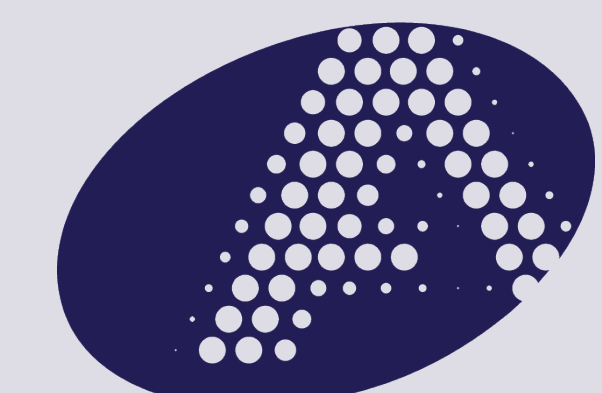


Performance Assessment of ATCC® Quantitative Synthetic Analytical Reference Material for Zika Virus



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Credible leads to Incredible®

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Background and Introduction

Zika virus (ZIKV) is an enveloped, single-stranded RNA arbovirus transmitted primarily by *Aedes aegypti* mosquitoes, sexual contact, or from mother to fetus during pregnancy. ZIKV is classified into 2 major genotypes: African and Asian. Most infections are asymptomatic or mild, with rash, fever, conjunctivitis, headache, malaise, and muscle or joint pain. However, infections during pregnancy are linked to severe congenital abnormalities like microcephaly. ZIKV infection has also been associated with neurological complications such as Guillain-Barré syndrome, though these outcomes are rare.

Accurate identification is critical for diagnosis because ZIKV symptoms resemble other viral infections. While culture-based approaches can be used for detection, viral growth and purification are tedious, slow, and costly. PCR-based methods provide sensitive, rapid alternatives but require high-quality reference materials. To meet this need, ATCC developed an analytical reference material (ARM) for ZIKV in the form of a quantitative synthetic RNA (ATCC® VR-3252SD™). A proprietary strategy was utilized to construct a synthetic that incorporates genes typically targeted in various assays for viral detection and identification. A synthetic ARM is beneficial as it contains all relevant genes but is BSL-1. In this study, the synthetic ZIKV RNA was evaluated as an ARM across published qRT-PCR assays from the CDC, WHO, and Faye *et al.* 2013.⁴⁻⁶

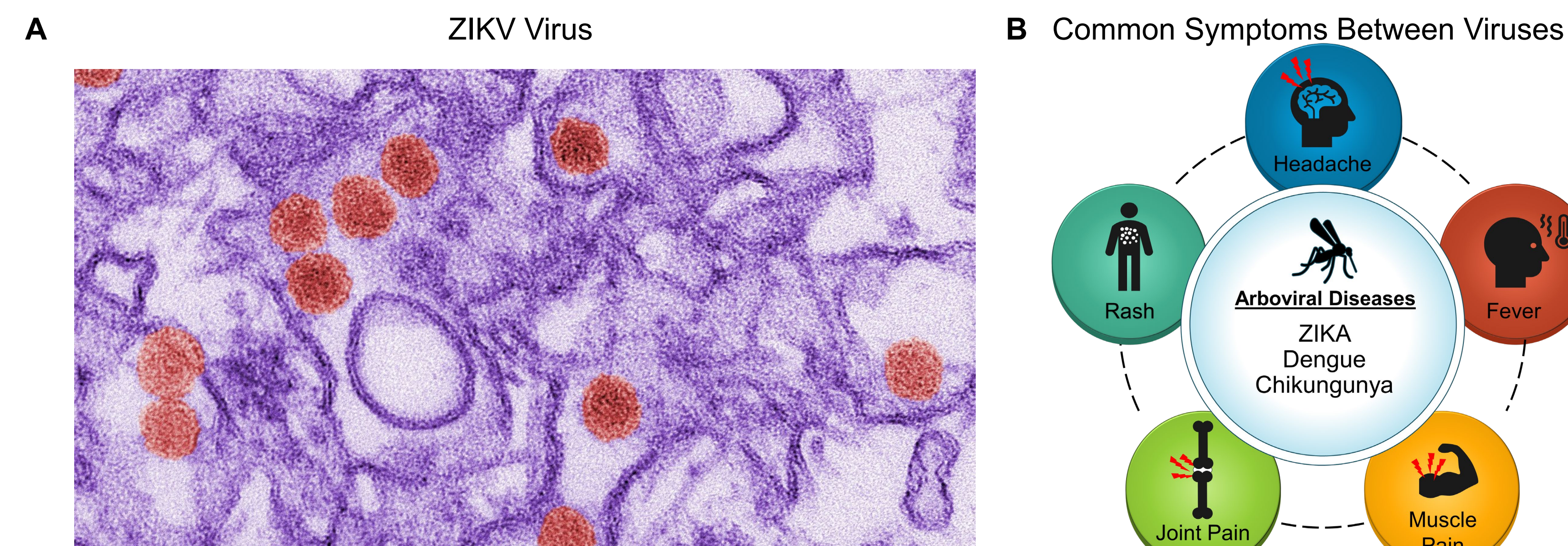


Figure 1: ZIKV morphology and overlapping clinical symptoms. (A) TEM image of ZIKV virus; photo courtesy of Cynthia Goldsmith. (B) the overlap in symptoms with 2 other viruses that circulate in the same geographic regions as ZIKV.

ATCC Synthetic Molecular Standards

ATCC® VR-3252SD™

Quantitative Synthetic Zika Virus from RNA

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

Target Genes

prM | E | NS1 | NS2B | NS3 | NS4B | NS5

Key features

Stabilized

Quantitated

Biosafety Level 1

Materials and Methods

Quantitative Synthetic RNA

ATCC® designed a synthetic RNA construct to represent the ZIKV genome (ATCC® VR-3252SD™) and serve as an ARM for molecular assays. This ARM comprises fragments from the following genomic regions: Membrane glycoprotein precursor M, Envelope, NS1, NS2B, NS3, NS4B, and NS5 regions. The ARM is authenticated via next-generation sequencing, quantified via digital-based PCR, and stabilized in a proprietary storage buffer enabling a 5-year long shelf life.

qRT-PCR Assay

qRT-PCR assays were performed using the Bio-Rad CFX Opus 96 Real-Time PCR Detection System (Bio-Rad) according to the manufacturer's instructions with slight modifications.

Synthetic Design

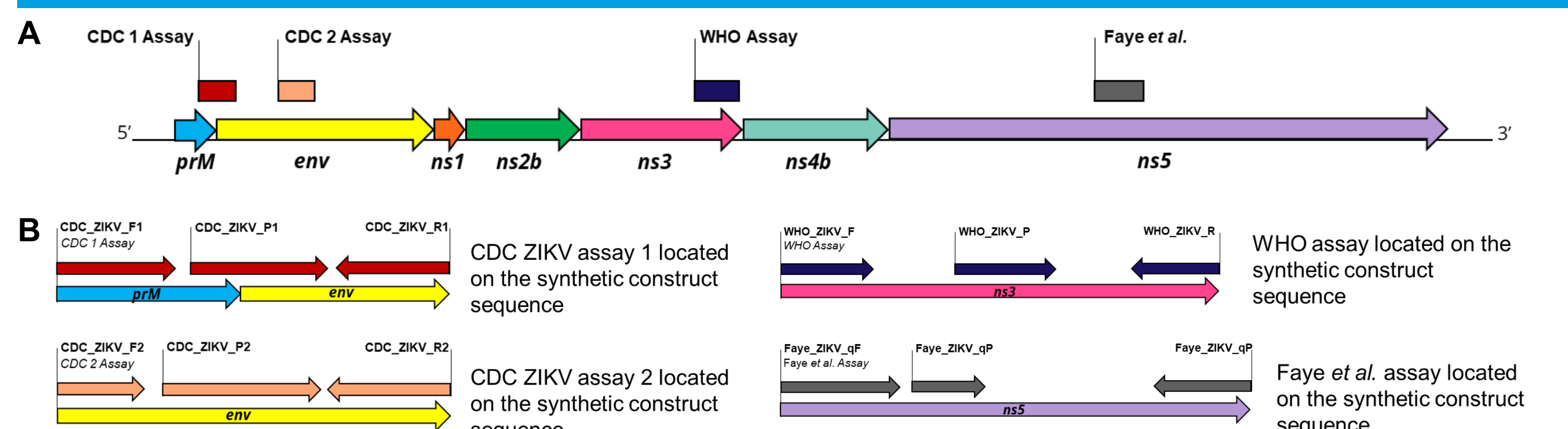


Figure 2: Synthetic design of the ZIKV synthetic analytical reference material. Gene segments incorporated in the synthetic construct and the associated diagnostic assay targets, showing (A) the full construct with relative assay target locations and (B) the position of each assay target within the corresponding gene segment.

Results

Synthetic ARM was compatible with Faye *et al.* Assay

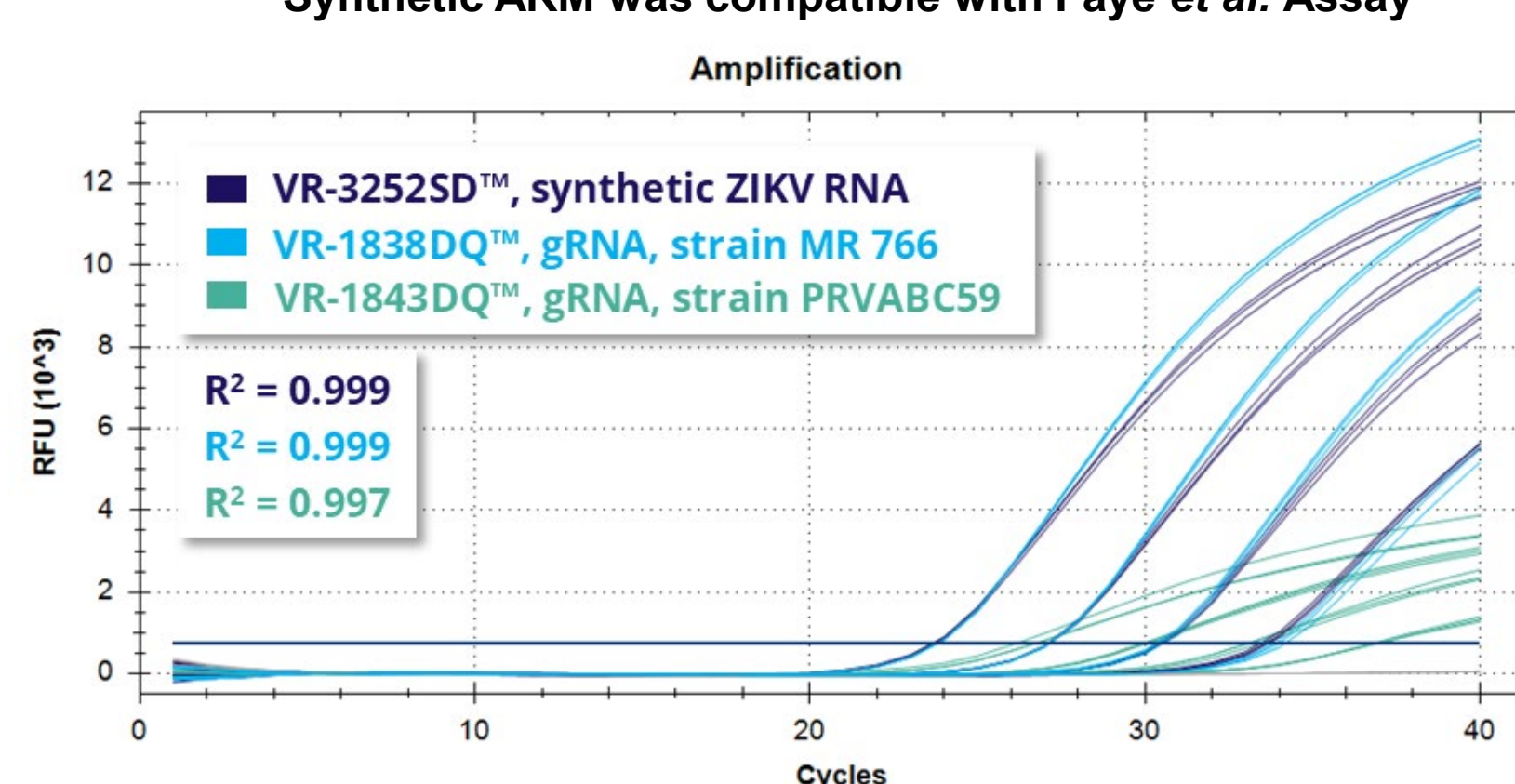


Table 1: Summary of data for Faye *et al.* assay.

Template	Mismatches F / R / P	Conc. (copies/rxn)	Mean Cq	SD	ΔCq
ATCC® VR-3252SD™	0 / 0 / 0	50000	24.01	0.05	n/a
		5000	27.35	0.04	3.34
		500	30.75	0.08	3.40
		50	33.88	0.14	3.13
ATCC® VR-1838DQ™	0 / 0 / 0	50000	24.08	0.01	n/a
		5000	27.36	0.03	3.29
		500	30.68	0.13	3.32
		50	34.26	0.15	3.58
ATCC® VR-1843DQ™	0 / 0 / 1	50000	27.05	0.35	n/a
		5000	30.51	0.09	3.46
		500	33.71	0.21	3.20
		50	37.57	0.12	3.86
NTC	n/a	0	0	0	n/a

Synthetic ARM was compatible with WHO Assay

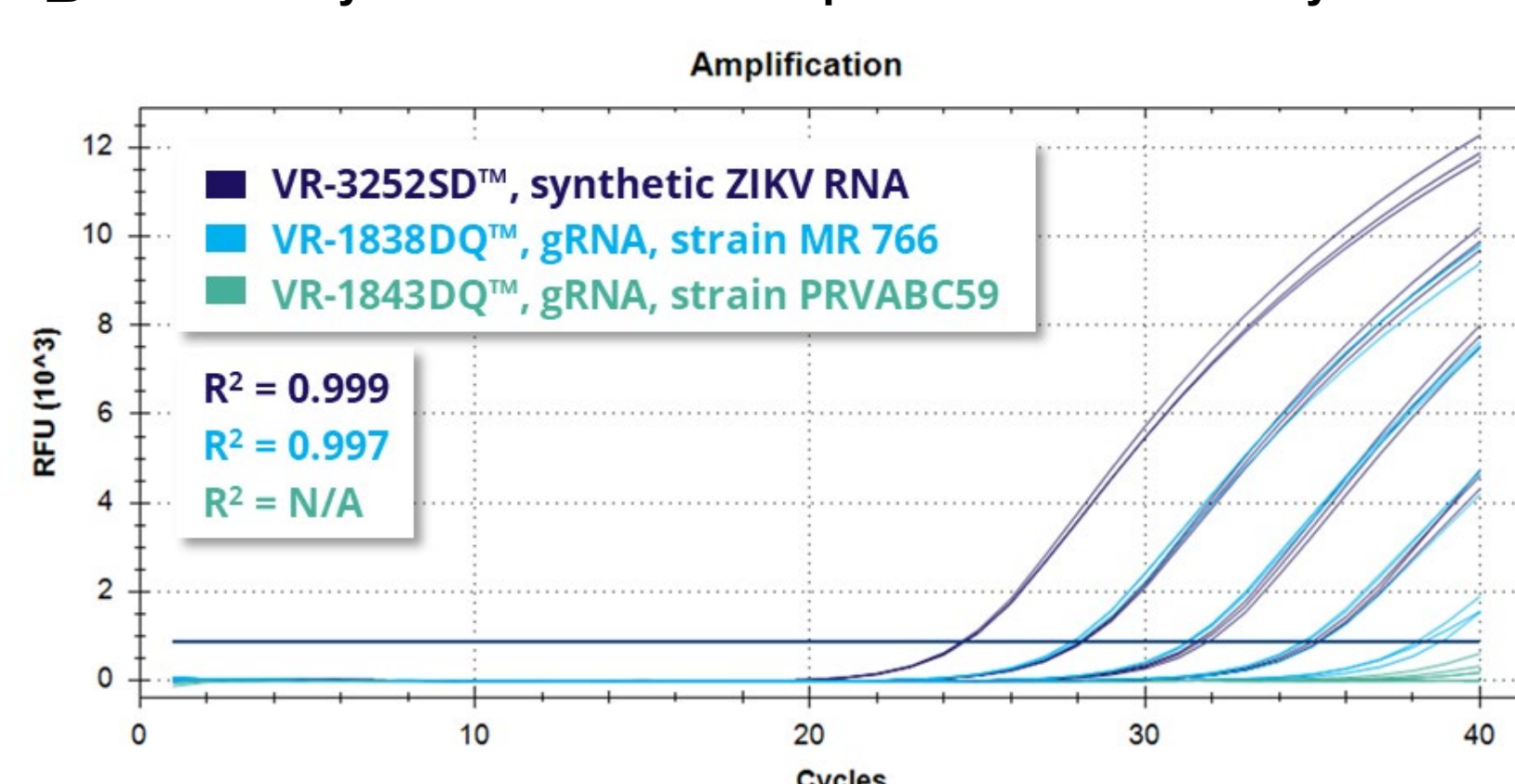


Table 2: Summary of data for WHO assay.

Template	Mismatches F / R / P	Conc. (copies/rxn)	Mean Cq	SD	ΔCq
ATCC® VR-3252SD™	0 / 0 / 0	50000	24.60	24.60	n/a
		5000	28.14	28.14	3.54
		500	31.71	31.71	3.57
		50	35.14	35.14	3.44
ATCC® VR-1838DQ™	0 / 0 / 0	50000	27.90	27.90	n/a
		5000	31.29	31.29	3.39
		500	34.92	34.92	3.63
		50	38.44	38.44	3.52
ATCC® VR-1843DQ™	2 / 3 / 2	50000	0	0	n/a
		5000	0	0	n/a
		500	0	0	n/a
		50	0	0	n/a
NTC	n/a	0	0	0	n/a

Figure 3: 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, and (B) an assay targeting NS3 from the WHO (Tappe *et al.*, 2014). The cycling conditions for both assays were 50°C for 15 min, 95°C for 2 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec.

Results (continued)

Synthetic ARM was compatible with CDC 1 Assay

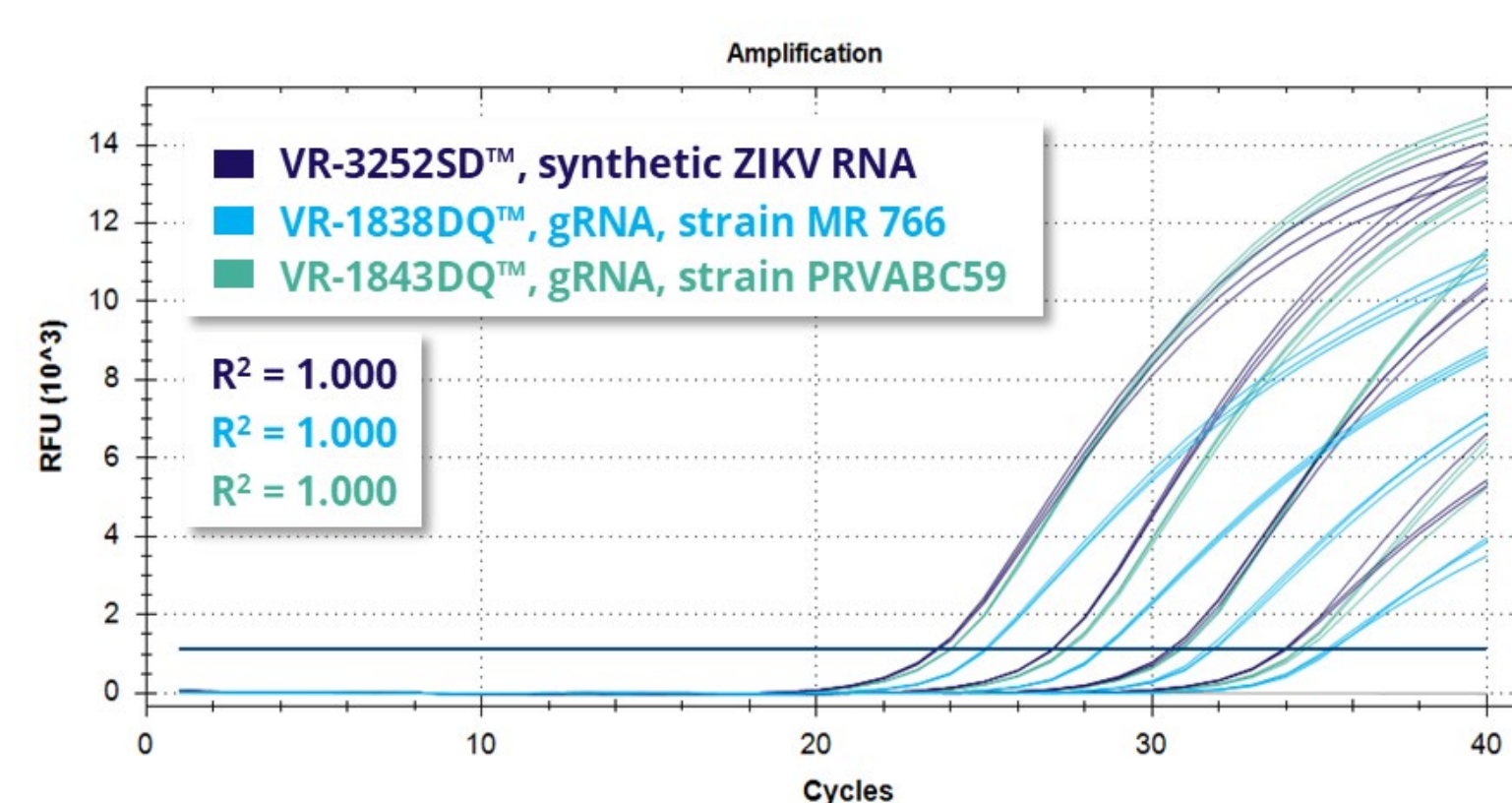


Table 3: Summary of data for CDC 1 assay.

Template	Mismatches F / R / P	Conc. (copies/rxn)	Mean Cq	SD	ΔCq
ATCC® VR-3252SD™	0 / 0 / 0	50000	23.57	0.05	n/a
		5000	27.03	0.02	3.46
		500	30.57	0.08	3.54
		50	33.96	0.06	3.39
ATCC® VR-1838DQ™	0 / 3 / 2	50000	25.04	0.04	n/a
		5000	28.50	0.05	3.46
		500	31.74	0.12	3.25
		50	35.30	0.10	3.56
ATCC® VR-1843DQ™	0 / 0 / 0	50000	23.99	0.02	n/a
		5000	27.41	0.05	3.42
		500	30.84	0.06	3.43
		50	34.43	0.11	3.59
NTC	n/a	0	0	0	n/a

Synthetic ARM was compatible with CDC 2 Assay

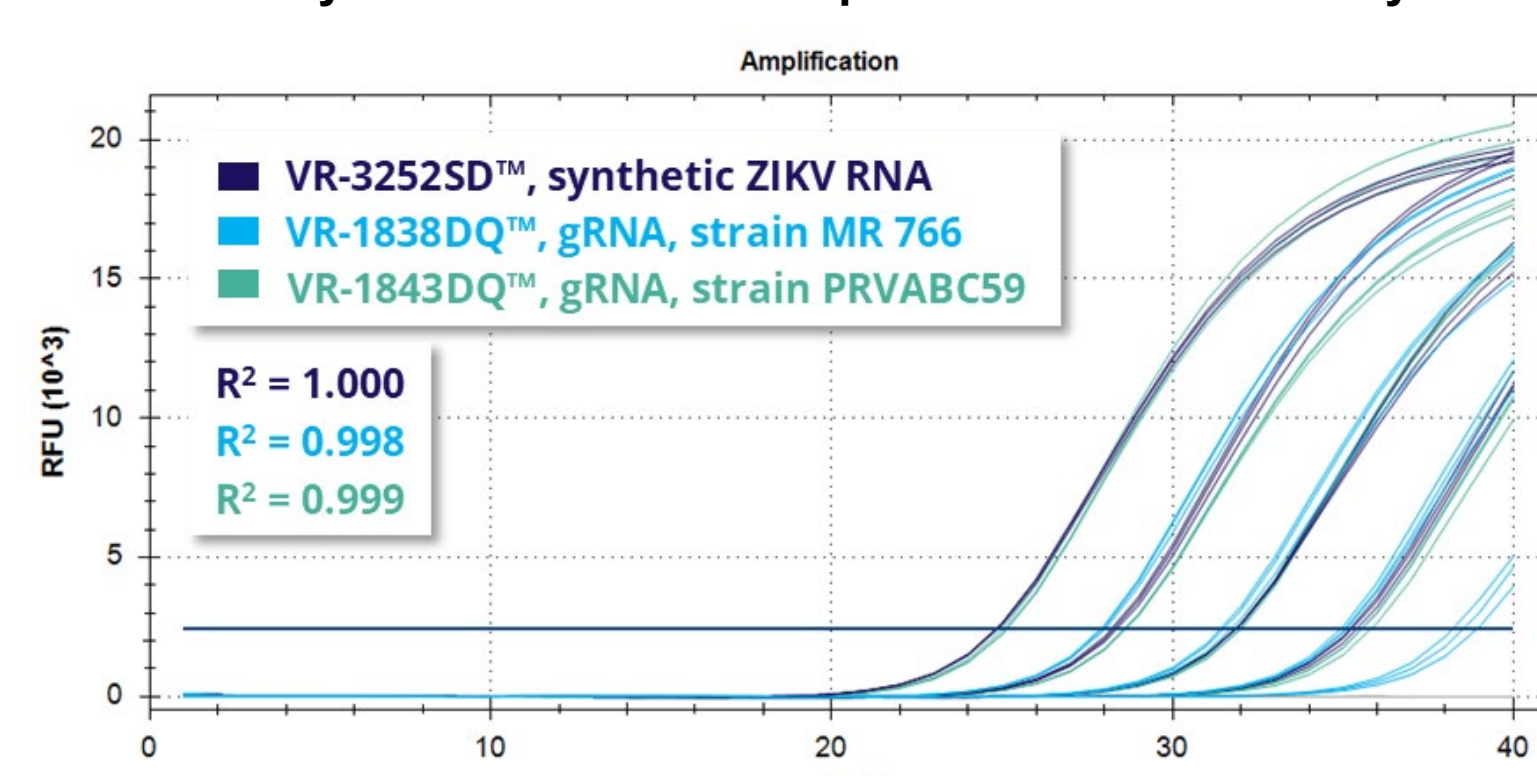


Table 4: Summary of data for CDC 2 assay.

Template	Mismatches F / R / P	Conc. (copies/rxn)	Mean Cq	SD	ΔCq
ATCC® VR-3252SD™	0 / 0 / 0	50000	24.83	24.83	n/a
		5000	28.27	28.27	3.44
		500	31.83	31.83	3.56
		50	35.27	35.27	3.44
ATCC® VR-1838DQ™	2 / 1 / 1	50000	27.94	27.94	n/a
		5000	31.52	31.52	3.58
		500	35.02	35.02	3.50
		50	38.54	38.54	3.52
ATCC® VR-1843DQ™	0 / 0 / 1	50000	25.08	25.08	n/a
		5000	28.59	28.59	3.51
		500	31.93	31.93	3.34
		50	35.62	35.62	3.69
NTC	n/a	0	0	0	n/a

Figure 4: 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, B) two assays targeting ENV from the CDC (Lanciotti *et al.*, 2008). The cycling conditions for both assays were 50°C for 15 min, 95°C for 2 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec.

Conclusions

- These data demonstrate that the synthetic ZIKV RNA ARM (ATCC® VR-3252SD™) can be used as a control for assay development, verification, and validation.
- The ARM was manufactured under ISO 13485 guidance and can be used to determine the viral load of unknown ZIKV samples through the generation of a standard curve.
- The ARM is compatible with numerous published assays⁴⁻⁶ and exhibited minimal variability, as evident from the slope and R^2 values.
- Together, these results support that this ARM is a high-quality, standardized control material.



Order the synthetic Zika virus RNA

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