TABLE S1 HEV sequence variants selected for evaluation by HEV RT-qPCR

GenBank accession no. [a]	HEV GT	Forward primer GGTGGTTTCTGGGGTGAC	Probe G*TGATTCTCAGCCCTTCG	Reverse primer TTCATCCAACCAACCCCT	Nucleotide position
AF060669	3a	**GGTGGTTTCTGGGGTGAC**agggt	**TGATTCTCAGCCCTTCG**ccctcccctata	**TTCATCCAACCAACCCCT**	5311 - 5380
M74506	2a	·····c·····	·····aa·····	·····	5231 - 5300
GU119960	U	A····c·····	·····	·····	5299 - 5368
AF060668	3a	···A·····	·····	·····	5246 - 5315
FJ457024	U	·····c·····	·····aa·····	·····T	5261 - 5330
AB369688	4	·····c·····	·····a·····	·····T····	5279 - 5348
AB425831	3	·····C·····	·····	·····	5286 - 5355
AB089824	3a	·····C·····	·····	·····	5304 - 5373
DQ022752	U	·····c·····	·····T·····	·····	129 - 198
AF058684	1a	·····c·····	·····C·····aa·····	·····	247 - 316
AB075971	U	·····c·····	·····A·····	·····	234 - 303
AB222183	3	·····c·····	·····T·····	·····	5286 - 5355
FJ998008	U	A·····	·····C·····	·····	5261 - 5330
DQ279091	4	A····c·····	·····A·····	·····	5297 - 5366
AY575857	U	·····	·····C·····	·····G·····	5302 - 5371
DQ079627	U	·····C·C·····	·····	·C·····	115 - 184

[a] HEV sequences selected from a comprehensive multiple sequence alignment (n = 203) based on high-level nucleotide mismatching within primer and/or probe binding regions.

GT, genotype; U, unknown.

TABLE S2 HEV sequence variant detection by HEV RT-qPCR

GenBank accession no. [a]	HEV GT	Oligonucleotide concentration (copies/reaction)	No. of forward primer mismatches	No. of probe mismatches	No. of reverse primer mismatches	Amplification curve (cycles)	Dissociation peak T_m (°C)
AF060669	3a	1,000	0	0	0	33.8	68.2
M74506	2a	1,000	0	0	0	33.5	68.2
GU119960	U	1,000	1	0	0	32.8	68.2
AF060668	3a	1,000	1	0	0	34.0	68.2
FJ457024	U	1,000	0	0	1	33.7	68.2
AB369688	4	1,000	0	0	1	33.6	68.2
AB425831	3	1,000	0	1	0	36.1	59.2
AB089824	3a	1,000	0	1	0	35.5	61.2
DQ022752	U	1,000	0	1	0	38.8	57.7
AF058684	1a	1,000	0	1	0	34.4	64.0
AB075971	U	1,000	0	1	0	36.1	61.2
AB222183	3	1,000	0	1	0	37.8	61.8
FJ998008	U	1,000	1	1	0	35.7	62.6
DQ279091	4	1,000	1	1	0	36.2	61.8
AY575857	U	1,000	0	1	1	35.0	63.3
DQ079627	U	1,000	0	2	1	38.4	59.1

[a] DNA oligonucleotides were synthesized based on corresponding nucleotide sequences, PAGE purified, spectrophotometrically quantified and diluted in 10 mM TRIS, pH 9.0 prior to testing.

GT, genotype; U, unknown; T_m, melting temperature.

TABLE S3 Limit of detection for HEV RT-qPCR

HEV RNA (IU/ml) [a]	HEV RNA (log_{10} IU/ml)	No. of replicates tested	No. of replicates detected	% Detected (95% CI)
500	2.70	12	12	100.0 (73.5, 100.0)
200	2.30	12	12	100.0 (73.5, 100.0)
50	1.70	12	12	100.0 (73.5, 100.0)
20	1.30	12	9	75.0 (42.8, 94.5)
10	1.00	12	8	66.7 (34.9, 90.1)
0	-	12	0	0.0 (0.0, 26.5)

Probit value (95% detection rate) = 25.2 IU/ml (95% CI; 19.2, 44.1)

[a] WHO International Standard for HEV RNA, code 6329/10, diluted in normal human serum.
CI, confidence interval.

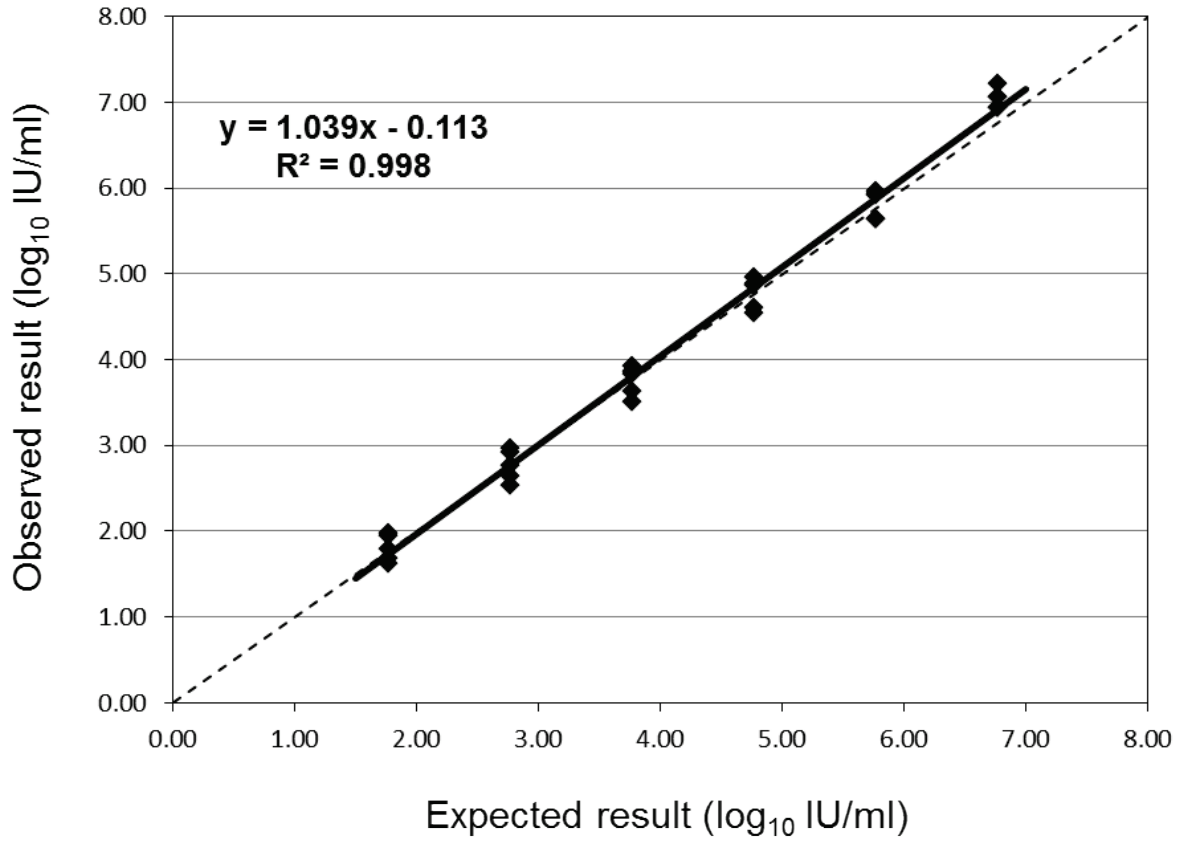

FIG S1 HEV RT-qPCR linearity with serial 10-fold dilutions of SynTura HEV (Thermo Scientific Quality Controls) prepared in normal human serum and tested five times each.

TABLE S4 SynTura HEV calibrator and control performance

HEV RT-qPCR run no.	Standard curve		Assay control results				
	Slope	R^2	High positive		Low positive		Negative
			IU/ml	$\log_{10}$ IU/ml	IU/ml	$\log_{10}$ IU/ml	
1	-3.37	0.9999	62,800	4.80	584	2.77	Not detected
2	-3.38	0.9997	70,100	4.85	649	2.81	Not detected
3	-3.33	0.9999	71,900	4.86	575	2.76	Not detected
4	-3.31	0.9990	66,800	4.82	711	2.85	Not detected
5	-3.31	0.9998	62,000	4.79	628	2.80	Not detected
6	-3.30	0.9994	64,400	4.81	487	2.69	Not detected
7	-3.32	0.9992	59,000	4.77	636	2.80	Not detected
8	-3.30	0.9998	64,700	4.81	736	2.87	Not detected
9	-3.34	1.0000	67,900	4.83	674	2.83	Not detected
10	-3.34	0.9992	81,200	4.91	621	2.79	Not detected
Mean	-3.33	0.9996	67,100	4.83	630	2.80	-
SD	0.03	0.0004	6,280	0.04	71	0.05	-