		2 step RT-PCR of HCV RNA in samples ***WITHOUT*** ThermaStop-RT[1]				2 step RT-PCR of HCV RNA in samples ***WITH*** ThermaStop-RT[1]			
Reverse Transcriptase	**Company**	mean Cq in RT buffer	mean Cq in PCR buffer	specific product yield	non-specific product	TS-RT units for specificity	mean Cq in RT buffer	specific product yield	non specific product[2]
SuperScript™ III	Invitrogen	29	ND	++	+++	1	29	++++	–
igScript™	Intact Genomics	29	29	++	++	2	29	++++	–
WarmStart® RTx[3]	NEB	31	33	++	++	2	31	++++	–
SydScript	SydLabs	29	ND	+	++	0.5	30	+++	–
SuperScript IV	Invitrogen	no Cq	ND	–	+++	2	29	+++	±
SunScript™	Expedion	no Cq	no Cq	–	++	1	30	+++	±
e™ AMV	MilliporeSigma	30	ND	+	++++	2	32	+++	±
SOLIScript	Solis BioDyne	31	ND	++	+	1	32	+++	±
UltraScript	PCR Biosystems	33	ND	++	±	1	36	++	–
TGIRT®[4]	InGex	no Cq	no Cq	–	++++	2	33	+	++

1Two-Step RT-PCR was done using an HCV Armored RNA target in a background of human leukocyte total RNA. The HCV sequence was from the 5'UTR of the virus and has moderate secondary structure. Each reverse transcription sample contained about 1,000 targets based on the manufacturer's estimates and the buffer recommended by the reverse transcriptase supplier was used. In some cases, reverse transcription was also tested using Invitrogen PCR buffer with 3 mM magnesium. **Reverse transcription was done at 55°C for 30 minutes.** Each RT sample was used to prepare 4 PCR samples.

2The result "±" under non-specific product indicates the presence of a band the size of primer-dimer, but no other non-specific products.

3WarmStart RTx from New England Biolabs is an MMLV-derived transcriptase with RNase H activity that is optimized for LAMP and includes an aptamer for hot start.

4TGIRT is a Group II reverse transcriptase and the manufacturer's protocol is optimized for RNA Seq. The buffer has extremely high salt concentrations and no specific amplification was observed after RT in that buffer, although adjustments were made for the PCR step. Low level probe signal was observed after RT in PCR buffer samples containing 1 or 2 units of ThermaStop-RT.