

$$\text{copy number (molecules}/\mu\text{L)} = \{[\text{concentration (ng}/\mu\text{L)} \times \text{dilution factor} \times \text{RNA template volume } (\mu\text{L)}] \times [6.022 \times 10^{23} \text{ (molecules/mol)}]\} / [\text{length of amplicon} \times 660 \text{ (g/mol)} \times 10^9 \text{ (ng/g)}]$$

**Figure S1** Calculation to determine dsRNA copy number per reaction for the NSP3 gene qRT-PCR assay using the EMD One-Step RT-PCR Master Mix Kit.

**Table S1** Rotavirus A detection results<sup>a,b</sup> and NSP3 gene qRT-PCR assay  $C_T$  values using the ABI GeneAmp EZ *rTth* RNA PCR Kit and EMD One-Step RT-PCR Master Mix Kit, using identical clinical samples (run in duplicate: *nA* & *nB*).

Sample #	Sample $C_T$ values		Rotavirus A Detection	
	ABI GeneAmp EZ <i>rTth</i> RNA PCR Kit	EMD One-Step RT-PCR Master Mix Kit	ABI GeneAmp EZ <i>rTth</i> RNA PCR Kit	EMD One-Step RT-PCR Master Mix Kit
1A	Negative	Negative	Negative	Negative
1B	Negative	Negative		
2A	Negative	Negative	Negative	Negative
2B	Negative	Negative		
3A	Negative	Negative	Negative	Negative
3B	Negative	Negative		
4A	Negative	Negative	Negative	Negative
4B	Negative	Negative		
5A	Negative	Negative	Negative	Negative
5B	Negative	Negative		
6A	Negative	Negative	Negative	Negative
6B	Negative	Negative		
7A	Negative	Negative	Negative	Negative
7B	Negative	Negative		
8A	Negative	Negative	Negative	Negative
8B	Negative	Negative		
9A	38.388	35.087	Negative	Positive
9B	Negative	37.723		
10A	Negative	Negative	Negative	Negative
10B	Negative	Negative		
11A	Negative	Negative	Negative	Negative
11B	Negative	Negative		
12A	Negative	Negative	Negative	Negative
12B	Negative	Negative		
13A	Negative	Negative	Negative	Negative
13B	Negative	Negative		
14A	Negative	Negative	Negative	Negative
14B	Negative	Negative		
15A	Negative	35.025	Negative	Positive
15B	Negative	38.419		
16A	34.145	33.444	Positive	Positive
16B	36.843	34.135		
17A	Negative	Negative	Negative	Negative
17B	Negative	Negative		
18A	Negative	Negative	Negative	Negative
18B	Negative	Negative		

19A	Negative	Negative	Negative	Negative
19B	Negative	Negative	Negative	Negative
20A	Negative	Negative	Negative	Negative
20B	Negative	Negative	Negative	Negative
21A	Negative	Negative	Negative	Negative
21B	Negative	Negative	Negative	Negative
22A	Negative	Negative	Negative	Negative
22B	Negative	Negative	Negative	Negative
23A	Negative	35.246	Negative	Positive
23B	Negative	37.101	Negative	Positive
24A	Negative	Negative	Negative	Negative
24B	Negative	Negative	Negative	Negative
25A	Negative	Negative	Negative	Negative
25B	Negative	Negative	Negative	Negative
26A	Negative	Negative	Negative	Negative
26B	Negative	Negative	Negative	Negative
27A	Negative	Negative	Negative	Negative
27B	Negative	Negative	Negative	Negative
28A	28.426	28.208	Positive	Positive
28B	28.522	28.126	Positive	Positive
29A	Negative	Negative	Negative	Negative
29B	Negative	Negative	Negative	Negative
30A	Negative	Negative	Negative	Negative
30B	Negative	Negative	Negative	Negative
31A	Negative	Negative	Negative	Negative
31B	Negative	Negative	Negative	Negative
32A	Negative	Negative	Negative	Negative
32B	Negative	Negative	Negative	Negative
33A	Negative	Negative	Negative	Negative
33B	Negative	Negative	Negative	Negative
34A	Negative	37.699	Negative	Positive
34B	Negative	34.426	Negative	Positive
35A	Negative	Negative	Negative	Negative
35B	Negative	Negative	Negative	Negative
36A	Negative	Negative	Negative	Negative
36B	Negative	Negative	Negative	Negative
37A	Negative	Negative	Negative	Negative
37B	Negative	39.473	Negative	Negative
38A	Negative	Negative	Negative	Negative
38B	Negative	Negative	Negative	Negative
39A	Negative	Negative	Negative	Negative
39B	Negative	Negative	Negative	Negative
40A	Negative	Negative	Negative	Negative
40B	Negative	Negative	Negative	Negative
41A	Negative	Negative	Negative	Negative

41B	Negative	Negative		
42A	Negative	Negative		
42B	Negative	Negative	Negative	Negative
43A	Negative	Negative		
43B	Negative	Negative	Negative	Negative
44A	Negative	Negative		
44B	Negative	Negative	Negative	Negative
45A	Negative	Negative		
45B	Negative	Negative	Negative	Negative
46A	Negative	Negative		
46B	Negative	Negative	Negative	Negative
47A	Negative	Negative		
47B	Negative	Negative	Negative	Negative
48A	Negative	38.988		
48B	Negative	38.454	Negative	Positive
49A	Negative	Negative		
49B	Negative	Negative	Negative	Negative
50A	Negative	Negative		
50B	Negative	Negative	Negative	Negative
51A	28.239	28.460		
51B	28.319	28.266	Positive	Positive
52A	37.410	32.148		
52B	Negative	32.309	Negative	Positive
53A	Negative	Negative		
53B	Negative	Negative	Negative	Negative
54A	14.422	14.066		
54B	14.582	14.150	Positive	Positive
55A	16.230	15.884		
55B	16.247	15.874	Positive	Positive
56A	13.839	13.591		
56B	13.912	13.621	Positive	Positive
57A	16.889	16.685		
57B	16.961	16.639	Positive	Positive
58A	20.003	19.742		
58B	20.139	19.604	Positive	Positive
59A	12.984	12.292		
59B	13.025	12.146	Positive	Positive
60A	17.291	17.032		
60B	17.320	17.087	Positive	Positive
61A	20.361	20.078		
61B	20.446	20.209	Positive	Positive
62A	12.156	12.047		
62B	12.182	12.045	Positive	Positive
63A	15.549	15.308		
63B	15.568	15.345	Positive	Positive

64A	21.738	21.583	Positive	Positive
64B	21.902	21.523		
65A	29.284	29.914	Positive	Positive
65B	30.037	29.916		
66A	26.749	27.005	Positive	Positive
66B	26.799	26.920		
67A	29.011	29.320	Positive	Positive
67B	29.134	28.857		
68A	33.297	34.140	Positive	Positive
68B	33.901	34.322		
69A	30.513	30.849	Positive	Positive
69B	30.808	30.600		
70A	26.538	26.494	Positive	Positive
70B	26.638	26.523		
71A	15.006	13.464	Positive	Positive
71B	15.088	13.449		
72A	20.786	19.077	Positive	Positive
72B	21.037	19.064		
73A	33.424	32.392	Positive	Positive
73B	34.280	32.746		
74A	16.496	15.123	Positive	Positive
74B	16.603	15.243		
75A	19.607	18.163	Positive	Positive
75B	19.695	18.283		
76A	16.460	14.801	Positive	Positive
76B	16.530	14.896		
77A	15.918	14.293	Positive	Positive
77B	16.102	14.510		
78A	22.045	21.085	Positive	Positive
78B	22.385	21.195		
79A	14.455	13.232	Positive	Positive
79B	14.465	13.248		
80A	16.169	14.748	Positive	Positive
80B	16.237	14.767		
81A	16.143	14.700	Positive	Positive
81B	16.252	14.619		
82A	14.295	13.010	Positive	Positive
82B	14.464	12.932		
83A	20.182	18.738	Positive	Positive
83B	20.382	18.669		
84A	Negative	38.110	Negative	Positive
84B	Negative	36.595		
85A	19.180	17.856	Positive	Positive
85B	19.217	17.732		
86A	Negative	Negative	Negative	Negative

86B	Negative	37.266		
87A	13.727	12.592	Positive	Positive
87B	13.778	12.672		
88A	21.311	20.135	Positive	Positive
88B	21.372	20.090		
89A	Negative	36.192	Negative	Negative
89B	Negative	Negative		
90A	13.365	12.064	Positive	Positive
90B	13.394	12.100		
91A	22.063	20.409	Positive	Positive
91B	22.153	20.404		
92A	Negative	35.998	Negative	Negative
92B	Negative	Negative		
93A	32.289	32.159	Positive	Positive
93B	35.154	32.250		
94A	41.430	35.268	Negative	Positive
94B	Negative	36.845		
95A	33.997	33.116	Positive	Positive
95B	34.605	33.069		
96A	30.162	29.827	Positive	Positive
96B	30.660	29.579		
97A	34.185	33.470	Positive	Positive
97B	42.399	33.591		
98A	35.342	35.273	Positive	Positive
98B	37.564	37.142		
99A	30.845	37.115	Positive	Positive
99B	37.071	34.767		
100A	26.424	26.573	Positive	Positive
100B	26.666	26.168		
101A	21.038	21.256	Positive	Positive
101B	21.169	21.129		
102A	33.852	34.012	Positive	Positive
102B	34.607	34.128		
103A	34.144	34.455	Positive	Positive
103B	37.466	33.965		
104A	20.031	19.586	Positive	Positive
104B	20.043	19.844		
105A	37.274	Negative	Negative	Negative
105B	Negative	39.213		
106A	21.049	21.047	Positive	Positive
106B	21.218	21.027		
107A	37.166	36.051	Positive	Positive
107B	41.862	36.252		
108A	23.072	22.853	Positive	Positive
108B	23.079	23.137		

109A	Negative	Negative	Negative	Negative
109B	Negative	39.490		
110A	Negative	34.381	Negative	Positive
110B	Negative	34.885		
111A	Negative	37.494	Negative	Positive
111B	Negative	38.028		
NTC 1	Negative	Negative	Negative	Negative
NTC 2	Negative	Negative	Negative	Negative
NTC 3	Negative	Negative	Negative	Negative
NTC 4	Negative	Negative	Negative	Negative
NTC 5	Negative	Negative	Negative	Negative
NTC 6	Negative	Negative	Negative	Negative
NTC 7	Negative	Negative	Negative	Negative
NTC 8	Negative	Negative	Negative	Negative
NTC 9	Negative	Negative	Negative	Negative
NTC 10	Negative	Negative	Negative	Negative
Negative Control 1A	Negative	Negative	Negative	Negative
Negative Control 1B	Negative	Negative		
Negative Control 2A	Negative	Negative	Negative	Negative
Negative Control 2B	Negative	Negative		
Negative Control 3A	Negative	Negative	Negative	Negative
Negative Control 3B	Negative	Negative		
Negative Control 4A	Negative	Negative	Negative	Negative
Negative Control 4B	Negative	Negative		
Negative Control 5A	Negative	Negative	Negative	Negative
Negative Control 5B	Negative	Negative		
Wa Strain (+) Control 1A	18.216	17.622	Positive	Positive
Wa Strain (+) Control 1B	18.292	17.285		
Wa Strain (+) Control 2A	18.296	18.166	Positive	Positive
Wa Strain (+) Control 2B	18.471	18.627		

Wa Strain (+) Control 3A	17.370	17.162	Positive	Positive
Wa Strain (+) Control 3B	17.389	17.407		
Wa Strain (+) Control 4A	17.603	17.618	Positive	Positive
Wa Strain (+) Control 4B	17.894	17.684		
Wa Strain (+) Control 5A	18.017	17.392	Positive	Positive
Wa Strain (+) Control 5B	18.066	17.421		

<sup>a</sup> Samples determined to be positive for Rotavirus A only if both replicates showed NSP3 amplification with a calculated  $C_T$  value.

<sup>b</sup> Comparison of  $C_T$  values between ABI and EMD kits [t-Test (paired)] used the average  $C_T$  value per sample. The EMD kit showed significantly lower  $C_T$  values,  $p < 0.0001$ .



**Table S2** Rotavirus A detection results<sup>a</sup> and measures of agreement for the NSP3 gene qRT-PCR assay when comparing the EMD One-Step RT-PCR Master Mix Kit to the ABI GeneAmp EZ *rTth* RNA PCR Kit, using identical clinical samples.

		ABI GeneAmp EZ <i>rTth</i> RNA PCR Kit (non-reference standard)		Total
		Positive	Negative	
EMD One-Step RT-PCR Master Mix Kit (new test)	Positive	$a = 52$	$b = 10$	62
	Negative	$c = 0$	$d = 49$	49
Total		52	59	
Positive Percent Agreement = $100 \times [a/(a+c)]$		100.00% (95% CI 93.12% - 100.00%)		
Negative Percent Agreement = $100 \times [d/(b+d)]$		83.05% (95% CI 71.54% - 90.52%)		
Overall Percent Agreement = $100 \times [(a+d)/(a+b+c+d)]$		90.99% (95% CI 80.49% - 98.75%)		

<sup>a</sup> Samples determined to be positive for Rotavirus A only if both replicates showed NSP3 amplification along with a calculated  $C_T$  value.