

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Yu AC-H and Lau L-T. Boosting the Sensitivity of Real-Time Polymerase-Chain-Reaction Testing for SARS. *N Engl J Med* 2003;350:1577-9.

Supplementary Appendix 1

SARS-CoV one-step RT-PCR for enhanced real-time PCR detection

In order to explore the possibility of improving sensitivity of our current enhanced real-time PCR (ERT) method, ERT was carried out as previously described,¹ except that reverse transcription (RT) and target pre-amplification by PCR were combined to a single step.

One-step RT-PCR

A known amount of SARS-CoV nucleic acid¹ was used for preparing 10-fold serial dilutions (10^{-2} to 10^{-4}). For each dilution, ERT assays with one-step RT-PCR or separate RT and PCR steps were performed. The PCR primer pair used was derived from the membrane protein (MP) gene region of the SARS-CoV genome (GenBank: AY278554), having sequences of 5'ACCGCTCATGGAAAGTGAAC and 5'CTACCGGCGTGGTCTGTATT. Regular ERT was carried out according to described method.¹ ERT with one-step RT-PCR was performed as follows: 10 μ l of each diluted nucleic acid was added to a RT-PCR mixture (final volume of 50 ml), containing 1X reaction mix (Superscript one-step RT-PCR kit, Invitrogen), 0.2 mM of each PCR forward and reverse primers, 3 mM MgSO₄, and RT/Platinum Taq mix (Superscript one-step RT-PCR kit, Invitrogen). RT-PCR was performed in a MasterCycler (Eppendorf) with the following temperature profile: initial incubation at 42°C for 30 min, followed by denaturation at 95°C for 5 min, 10 cycles of 95°C for 10 sec; 60°C for 10 sec (1°C reduction after each cycle); 72°C for 20 sec, a further 40 cycles of 95°C for 10 sec; 56°C for 10 sec; 72°C for 20 sec, and ending with an extension of 72°C for 5 min. RT-PCR products (2 μ l) were used in subsequent real-time PCR assay, with the same conditions as previously described.¹ Sequences of the real-time PCR primers and probe used were: 5'AAGGACCTGCCAAAAGAGATCA, 5'CGCTGCGACGCTCCTAAT, and 5'TGTGGCTACATCACGAACGCTTTCTTATTACA, respectively.

Reference

1. Lau LT, Fung YW, Wong FP, et al. A real-time PCR for SARS-coronavirus incorporating target gene pre-amplification. *Biochem Biophys Res Commun* 2003;312:1290-6.