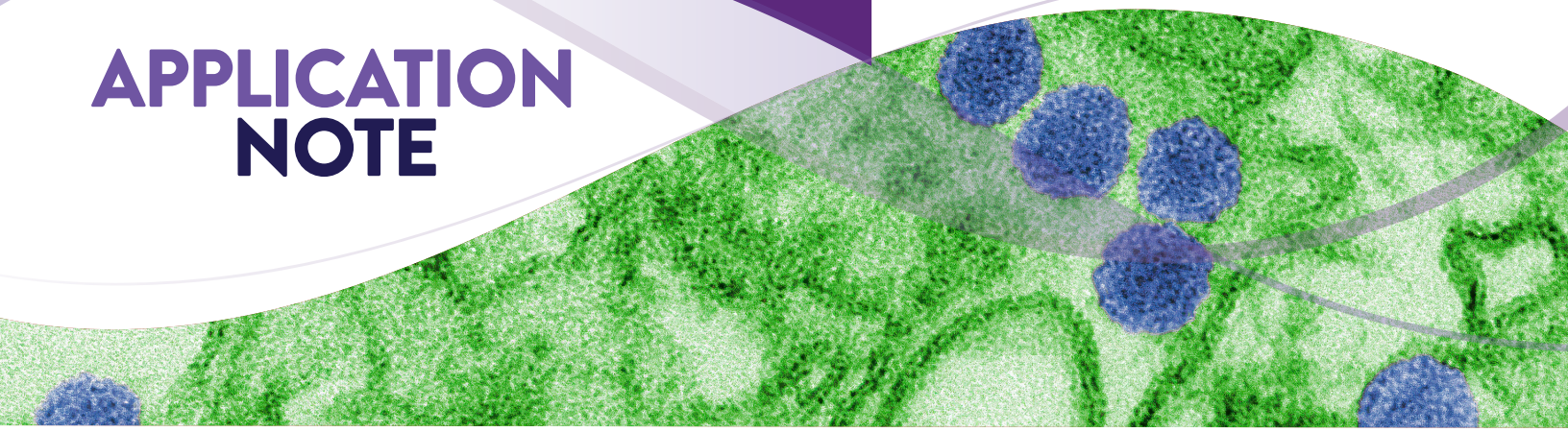
ATCC®

APPLICATION NOTE



# Performance Assessment of ATCC® Quantitative Synthetic Zika Virus RNA as an Analytical Reference Material

Authors: Holly A. Asbury, BS; Sydney McKnight, MS; Kyle Young, MBA; Victoria Knight-Connoni, PhD; Leka Papazisi, DVM, PhD

## Abstract

Zika virus (ZIKV), an arbovirus first identified in Uganda in 1947, has emerged as a global health concern due to recent outbreaks facilitated by the spread of *Aedes* mosquitoes. While most ZIKV infections are mild or asymptomatic, severe neurological complications can occur. Rapid and accurate identification of ZIKV is therefore essential, making robust molecular assays and the high-quality reference materials needed to validate them critical for effective diagnostics and surveillance. This study evaluates ATCC® Quantitative Synthetic Zika virus RNA (ATCC® VR-3252SD™) as an analytical reference material for qRT-PCR assay development and validation. The synthetic RNA was tested across multiple established qRT-PCR protocols, demonstrating its suitability for reliable detection and quantification of ZIKV when benchmarked against genomic RNA (gRNA) from both genetic lineages of ZIKV.

## Introduction

Zika virus (ZIKV) is an arbovirus that was first isolated in 1947 from a rhesus monkey in the Zika forest of Uganda and shortly thereafter from *Aedes africanus* mosquitoes. For decades following its discovery, ZIKV has been detected in mosquito and human populations in Africa and Southeast Asia.[1] In the past two decades, however, ZIKV has emerged as a significant global public concern, driven by a series of widespread outbreaks across the world. Facilitated by increasing international travel and the expanding geographic range of *Aedes* mosquito vectors driven by climate change, ZIKV has caused major outbreaks in Micronesia in 2007, French Polynesia in 2013-2014, and the Americas in 2015-2016—most notably in Brazil, Puerto Rico, and the US Virgin Islands.[2] ZIKV infection is typically mild, presenting with fever, rash, or conjunctivitis, and approximately 80% of people infected are asymptomatic. However, as observed during the 2015-2016 outbreak, Zika can, in rare cases, lead to severe neurological complications like Guillain-Barré syndrome, encephalopathy, and other disorders.[3]

Currently two genetic lineages of ZIKV are recognized: the "African lineage" and the "Asian lineage." The MR 766 strain from Uganda is considered the "classical strain" and part of the "African lineage." Most cases outside of Africa, including those reported during the North and South American outbreaks in 2015-2016, are caused by the "Asian lineage" of viruses.[4] As the *Aedes* mosquitoes continue to spread farther north with the warming climate across the globe, the risk of ZIKV or other arboviral infections is expected to increase, underscoring the importance of robust diagnostic and surveillance systems.

PCR-based methods remain central to ZIKV detection because of their high sensitivity and rapid turnaround time. However, the development and validation of these assays depend on high-quality reference materials. Here, we demonstrate the utility of the ATCC® Quantitative Synthetic Zika virus RNA (ATCC® VR-3252SD™) as a robust analytical reference material for qRT-PCR assay development and validation, and we compare amplification performance to gRNA from both ZIKV genetic lineages.

## Materials and Methods

Quantitative Synthetic Zika virus RNA (ATCC® VR-3252SD™) comprises defined RNA fragments derived from multiple regions of the ZIKV genome, including the membrane glycoprotein precursor M, envelope (ENV), NS1, NS2B, NS3, NS4B, and NS5 genes—segments commonly targeted in diagnostic assays. This preparation was manufactured under ISO 13485 guidance, authenticated by next-generation sequencing, quantified via digital-based PCR, and stabilized in a proprietary storage buffer, which enables a 5-year-long shelf life and ensures consistent results. In this study, we compared amplification performance of the synthetic RNA with that of genomic RNA (gRNA) extracted from two ZIKV strains (Table 1): strain MR 766, representing the "African lineage," and strain PRVABC59, a Puerto Rican isolate from 2015, representing the "Asian lineage".

The functionality of the synthetic ZIKV RNA as an analytical reference material (ARM) was verified using qRT-PCR assays from the Centers for Disease Control and Prevention (CDC),[1] Pasteur Institute of Dakar,[5] and World Health Organization (WHO)[6] (Table 2). These experiments were performed on the CFX Opus Real-Time PCR System (Bio-Rad), with amplification achieved using the Invitrogen SuperScript III Platinum One-Step qRT-PCR Kit (Thermo Fisher Scientific). Cycling parameters and oligonucleotide concentrations emulated the source publication of each assay. All oligonucleotides, except the TaqMan probe described in Faye *et al.*, 2013, were ordered from Integrated DNA Technologies; TaqMan probes were tagged with 6-FAM and double-quenched. The TaqMan probe from the Faye *et al.*, 2013, assay was procured from Thermo Fisher Scientific, and was labeled with the 6FAM dye and a minor groove-binding (MGB) quencher. Synthetic and genomic RNA templates were tested at concentrations ranging from 50-50,000 genome copies/reaction (GC/rxn).

**Table 1: ATCC® items featured in this study**

| ATCC® item | Description |
|---|---|
| VR-3252SD™ | Quantitative Synthetic Zika virus RNA |
| VR-1838DQ™ | Quantitative Genomic RNA from Zika virus strain MR 766 |
| VR-1843DQ™ | Quantitative Genomic RNA from Zika virus strain PRVABC59 |

**Table 2: Published qRT-PCR assays assessed for product compatibility in this study**

| Publication source | Assay target |
|---|---|
| Faye *et al.* (Pasteur Institute of Dakar), 2013[5] | NS5 |
| Lanciotti *et al.* (CDC), 2008[1] | ENV |
| Tappe *et al.* (WHO), 2014[6] | NS3 |

## Results

Comparative amplification results for the qRT-PCR assay targeting NS5 (Figure 1A) described by Faye *et al.*, 2013,[5] demonstrate efficient and comparable performance of the synthetic RNA and MR 766 gRNA in terms of Ct values, $R^2$ values, and fluorescence levels. The dilutions of PRVABC59 gRNA were amplified approximately three cycles later than those of the synthetic RNA and MR 766 gRNA, with comparatively lower fluorescent signals, although the $R^2$ value remained comparable.

For the two CDC assays targeting ENV (Lanciotti *et al.*, 2008[1]), dilutions of the synthetic RNA and gRNA from both strains amplified comparably, with $R^2$ values indicating high linearity ($R^2$ = 0.998-1.000 for all templates across both assays). However, amplification of MR 766 gRNA was delayed by about one cycle in the first ENV assay (Figure 1B) and by about three cycles in the second (Figure 1C) relative to the synthetic RNA and PRVABC59 gRNA.

With the WHO assay targeting NS3 (Tappe *et al.*, 2014[6]), we observed comparable amplification between the synthetic RNA and MR 766 gRNA, but little to no amplification of PRVABC59 gRNA (Figure 1D). These amplification discrepancies between the two gRNAs may be due to mismatches with the primer and probe sequences, which affect amplification efficiency. An in silico mismatch analysis was performed (Table 3), where we summarize the experimentally verified compatibility of each of the three ZIKV RNA items with all four qRT-PCR assays. In cases where the gRNA sequence did not perfectly match the oligonucleotide sequences of an assay, amplification efficiency was diminished, or, in the case of the WHO NS3 assay with gRNA from strain PRVABC59, no significant amplification was observed.

These results present a potential advantage for using the synthetic RNA as a control for either ZIKV genetic lineage, as it is compatible with all four qRT-PCR assays irrespective of lineage-specific genomic variation. Collectively, the data from this study indicate that the synthetic ZIKV RNA (ATCC® VR-3252SD™) is well designed and suitable for assay development and validation, exhibiting qRT-PCR performance comparable to native ZIKV gRNA while maintaining consistent compatibility across all assays tested.

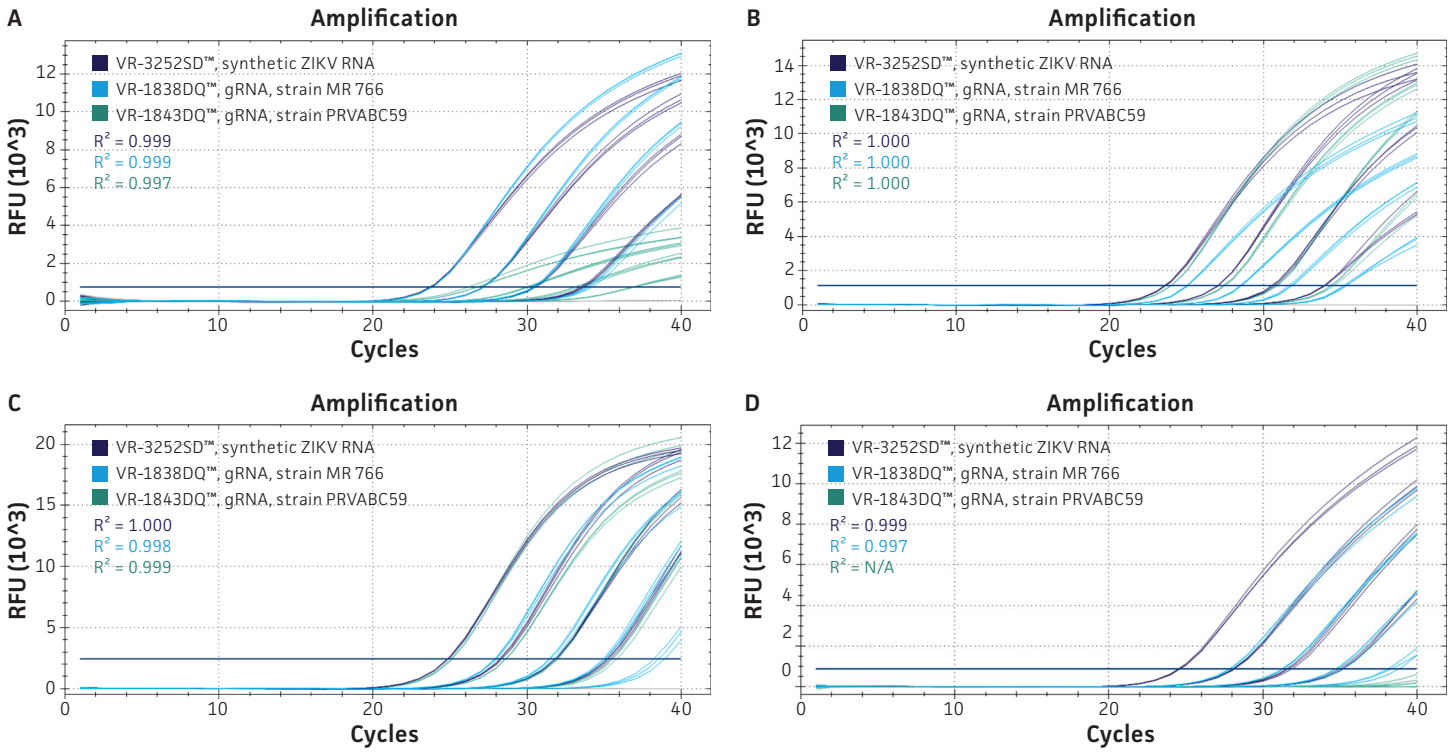



**Figure 1: Comparative qRT-PCR amplification of synthetic ZIKV RNA and gRNA.** qRT-PCR amplification curves generated with synthetic ZIKV RNA (ATCC® VR-3252SD™), gRNA from ZIKV strain MR 766 (ATCC® VR-1838DQ™), and gRNA from ZIKV strain PRVABC59 (ATCC® VR-1843DQ™) using (A) an assay targeting NS5 from Faye *et al.*, 2013,[5] (B, C) two assays targeting ENV from the CDC (Lanciotti *et al.*, 2008[1]), and (D) an assay targeting NS3 from the WHO (Tappe *et al.*, 2014[6]).

**Table 3: In silico mismatch analysis and experimentally verified assay compatibility of published qRT-PCR assays with ZIKV RNA items**

| | | | | In silico mismatches with sequence | | |
|---|---|---|---|---|---|---|
| **Publication Source** | **Assay Target** | **Primer or Probe** | **Sequence (5' to 3')** | **Synthetic RNA (VR-3252SD™)** | **MR 766 gRNA (VR-1838DQ™)** | **PRVABC59 gRNA (VR-1843DQ™)** |
| Faye *et al.*, 2013[5] (Pasteur Institute of Dakar) | NS5 | Forward Primer | AARTACACATACCARAACAAAGTGGT | 0 | 0 | 0 |
| | | Reverse Primer | TCCRCTCCCYCTYTGGTCTTG | 0 | 0 | 0 |
| | | Probe | CTYAGACCAGCTGAAG* | 0 | 0 | 1 |
| | | Experimentally verified assay compatibility | | + | + | +** |
| Lanciotti *et al.*, 2008[1] (CDC) | ENV | Forward Primer 1 | TTGGTCATGATACTGCTGATTGC | 0 | 0 | 0 |
| | | Reverse Primer 1 | CCTTCCACAAAGTCCCTATTGC | 0 | 3 | 0 |
| | | Probe 1 | CGGCATACAGCATCAGGTGCATAGGAG | 0 | 2 | 0 |
| | | Experimentally verified assay compatibility | | + | +** | + |
| | | Forward Primer 2 | CCGCTGCCCAACACAAG | 0 | 2 | 0 |
| | | Reverse Primer 2 | CCACTAACGTTCTTTTGCAGACAT | 0 | 1 | 0 |
| | | Probe 2 | | 0 | 1 | 1 |
| | | Experimentally verified assay compatibility | | + | +** | + |
| Tappe *et al.*, 2014[6] (WHO) | NS3 | Forward Primer | TGGAGATGAGTACATGTATG | 0 | 0 | 2 |
| | | Reverse Primer | GGTAGATGTTGTCAAGAAG | 0 | 0 | 3 |
| | | Probe | CTGATGAAGGCCATGCACACTG | 0 | 0 | 2 |
| | | Experimentally verified assay compatibility | | + | + | -*** |

*This oligo was modified from the source publication (last nucleotide changed from R to G) since the MGB probe could not be manufactured with a terminal degenerate base.
**Decreased amplification efficiency compared to templates with 0 mismatches with the oligonucleotide sequences.
*** No amplification

## Conclusions

The findings from this study confirm that the ATCC® Quantitative Synthetic Zika virus RNA (ATCC® VR-3252SD™) is a safe and reliable ARM for qRT-PCR assay development, verification, and validation. Across four widely used molecular assays, the synthetic RNA consistently produced amplification profiles comparable to native ZIKV gRNA from both the "African lineage" and "Asian lineage." Importantly, its compatibility across assays, despite lineage-specific sequence variation that affected the performance of some gRNA templates, highlights it utility as a lineage-agnostic control material. Together, these results support the use of the synthetic RNA preparation as a high-quality, standardized control material for the development and validation of diagnostic tools for detecting either genetic lineage of ZIKV.

## References


1. Lanciotti RS, *et al.* Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis. 14(8): 1232-9, 2008. PubMed: 18680646
2. Lura T, *et al.* A validated triplex RT-qPCR protocol to simultaneously detect chikungunya, dengue and Zika viruses in mosquitoes. J Vector Borne Dis 59(3): 198-205, 2022. PubMed: 36511035
3. Centers for Disease Control and Prevention (CDC). Clinical Signs and Symptoms of Zika Virus Disease. Accessed January 2026. https://www.cdc.gov/zika/hcp/clinical-signs/index.html
4. Beaver JT, *et al.* Evolution of Two Major Zika Virus Lineages: Implications for Pathology, Immune Response, and Vaccine Development. Front Immunol 9: 1640, 2018. PubMed: 30072993
5. Faye O, *et al.* Quantitative real-time PCR detection of Zika virus and evaluation with field-caught mosquitoes. Virol J 10: 311, 2013. PubMed: 24148652
6. Tappe D, *et al.* First case of laboratory-confirmed Zika virus infection imported into Europe, November 2013. Euro Surveill 19(4): 20685, 2014. PubMed: 24507467



10801 University Boulevard
Manassas, Virginia 20110-2209

703.365.2700 703.365.2701 sales@atcc.org www.atcc.org



**ZIKA-032026-v01**