

Supplementary table 1. Available sequences of primers and hydrolysis probes used in this study.

Type of assay	Sequence (5' to 3')*		
	Forward primer(s)	Probe	Reverse primer
Panenterhino/Ge/08 assay	AGCCTGCGTGGCKGCC CYlnaAGCClnaTGCGTGG3	FAM-CTCCGGCCCTGAATGYGGCTAA-TAMRA	GAAACACGGACACCCAAAGTAGT
CDV assay	GCTACCCAAGAAACCGTCATTG	VIC-CG TTCAGGGAGTCCAGGACTACGTCAAC-TAMRA	GCATGGCAGGGACGAGTT
HRV-A16-specific assay	GACAAAGAAACCATCTCAAATGCA	FAM-AGCATGTCTTATCCTTG	GACCATTGTGCCACATTAAGTGA

* K=G or T; Y=C or T; ln, locked nucleic acid.

Supplementary table 2. One-step versus two-step HRV RNA quantification by real-time RT-PCR in respiratory specimens.

Specimen	Genotype	Log (RNA copies/ml)						Two-step minus one-step	One-step/two-step % CV
		One-step			Two-step				
		Exp1	Exp2	Mean	Exp1	Exp2	Mean		
L45-1009-U	HRV-A1	5.99	6.02	6	5.54	6.14	5.84	-0.17	0.11
M65-1008-U	HRV-C	5.34	5.3	5.32	5.75	5.87	5.81	0.49	0.35
H71-0110-U	HRV-C	4.7	5.08	4.89	5.73	5.99	5.86	0.97	0.69
M55-0110-U	HRV-C	5.64	5.75	5.7	6.41	6.61	6.51	0.82	0.57
J56-1209-U		6.79	6.74	6.77	7.34	7.7	7.52	0.75	0.53
F61-1209-U	HRV-C	7.51	7.4	7.45	7.06	7.49	7.28	-0.18	0.12
T53-0110-U	HRV-A7	6.76	6.59	6.68	6.65	6.97	6.81	0.14	0.09
C75-0109-U	HRV-C	5.15	5.31	5.23	5.43	5.85	5.64	0.41	0.29
B53-0510-U		5.37	6.72	6.04	5.76	6.1	5.93	-0.12	0.08
G49-1008-U	HRV-A9	5.72	5.5	5.61	5.44	5.84	5.64	0.03	0.02
S39-0908-U	HRV-A58	5.71	5.38	5.55	5.58	6.09	5.84	0.29	0.21
F78-1008-U		5.02	6.5	5.76	4.8	5.61	5.21	-0.56	0.39
D47-0209-U	HRV-C	5.46	5.75	5.6	5.23	6.29	5.76	0.15	0.11
G82-0309-U	HRV-C	6.42	6.13	6.28	7.05	7.41	7.23	0.96	0.67
M65-0109-U	HRV-C	6	6.71	6.36	5.46	7.15	6.3	-0.05	0.04
C41-0909-U	HRV-A20	6.51	6.35	6.43	6.76	6.94	6.85	0.42	0.3
C47-1009-U	HRV-A10	5.58	5.28	5.43	5.59	5.83	5.71	0.28	0.2
S68-0909-U	HRV-A68	6.28	5.97	6.13	6.65	6.9	6.77	0.65	0.45
M41-1108-U	HRV-A51	5.41	4.96	5.19	5.31	5.58	5.45	0.26	0.18
B53-1109-U	HRV-A62	5.85	5.91	5.88	5.3	6.15	5.72	-0.16	0.11
B53-1209-U	HRV-A62	4.98	4.94	4.96	5.07	5.32	5.2	0.23	0.17
C70-0510-U		6.65	6.72	6.68	5.49	5.86	5.67	-1.01	0.71
G44-0410-U		5.95	6.05	6	6.4	6.87	6.64	0.64	0.45
B53-0410-U		5.53	5.71	5.62	5.5	6.28	5.89	0.27	0.19
V74-0510-U		6.78	6.93	6.86	7.37	7.69	7.53	0.68	0.47
C67-0410-U		5.07	5.29	5.18	5.4	5.97	5.68	0.51	0.35
C67-0410-U		5.26	5.31	5.29	5.82	6.07	5.94	0.66	0.46
D46-0410-U		5.18	5.22	5.2	4.73	5.28	5	-0.19	0.14
G49-0410-U		4.72	4.79	4.75	5.15	5.62	5.38	0.63	0.45
B54-0710-U		5.94	6.17	6.06	5.91	6.34	6.13	0.07	0.05
A77-0810-U		5.78	5.08	5.43	6.04	5.75	5.9	0.47	0.33
B55-0910-U		5.5	5.58	5.54	5.64	6.17	5.91	0.36	0.26
V74-1010-U		5.51	5.82	5.66	4.86	5.27	5.07	-0.6	0.42
M41-1010-U		4.87	4.97	4.92	4.73	5.19	4.96	0.04	0.03
B55-1010-U		4.45	4.43	4.44	4.47	4.66	4.57	0.13	0.09
J63-0111-U		4.5	4.52	4.51	5.09	5.75	5.42	0.91	0.64
J56-1209-U		4.65	4.66	4.66	4.31	4.85	4.58	-0.08	0.06
H88-1210-U		6.2	5.95	6.08	6.5	6.31	6.4	0.33	0.23
G44-0111-U		6.43	6.8	6.61	6.97	5.06	6.01	-0.6	0.42
H88-0810-U		4.39	4.81	4.6	4.8	5.64	5.22	0.62	0.44

C67-0908-U	HRV-B6	5.17	5.32	5.24	4.98	5.45	5.22	-0.03	0.01
B68-0509-U	HRV-C	5.91	6	5.96	4.96	5.44	5.2	-0.76	0.54
M41-0909-U		6.64	6.76	6.7	6.61	7.27	6.94	0.24	0.17
B53-0210-U	HRV-A62	5.12	5.07	5.1	4.94	5.99	5.46	0.37	0.25
M65-0109-L		5.23	5.24	5.24	4.97	4.59	4.78	-0.46	0.33
F78-1008-L		7.36	7.5	7.43	6.94	7.06	7	-0.43	0.3
C47-1009-L	HRV-A10	5.75	5.83	5.79	5.7	6.02	5.86	0.07	0.05
B54-0710-L		7.34	7.43	7.38	7.25	7.58	7.42	0.03	0.03
B53-0510-L		5.56	5.84	5.7	5.22	5.52	5.37	-0.33	0.23
M41-0710-L		5.63	5.88	5.76	4.99	5.43	5.21	-0.55	0.39

Specimens are designated by the first letter of their last name followed by the last two numbers of their birth year (eg. B48), the month and year of sample collection (eg. 0209), and the site of sampling (U, upper respiratory tract; L, lower respiratory tract).

HRV species and genotype are indicated when sequences are available.

Supplementary Table 3. Inter-assay variability and difference in cell amount analyzed in 12 respiratory samples.

Specimen	Inter-assay variability						Estimation of sample cell content		
	One-step log(RNA copies/ml)			Two-step log(RNA copies/ml)			Panente-rhino/Ge/08 mean Ct value	GAPDH RNA Ct value	CELL control Ct value
	Exp1	Exp2	%CV	Exp1	Exp2	%CV			
M65-1008-U	5.34	5.31	0.37	5.75	5.58	2.08	31.06	34.99	32.8
J56-1209-U	6.79	6.57	2.35	7.34	7.48	1.31	26.53	32.35	29.42
F61-1209-U	7.51	7.31	1.89	7.06	8.00	8.84	23.85	36.12	29.81
T53-0110-U	6.76	6.48	2.96	6.65	7.21	5.67	26.68	36.7	29.49
G49-1008-U	5.72	5.61	1.29	5.44	6.21	9.32	30.18	37.33	31.76
M65-0109-U	6	5.69	3.75	5.46	6.33	10.42	29.86	34.32	32.95
M41-1108-U	5.41	5.14	3.56	5.31	5.74	5.53	31.68	34.88	31.44
B53-1109-U	5.85	5.89	0.43	5.30	6.46	13.98	29.1	35.28	31.53
G49-0410-U	4.72	4.87	2.22	5.15	5.64	6.44	35.75	34.15	33.93
B54-0710-U	5.94	5.96	0.25	5.91	6.41	5.73	28.44	Undetected	33.83
H88-0810-U	4.39	4.58	3.04	4.80	5.38	8.11	38.83	35.15	28.57
B54-0710-L	7.34	7.62	2.65	7.25	7.67	3.97	22.85	32.13	29.58

Columns 2 to 7: one-step and two-step inter-assay variation in HRV RNA quantification.

Columns 8 to 10: housekeeping gene GAPDH Ct, and CELL control r-gene Ct values.

Supplementary Table 4. Genetic variability-related variation in HRV nucleic acid

quantification expressed in % CV without taking into account (A) and taking into account (B) an HEV 5'UTR sequence.

A.

Plasmid dilution (copies/reaction)	Log (copies/ml)			%CV	Mean % CV
	HRV-A16	HRV- B37/A16	HRV- Ca/A16		
1000000	8.25	8.55	8.13	2.57	5.22
100000	7.08	7.56	7	4.23	
10000	5.96	6.57	5.96	5.71	
1000	4.97	5.41	4.84	5.88	
100	4.11	4.28	3.68	7.69	

B.

Plasmid dilution (copies/reaction)	Log (copies/ml)				% CV	Mean % CV
	HRV-A16	HRV- B37/A16	HRV- Ca/A16	HEV- A71/A16		
1000000	8.25	8.55	8.13	7.48	5.58	6.98
100000	7.08	7.56	7	6.37	7.01	
10000	5.96	6.57	5.96	5.34	8.44	
1000	4.97	5.41	4.84	4.51	7.52	
100	4.11	4.28	3.68	3.95	6.38	

HRV-16: plasmid encoding a full-length HRV-A16 genome.

HRV-B37/A16: plasmid encoding an HRV-B37 5'UTR in an HRV-A16 background.

HRV-Ca/A16: plasmid encoding an HRV-Ca 5'UTR in an HRV-A16 background.

HEV-A71/A16: plasmid encoding an HEV-A71 5'UTR in an HRV-A16 background.