutu Virus (**USUV**)	Spiked-in Control	Plasma & Serum	MagNAV	Italy
Crimean Congo Hemorrhagic Fever Virus (**CCHFV**)	Clinical	Serum	QIAV	Türkiye

Table 1 *Overview of experimental setup and samples*

The clinical samples were initially characterized using in-house reference standards that were used for routine testing.

i. University of Padova, Italy - Roche's MagNA Pure 96 Viral NA Kit (MagNAV)

ii. Sivas Hospital, Türkiye - in-house RNA extraction protocol

Nevertheless, for the present comparative study, extractions performed in Turkey were done using Qiagen's QIAamp® Viral RNA Kit (QIAV) whereas, in Padova, the same Roche automated extraction workflow was used (refer to Table 1).

All three experimental protocols were performed according to the manufacturer's instructions and are depicted in Figure 1. Separation of the magnetic nanoparticles in SXV workflow was done using the magnetic rack (cat.no. MAG-12, MAG-4), which is available from Xpedite Diagnostics GmbH.

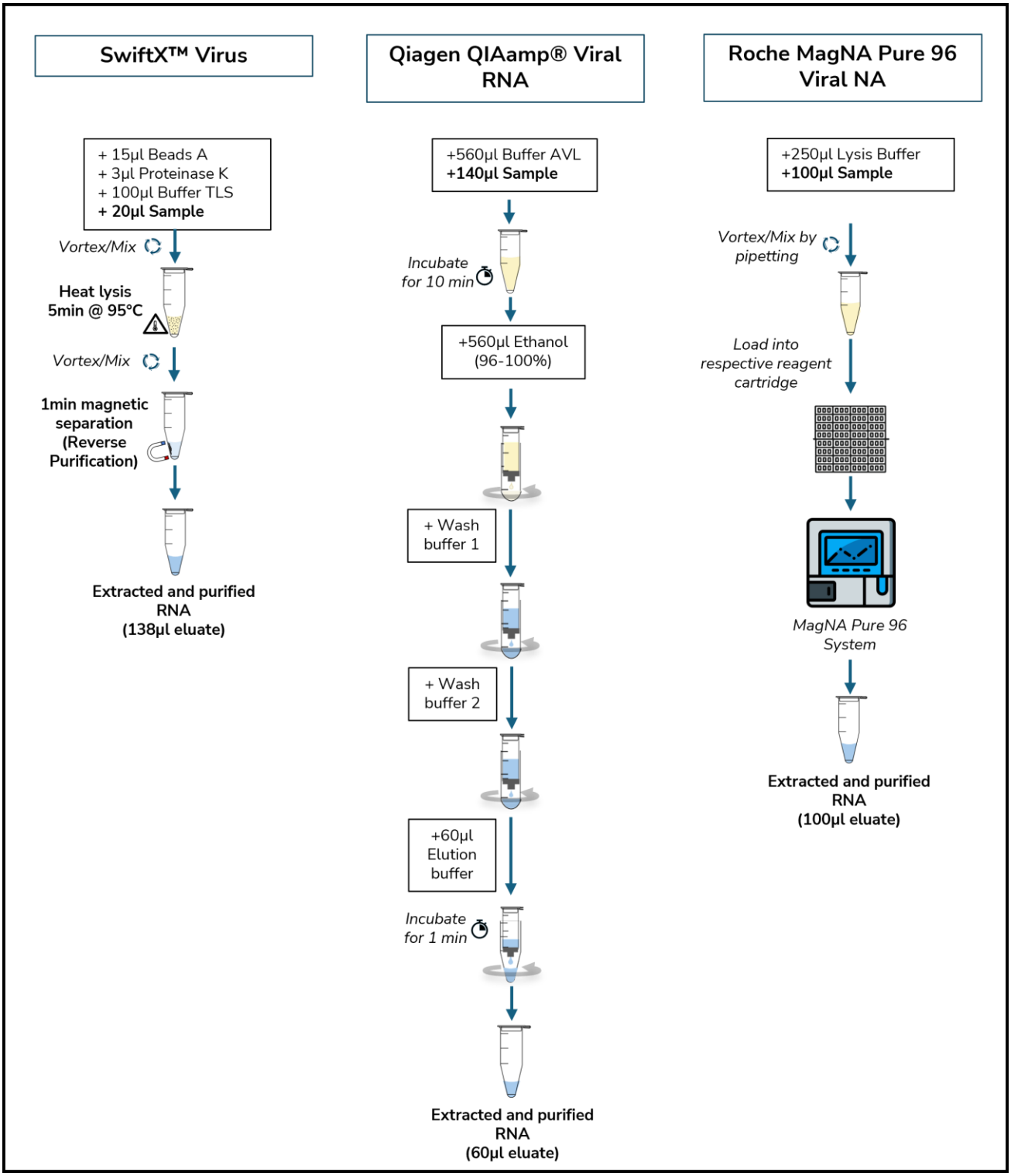

Figure 1 Comparison of experimental workflows for SXV vs Reference Protocols. Icons were either created using MS PowerPoint or taken from https://www.freepik.com/

The extracted nucleic acids were quantified using real-time RT-PCR assays either developed in-house (with TaqPath 1-Step RT-qPCR Master mix at Padova, Italy) or commercial assay kits (Bioeksen CCHFV RT-qPCR Kit CE-IVD at Sivas, Türkiye).

Results

A total of 171 samples were processed for this study, with 139 clinical samples containing 73 positives and 66 negatives, whereas the spike-in controls amounted to 32 samples, combining replicate values. RT-qPCR data for spike-in samples, performed specifically for mosquito-borne viruses, demonstrated statistically concordant relations between SXV and MagNAV methods, even for heavily diluted samples. (Refer to Figure 2). The RNA recovery from CHIKV, YFV, and USUV spike-ins is consistent with both methods, exhibiting a strong correlation ($r > 0.9$) whereas DENV extraction dataset with SXV highlights a single outlier, which after correction indicates a strong correlation to MagNAV ($r > 0.9$) as well. The relational trends with the corrected Pearson coefficients (r) can be observed in Figure 2.

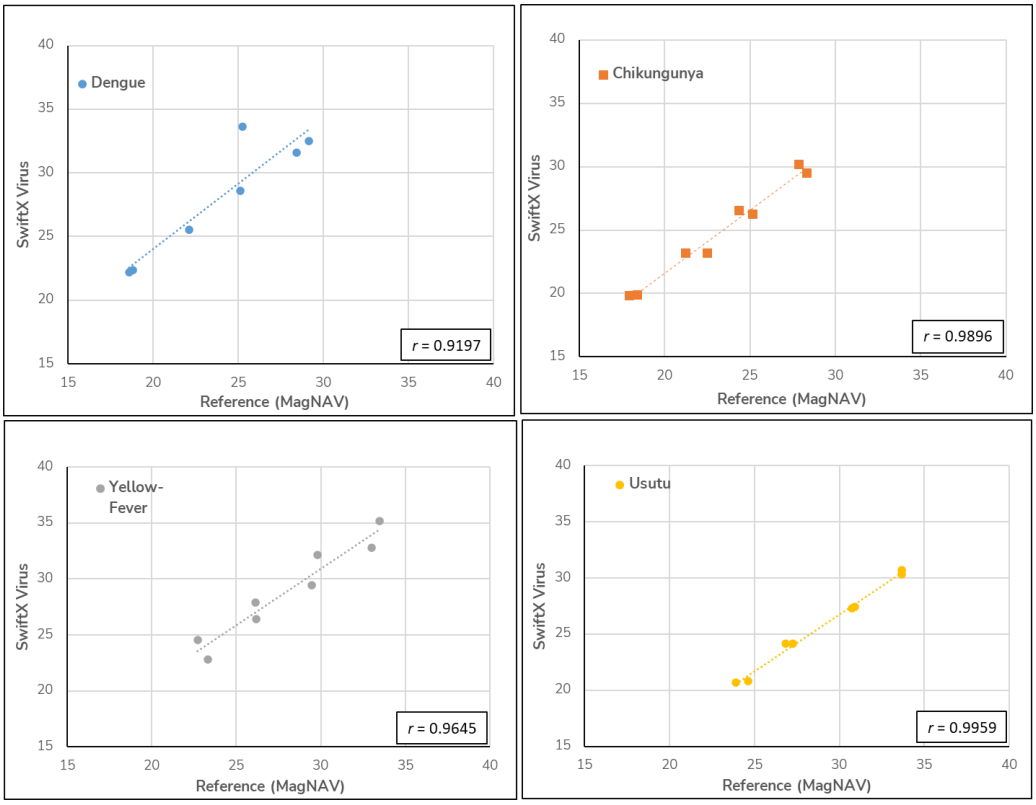

Figure 2 *SXV vs MagNAV correlation for spike-in controls. The Pearson's coefficient (**r**) is provided at the bottom-right of each graph to validate the correlations. R value is calculated from https://www.socscistatistics.com/*

In the case of clinical samples, all positives and negatives for DENV (serotypes 1, 2, and 3) and CCHFV were correctly identified by all comparative methods – SXV & MagNAV (DENV) and SXV & QIAV (CCHFV), with DENV samples showing higher concordance (11 positives), compared to CCHFV (50 positives) as shown in Figure 3.

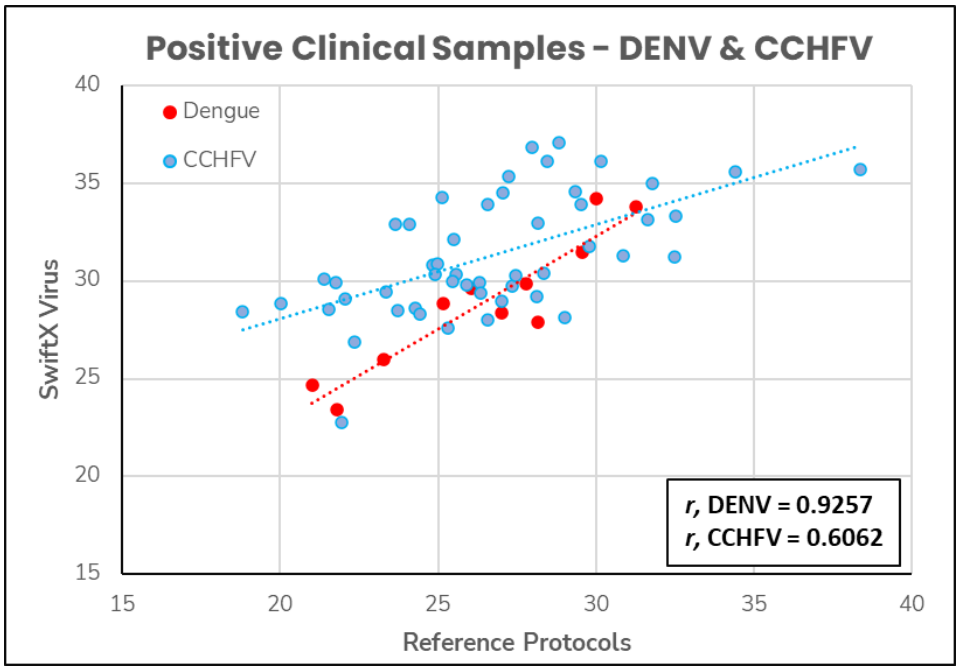

Figure 3 *Clinical sample correlation - SXV vs Reference Protocols*

On the other hand, WNV (serotypes 1&2) clinical samples extracted with SXV and MagNAV protocols were mostly indicated as negative. Although MagNAV extraction system was used to initially characterize the chosen clinical samples, only one sample was rendered positive in the current study as opposed to SXV which showed five positives (Refer to Table 2).

N = 18		MagNA Pure 96 Viral NA	
		Pos	Neg
SwiftX™ Virus Kit	Pos	1	4
	Neg	0	13

Table 2 *Concordance Matrix for WNV clinical samples*

This discordance was corrected by verifying the current sample positivity or negativity with the pre-characterized diagnostic results for the final diagnostic performance evaluation.

Discussion

This study introduces a simple yet rapid workflow for arboviral RNA extraction, while also validating the sensitivity and specificity of SwiftX™ Virus against routine reference standards such as Qiagen's QIAamp® Viral RNA Kit (silica column-based) and Roche's MagNA Pure 96 Viral NA Kit (magnetic bead-based).

Although both plasma and serum were used as sample matrices for spiked controls, no notable effect or variation was introduced in the extraction process rendering SXV results highly comparable with reference protocols. This observation provides compelling evidence for the robustness of the SwiftX™ Virus protocol in handling complex sample matrices. The novel reverse purification technique, mediated by the paramagnetic nanoparticles in addition to proteinase K digestion effectively removes the high protein content in sera or the proteinaceous viral envelopes, allowing for PCR amplification of viral RNA.

SXV also yielded considerable RNA amounts from clinical samples containing a mixture of viral serotypes such as DENV 1, 2, 3 or WNV 1&2, correctly identifying between serotypes. DENV (with 11 positives and 10 negatives) and CCHFV (50 positives and 50 negatives) samples were accurately diagnosed but WNV extractions were inconclusive in comparison with the reference MagNAV. When compared with the initial diagnosis, which recorded 12 positives and 6 negatives, four samples which held a 'false positive' status with SXV vs MagNAV (see Table 2), were confirmed as true positives. Most of the frozen clinical samples reported high Ct values ranging from 29 – 38 during the primary diagnosis and have undergone one freeze-thaw cycle for this study, which could have contributed to the high number of negatives from the pre-determined positive samples for both SXV and MagNAV.

Based on all the RT-qPCR data obtained from collectively 171 samples, and correcting for the discordance in WNV samples, we statistically evaluated the dataset and calculated the diagnostic performance for SwiftX™ Virus as indicated in Table 3.

	Value	95% CI min	95% CI max
Diagnostic Sensitivity:	98.98%	94.45%	99.97%
Diagnostic Specificity:	100.00%	95.07%	100.00%
Positive Predictive Value:	100.00%	96.27%	100.00%
Negative Predictive Value:	99.75%	98.24%	99.96%
Diagnostic Accuracy:	99.80%	97.46%	100.00%

Table 3 *Diagnostic Performance of SwiftX Virus with 95% Confidence Intervals (CI). Calculated with MedCalc. PPV and NPV are based on an assumed disease prevalence of 20% among tested patients.*

Therefore, SwiftX™ Virus exhibits 98.98% clinical sensitivity, and 100% clinical specificity which exceeds the expected ≥ 90% sensitivity and ≥95% specificity upon comparison with reference protocols.

Literature:

[1] Brady OJ, Bastos LS, Caldwell JM, et al. Why the growth of arboviral diseases necessitates a new generation of global risk maps and future projections. PLOS Comput Biol. 2025;21(4):e1012771.

[2] World Health Organization. WHO guidelines for clinical management of arboviral diseases: dengue, chikungunya, Zika and yellow fever [Internet]. 2025 [cited 2025 Sep 22]. Available from: https://www.who.int/publications/i/item/9789240111110.

[3] Sigfrid L, Chan XHS, Kasbergen LMR. Prevalence, clinical management, and outcomes of adults hospitalised with endemic arbovirus illness in southeast Europe (MERMAIDS-ARBO): a prospective observational study - The Lancet Infectious Diseases [Internet]. 2025 [cited 2025 Sep 16]. Available from: https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(24)00655-8/fulltext.

[4] Lakshmanan K, Liu BM. Impact of Point-of-Care Testing on Diagnosis, Treatment, and Surveillance of Vaccine-Preventable Viral Infections. Diagnostics. 2025;15(2):123.

[5] Rutkowski N, Dong Y, Dimopoulos G. Field-deployable molecular diagnostic platform for arbovirus detection in Aedes aegypti. Parasit Vectors. 2020;13(1):489.

[6] Pan American Health Organization. Recommendations for Laboratory Detection and Diagnosis of Arbovirus Infections in the Region of the Americas [Internet]. Pan American Health Organization; 2023 [cited 2025 Sep 16]. Available from: https://iris.paho.org/handle/10665.2/57555.

[7] Kobialka RM, Ceruti A, Truyen U, et al. Comparative Evaluation of Extraction Protocols for Point-Of-Need Molecular Diagnostics. iLABMED. 2025;3(1):7–13.