INTERPRETATION OF RESULTS

All data from test controls must be examined prior to interpretation of patient specimen's results. If the controls are not valid, the patient results cannot be interpreted. Each assay must be performed with proper positive and negative controls. The **CII-ArboViroPlex rRT-PCR assay** provides positive, negative, extraction and rRT-PCR controls. All controls must yield the expected results for sample results to be interpretable (Table 1).

NTCs – Negative controls. The NTCs must be negative; a positive result for ZIKV, DENV, CHIKV, or WNV, or RNase P indicates contamination and all sample results must be disregarded.

Troubleshooting:

- Clean bench areas, tools and pipettes in all work areas with DNA AWAY, RNase AWAY, and bleach. Repeat assay using fresh reagent aliquots and/or a fresh NTC aliquots.
- If repeat testing fails again, contact the manufacturer for support.

Positive viral controls – Positive controls for ZIKV, DENV, CHIKV, and WNV must be positive yielding results within the expected Ct value range (Ct between 25 and 35). Negative results with one or more positive viral controls most likely indicate missing components in the reaction mix.

Troubleshooting: Repeat the assay. If repeat testing continues to generate negative result from one or more positive control(s), contact the manufacturer for support.

Extracted human specimen control (eHSC) – Assay performance control. The eHSC must give positive result for RNase P within the expected Ct value range (Ct between 25 and 35). A negative result most likely indicates missing components in the reaction mix. In addition, the eHSC must generate negative results for ZIKV, DENV, CHIKV, and WNV.

Troubleshooting:

- If RNase P is negative: Repeat the assay. If repeat testing continues to generate negative result for RNase P, contact the manufacturer for support.
- If eHSC is positive for viral signal: Clean bench areas, tools and pipettes in all work areas with DNA AWAY, RNase AWAY, and bleach. Repeat assay using fresh reagent aliquots. If repeat testing fails again, contact the manufacturer for support.

Human specimen extraction control (HSC) – Extraction control, must be extracted concurrently with test samples and is included as a sample during rRT-PCR. The HSC should generate a positive result for RNase P. In addition, the HSC must generate negative results for ZIKV, DENV, CHIKV, and WNV.

Troubleshooting:

- If RNase P in HSC is negative: The nucleic acid extraction might have failed only for the HSC sample but other results on that plate may reportable; if the eHSC on the plate is positive and no contamination by positive virus controls is indicated, then results from individual patient sample wells can be reported provided they yield a positive RNase P signal confirming that the extraction for that well worked (see also 'RNase P signal in samples' below).
- If HSC is positive for viral signal: Clean bench areas, tools and pipettes in all work areas with DNA AWAY, RNase AWAY, and bleach. Repeat extraction and assay using fresh reagent aliquots. If repeat testing continues to generate positive viral signal, contact the manufacturer for support.

RNase P in sample wells – RNase P should be positive for each sample to confirm successful extraction. If RNase P is negative in some sample wells, either these individual extractions failed (e.g. machine failure for the respective sample slots) or the samples may not contain enough RNase P for detection.

Troubleshooting:

- If RNase P is negative for a sample but a positive viral signal is recorded and the eHSC on the plate is positive for RNase P, the result for that sample can be reported. For samples without viral signal and RNase P signal sample extraction should be repeated from a new specimen aliquot.
- If the samples remain negative for RNase P, the result for these samples is inconclusive.
- If RNase P is negative in all sample wells and for eHSC, repeat assay using fresh reagent aliquots. If repeat testing continues to generate negative results for RNase P, contact the manufacturer for support

Table 1. Description of positive and negative controls used in **CII-ArboViroPlex rRT-PCR assay**.

Control Name		Purpose	Anticipated Result					
			ZIKV	DENV	CHIKV	WNV	RNase P	Expected Ct Values
NTC		Detect reagent or environment contamination during assay setup	-	-	-	-	-	No Detection
Positive viral controls	ZPC	Detect failure of viral primers, probes and other assay components, and/or pipetting errors during assay setup	+				-	23-35 Ct
	DPC		-	+				
	CPC		-		+			
	WPC		-			+		
HSC		1. Detect failure of RNase P primers and probe (compare to HSC)	-	-	-	-	+	Only RNase P Ct 25-35
		2. Detect failure of other assay components, and/or pipetting errors during assay setup (compare to positive viral controls)						
		3. Detect reagent or environmental contamination during assay setup (compare to NTC and HSC)						
eHSC		1. Detect failure in nucleic acid extraction protocol	-	-	-	-	+	Only RNase P Ct 25-35
		2. Detect failure of RNase P primers and probe (compare to eHSC)						
		3. Detect failure of other assay components, and/or pipetting errors during assay setup (compare to positive viral controls and eHSC)						
		4. Detect reagent or environmental contamination during assay setup (compare to NTC and eHSC)						

Interpretation of Patient Specimen Results:

The **CII-ArboViroPlex rRT-PCR assay** uses the testing algorithm described in table 4 and table 5. Nucleic acid is extracted from serum or urine and subjected to rRT-PCR analysis using the kit components described in the **CII-ArboViroPlex rRT-PCR assay** Instructions For Use document. Signal indicative of the presence of viral nucleic acid in a sample extract is considered to be evidence of viral infection, see Table 2, if the negative control does not show viral signal and all other controls work appropriately (Table 1). The absence of signal for viral nucleic acid does not exclude the possibility of infection.

Table 2. **CII-ArboViroPlex rRT-PCR assay** results interpretation and reporting instructions

ZIKV	DENV	CHIKV	WNV	RNase P*	Interpretation	Reporting
-	-	-	-	+	No ZIKV, CHIKV, DENV or WNV RNA detected	Report test result Note: If date of onset of symptoms is unknown or if patient is asymptomatic, confirmatory testing may be warranted**
-	-	-	-	-	Inconclusive results for ZIKV, CHIKV, DENV and WNV RNA detection since extraction of nucleic acid is	Inconclusive test; no result can be reported. Repeat extraction and rRT-PCR with fresh aliquot of specimen and reagents. If repeat testing is

					questionable (no RNase P signal)	again inconclusive, report inconclusive results.
+	-	-	-	+/-	ZIKV RNA detected but no CHIKV, DENV or WNV RNA detected	ZIKV positive
-	+	-	-	+/-	DENV RNA detected but no CHIKV, ZIKV or WNV RNA detected	DENV positive
-	-	+	-	+/-	CHIKV RNA detected but no DENV, ZIKV or WNV RNA detected	CHIKV positive
-	-	-	+	+/-	WNV RNA detected but no CHIKV, ZIKV or DENV RNA detected	WNV positive
+	+	-	-	+/-	ZIKV and DENV RNA detected but no CHIKV or WNV RNA detected	ZIKV and DENV positive
+	-	+	-	+/-	ZIKV and CHIKV RNA detected but no DENV or WNV RNA detected	ZIKV and CHIKV positive
+	-	-	+	+/-	ZIKV and WNV RNA detected but no CHIKV or DENV RNA detected	ZIKV and WNV positive
-	+	+	-	+/-	DENV and CHIKV RNA detected but no ZIKV or WNV RNA detected	DENV and CHIKV positive
-	+	-	+	+/-	DENV and WNV RNA detected but no ZIKV or CHIKV RNA detected	DENV and WNV positive
-	-	+	+	+/-	CHIKV and WNV RNA detected but no ZIKV or DENV RNA detected	CHIKV and WNV positive
+	+	+	-	+/-	ZIKV, DENV and CHIKV RNA detected but no WNV RNA detected	ZIKV, DENV and CHIKV positive
+	+	-	+	+/-	ZIKV, DENV and WNV RNA detected but no CHIKV RNA detected	ZIKV, DENV and WNV positive
+	-	+	+	+/-	ZIKV, CHIKV and WNV RNA detected but no DENV RNA detected	ZIKV, CHIKV and WNV positive
-	+	+	+	+/-	DENV, CHIKV and WNV RNA detected but no ZIKV RNA detected	DENV, CHIKV and WNV positive
+	+	+	+	+/-	ZIKV, DENV, CHIKV and WNV RNA detected	ZIKV, DENV, CHIKV and WNV positive

* RNase P should be positive, but negative results may be observed for samples with low RNase P; if RNase P is negative but a viral signal is obtained, the test is valid and data can be reported (specimen likely contained insufficient levels of RNase P for detection, but viral RNA was extracted).
** A patient-matched serum specimen is currently required for serological follow up testing of negative RT-PCR results per the CDC testing algorithm (found at http://www.cdc.gov/zika/index.html).

The user should follow **CII-ArboViroPlex rRT-PCR assay** results interpretation and reporting instructions (Table 2). All controls should yield the expected results in order to allow analysis of specimen data (Table 1). True positives should produce exponential curves with Ct values ≤38.00 (Figure 1). Samples that do not show exponential amplification or do not cross the threshold within 38 cycles are considered negative (Figure 2a, 2b).

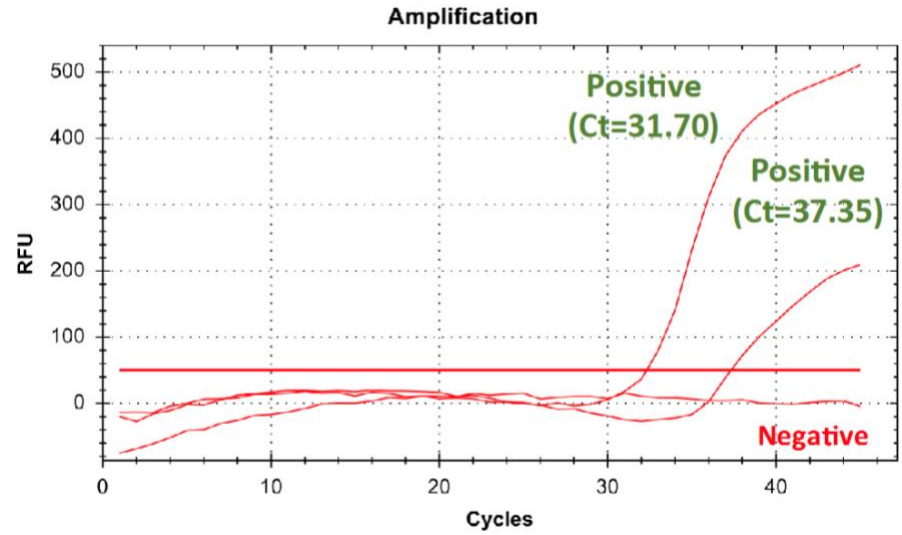
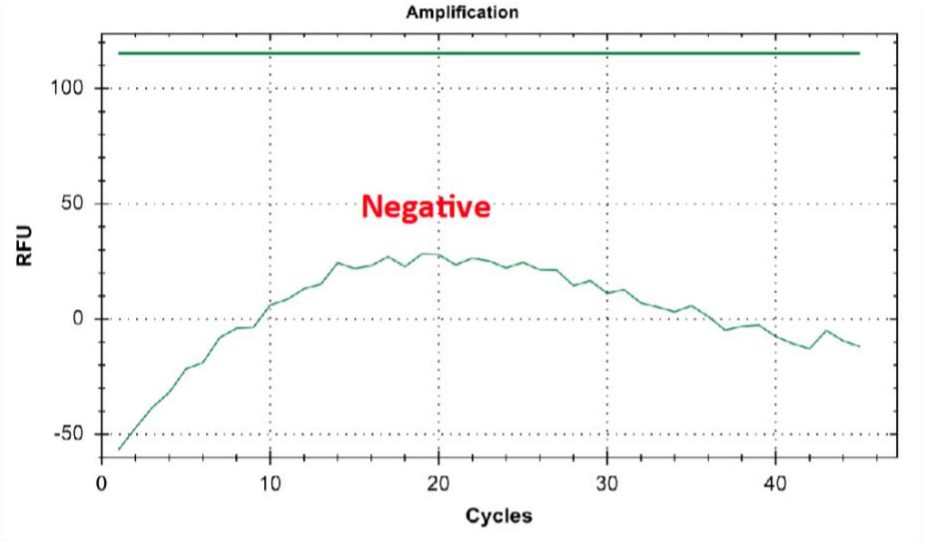


Figure 1. Determination of positive results in CII-ArboViroPlex rRT-PCR assay



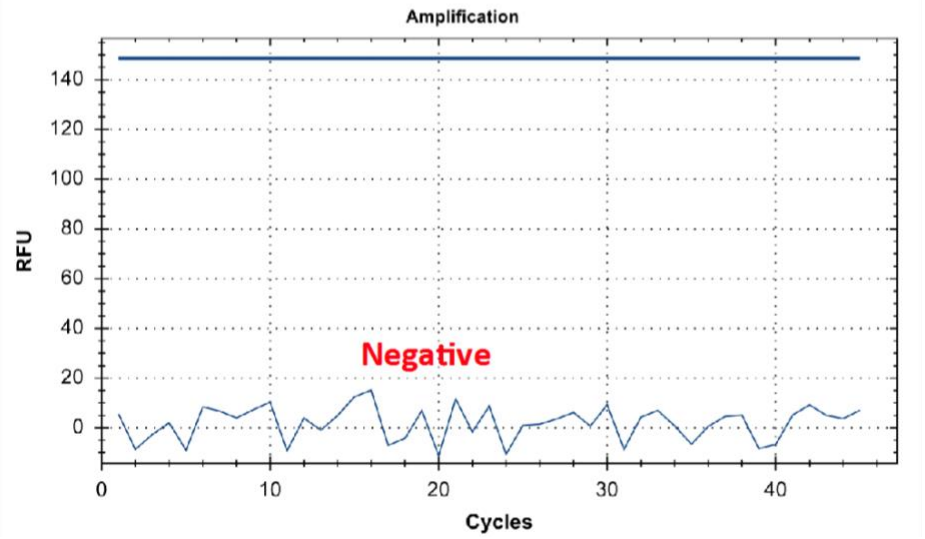


Figure 2a, 2b. Determination of negative results in **CII-ArboViroPlex rRT-PCR assay**

Examination of Patient Specimen Results:
Assessment of clinical specimen test results should be performed after the results of positive and negative controls have been examined and determined to be valid. If the PCR amplification curve based on increasing fluorescence for a primer/probe set crosses the threshold within (≤) 38.00 cycles, the result is positive. If the amplification curve for a primer probe set crosses the threshold above (>) 38 cycles, the result is negative.