Boosting the Sensitivity of Real-Time Polymerase-Chain-Reaction Testing for SARS

TO THE EDITOR: In view of recent concern about the recurrence of severe acute respiratory syndrome (SARS) in Guangdong, China, we would like to highlight the tremendous importance of sensitivity in testing for the SARS-associated coronavirus (SARS-CoV). Because the initial symptoms of SARS

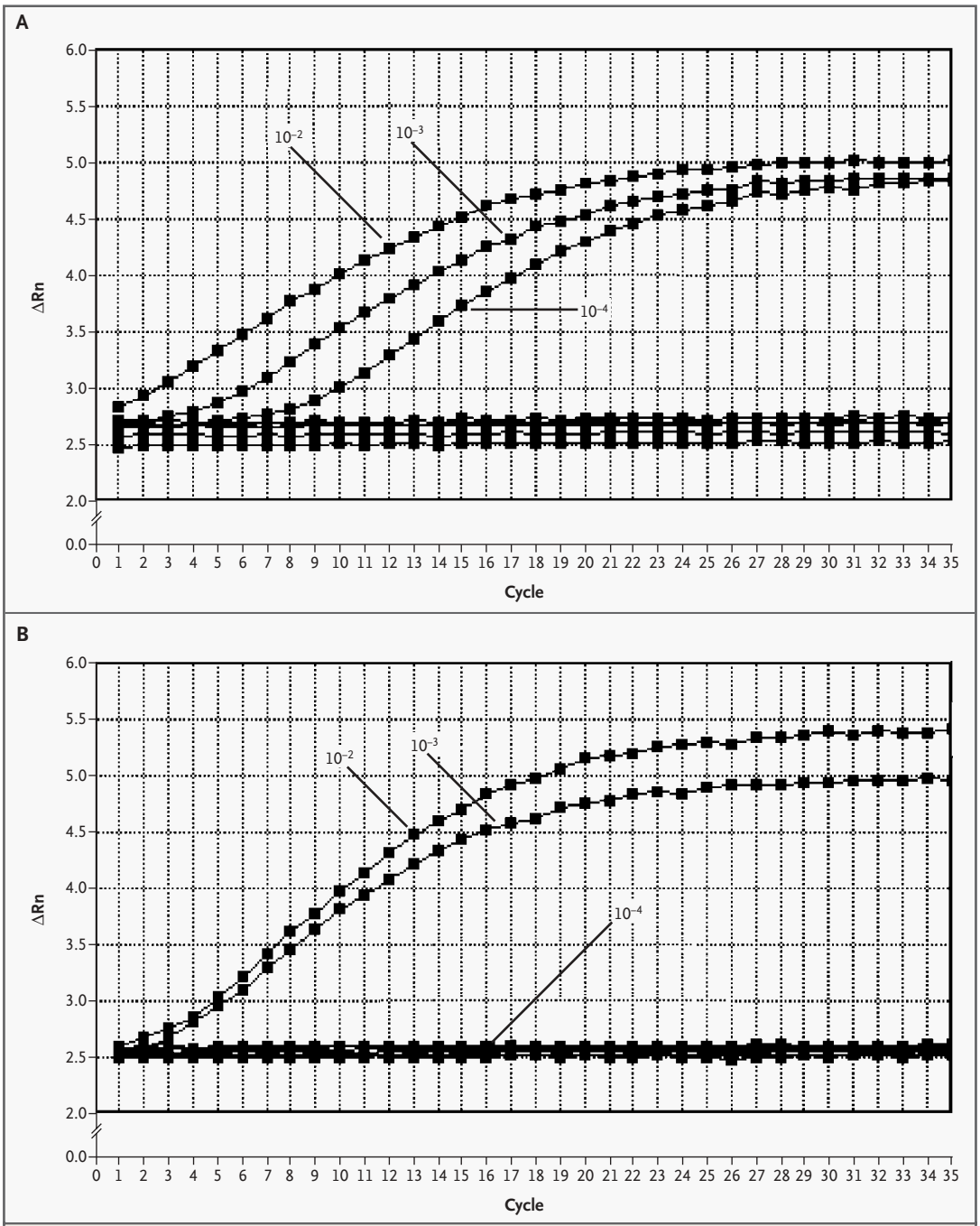

Figure 1. Results of Modified Enhanced Real-Time (ERT) Polymerase Chain Reaction (PCR) with One-Step Reverse-Transcriptase PCR (RT-PCR).

The sensitivity of the ERT technique is 10 times that of regular real-time PCR. Serial dilutions (10^{-2} to 10^{-4}) of a known amount of SARS-CoV nucleic acid (as previously described[1]) were prepared and used to compare the sensitivity of the ERT technique with one-step RT-PCR (Panel A) or separate RT and PCR steps (Panel B), followed by real-time PCR (TaqMan) under identical conditions. The change of normalized reporter signals (ΔRn) is calculated by normalizing the reporter signals with the fluorescent signals given by the passive reference. The region of the membrane-protein gene of the SARS-CoV was detected in this case. Positive signals are clearly displayed as prominent amplification curves on both real-time PCR plots, whereas negative signals remain unambiguously flat along the abscissas. The results clearly demonstrate that one-step RT-PCR boosts the sensitivity of the ERT technique by a factor of 10 as compared with the regular ERT technique.

are similar to those of other common respiratory diseases, making a specific diagnosis of SARS poses difficulties to medical professionals. Our enhanced real-time (ERT) polymerase-chain-reaction (PCR) method (first presented in June 2003 at a symposium on SARS[1]) has been designed for the detection of SARS-CoV with high sensitivity and easy-to-interpret results.[2] The power of the ERT technique has now been extensively explored with the development of ERT-based diagnostic tests for various infectious diseases, including avian influenza and foot-and-mouth disease.

Since the first report of ERT results for SARS,[2] the ERT technique has been modified to increase its sensitivity for the detection of SARS-CoV by at least 10 times (Fig. 1). This improved sensitivity has been achieved by combining the reverse-transcriptase (RT) and PCR steps into a single step (described in Supplementary Appendix 1, available with the full text of this letter at www.nejm.org). In addition, the procedural change makes the diagnostic procedure more convenient. These salient features of one-step RT-PCR have thus far been overlooked by other researchers in this field. Because the single RT-PCR step and the subsequent real-time PCR step require only 35 cycles, the detection of SARS-CoV by the modified ERT technique yields results quickly and with higher sensitivity than regular real-time PCR assays reported to date.

As noted by the World Health Organization with respect to the shortcomings commonly seen in available diagnostic tests for SARS,[3] it is important to unify a molecular test for SARS that can provide sensitive, reliable, and accurate results. Currently, many research groups claim that their methods are accurate, but the way in which they evaluate accuracy is not clearly described.[4] The usefulness of an accurate test that lacks sensitivity has yet to be determined. Unless a unified molecular test for SARS with high sensitivity and reliability is available, we may face the risk of false negative test results, which would allow infected patients to slip into the community and avoid control measures set up to isolate carriers.

Over a year after the start of the 2003 SARS outbreak, many people are still struggling to recover from the physiological and psychological scars inflicted at that time. Identifying potential SARS-CoV carriers by a method with high sensitivity and reliability and as early as possible is crucial to avoid a repetition of the 2003 outbreak.

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