

Supplementary Materials: Prevalence of Rotavirus Genogroup A and Norovirus Genogroup II in Bassaseachic Falls National Park Surface Waters in Chihuahua, Mexico

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Table S1. Sample site from the Bassaseachic Falls National Park.

Sampling ID and Location		Geographical Coordinates		
Number	Sample site	MSL *	NORTH	WEST
1	Pozo Cascada de Basaseachi		28°07'59"	−108°15'00"
2	Cascada del Plan		28°07'59"	−108°15'00"
3	Río El Durazno	1,952	28°10'806"	−108°12'855"
4	Río Basaseachi	1,952	28°10'804"	−108°12'687"
5	Y. Punto de unión del Río Basaseachi y Rio el Durazno.	1,952	28°10'802"	−108°12'752"
6	Arroyo de Baquiriachi	2,022	28°12'680"	−108°13'341"
7	Aguaje de Basaseachi	2,052	28°13'992"	−108°12'453"
8	Arroyo de Las Estrellas	2,010	28°11'500"	−108°10'444"
9	Grifo CONANP	1,976	28°09'803"	−108°12'773"
10	Arroyo de Betorachi	1,932	28°10'928"	−108°11'067"
11	Arroyo de la laguna de oxidación	1,996	28°11'837"	−108°12'809"
12	Cahuisori	1,976	28°12'680"	−108°13'341"
13	Mirador de la Cascada		28°07'59"	−108°15'00"

* MSL: Meters above sea level

Table S2. Reverse Transcription conditions to detect Rotavirus.

	Reagent	[Initial]	[Final]	Volume
Step 1.	RNA	50 ng	23.8 ng	2 µL
	Con 2 (Oligo T4)	1 µm	0.23 µm	1 µL
	H ₂ O injectable (PISA)	-	-	1.2 µL
			Final volume =	4.2 µL
Thermocycler program: 1 cycle at 94°C for 5 minutes.				
Step 2.	RT Buffer (Promega)	5X	1 X	2 µL
	MgCl ₂ (Promega)	50 mM	2.5 mM	0.5 µL
	dNTP's (Promega)	2.5 mM	0.8 mM	3.2 µL
	RT enzyme (Promega)	200 U/µL	20 U	0.1 µL
			Final volume =	4.2 µL

Thermocycler program: 1 cycle at 94 °C for 30 min.

Table S3. Polimerase chaine reaction conditions to detect RV.

Reagent	[Initial]	[Final]	Volume
H ₂ O injectable (PISA)	-	-	16.3 µL
Buffer PCR (Promega)	10X	1X	2.5 µL
MgCl ₂ (Promega)	50 mM	2 mM	1 µL
Con 3 (Oligo T4)	25 µM	1 µM	1 µL
Con 2 (Oligo T4)	25 µM	1 µM	1 µL
dNTP's (Promega)	2.5 mM	0.4 mM	2 µL
Taq polymerase (Promega)	5 U/µL	0.04 U	0.2 µL
cDNA	-	-	1 µL
Final volume =			25 µL

Thermocycler program: 1 cycle at 94 °C for 90 s; 30 cycles at 94 °C for 30 s, 42 °C for 30 s and 72 °C for 40 s; and 1 final cycle at 72 °C for 7 min.

Table S4. RT conditions to detect Norovirus.

Reagent	[Initial]	[Final]	Volume
RNA	50 ng	12.5 ng	5 µL
JV13 (Oligo T4)	12 µm	2.5 µm	4 µL
H ₂ O injectable (PISA)	-	-	6.8 µL
RT Buffer (Promega)	5X	0.5 X	2 µL
MgCl ₂ (Promega)	50 mM	3 mM	1.2 µL
dNTP's (Promega)	25 mM	1 mM	0.8 µL
RT enzyme (Promega)	200 U/µL	2 U	0.2 µL
Final Volume =			20 µL

Thermocycler program: 1 cycle at 42 °C for 60 min.

Table S5. PCR conditions to detect Norovirus.

Reagent	[Initial]	[Final]	Volume
H ₂ O injectable (PISA)	-	-	8.2 µL
PCR Buffer (Promega)	5X	1X	4 µL
MgCl ₂ (Promega)	25 mM	1.5 mM	1.2 µL
JV12 (Oligo T4)	12 µM	0.75 µM	1.24 µL
dNTP's (Promega)	25 mM	0.2 mM	0.16 µL
Taq polymerase (Promega)	5 U/µL	0.04 U	0.2 µL
cDNA	-	-	5 µL
Final Volume =			20 µL

Thermocycler program: 1 cycle at 94 °C for 60 s; 40 cycles at 94 °C for 60 s, 37 °C for 30 s and 74 °C for 60 s; and 1 final cycle at 74 °C for 7 min.

Table S6. qPCR conditions to detect Rotavirus.

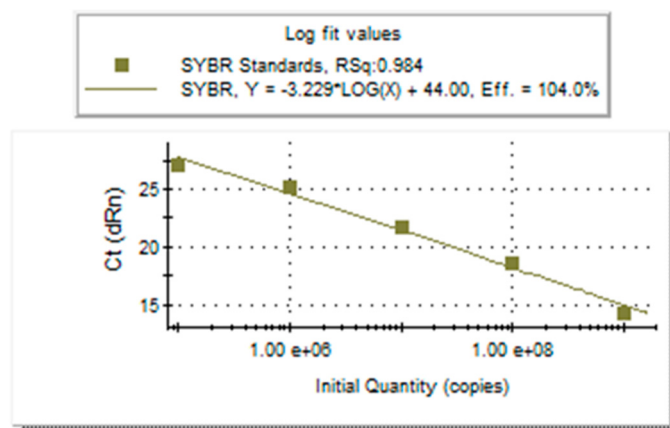
Reagent	[Initial]	[Final]	Volume
qPCR Buffer	2X	1X	10 µL
DYE ROX 30 nm	-	-	0.3 µL
IT-I/Con 2 (Fw) (Oligo T4)	4 µM	0.5 µM	2.5 µL
CON3 (Rw) (Oligo T4)	4 µM	0.5 µM	2.5 µL
Plasmidic DNA	-	-	1 µL
H ₂ O injectable (PISA)	-	-	3.7
Final Volume =			20 µL

Thermocycler program: 1 cycle at 95 °C for 3 min; 45 cycles at 95 °C for 30 s, 53 °C for 20 s and 72 °C for 30 s. Thermocycler program for dissociation curve: 1 cycle at 95 °C for 60 s, 53 °C for 30 s and 95 °C for 30 s.

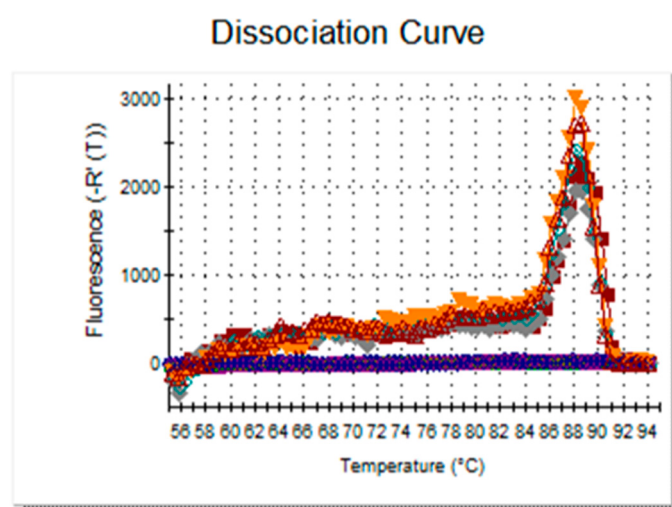
Table S7. qPCR conditions to detect Norovirus.

Reagent	[Initial]	[Final]	Volume
Buffer qPCR	2X	1X	10 µL
DYE ROX 30 nm	-	-	0.3 µL
JV12 (Fw) (Oligo T4, Irapuato, México)	4 µM	0.2 µM	1 µL
JV13 (Rw) (Oligo T4, Irapuato, México)	4 µM	0.2 µM	1 µL
Plasmidic DNA	-	-	1 µL
H ₂ O injectable (PISA, México)	-	-	6.7
Final Volume =			20 µL

Thermocycler program: 1 cycle at 95 °C for 3 min; 40 cycles at 95 °C for 10 s, 48 °C for 20 s and 60 °C for 42 s. Thermocycler program for dissociation curve: 1 cycle at 95 °C for 60 s, 48 °C for 30 s and 95 °C for 30 s.



(A)



(B)

Figure S1. Standard curve of the norovirus RdPd gene. (A) Lineal equation with a slope -3.229 ; (B) Products dissociation curve.

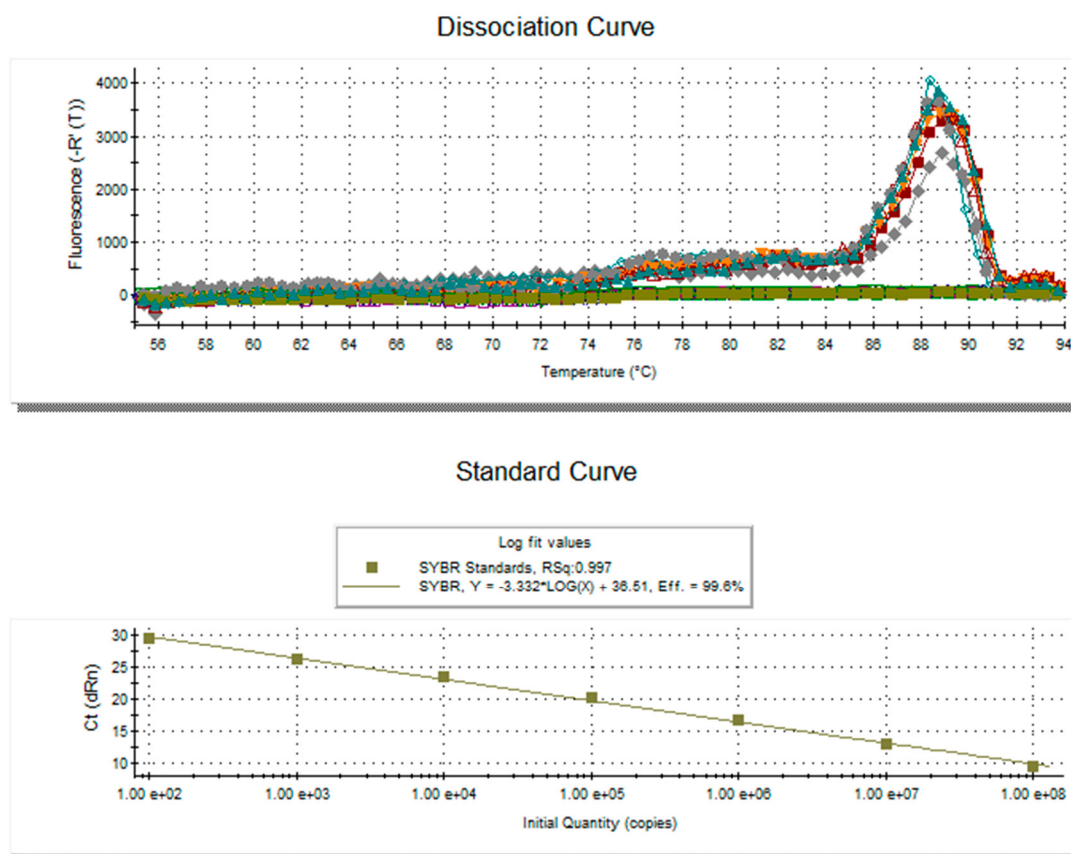


Figure S2. Standard curve of the four genes from rotavirus. (A) Lineal equation with a slope -3.332 ; (B) Products dissociation curve.



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