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Evaluation of Real-Time Reverse Transcriptase PCR and Real-Time Loop-Mediated Amplification Assays for Severe Acute Respiratory Syndrome Coronavirus Detection

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We compared the performance of a recently established real-time loop-mediated amplification (LAMP) assay with the one from a highly sensitive quantitative PCR assay. None of these assays produced false-positive results in this study. For samples isolated from patients within the first 3 days of disease onset, the detection rate of the quantitative PCR assay was higher (14 of 15 were positive) than the LAMP assay (9 of 15 were positive). By contrast, the detection rates of these assays toward specimens sampled from patients with more than 3 days of illness were similar (32 of 44 for PCR and 33 of 44 for LAMP were positive). The simpler operation of LAMP might be a possible solution for on-site diagnosis.

A novel human coronavirus (CoV) was identified as the etiological agent for severe acute respiratory syndrome (SARS) (7, 11, 18). The outbreak emerged in late 2002 and was brought under control through a concerted global effort, and by 5 July 2003, no further human–human transmission was taking place and the global outbreak was declared over (26). Although there is no evidence for the virus persisting in the human population (12), the identification of its precursor in animals (8) and the laboratory infection cases (16) have highlighted the possibility of the reemergence of SARS. Epidemiological studies have indicated that an early identification of human SARS cases is the key measure to prevent the spreading of the disease (5). Based on serological and molecular approaches, several laboratory diagnostic methods were developed for SARS diagnosis (1, 17, 21). Serological assays and enzyme immunoassays, such as detection of SARS CoV-specific antibodies (2) or viral antigens (3), were established. For the molecular approach, PCR-based methods are the major molecular assays for SARS CoV detection (21). Of these PCR-based assays, the quantitative reverse transcriptase PCR (RT-PCR) approach was shown to be the most sensitive method for early SARS detection (6, 19, 20, 24). Recently, non-PCR-based loop-mediated amplification (LAMP) tests were developed for SARS CoV detection (9, 22). This method depends on autocycling strand displacement DNA synthesis performed by a Bst DNA polymerase and is simple to use (15). The isothermal reaction relies on recognition of the target by six independent sequences, thereby making this kind of assay highly specific (15). In particular, a real-time LAMP assay for SARS CoV was shown to be 100-fold more sensitive than conventional RT-PCR assays (9). However, a direct comparison between the real-time LAMP assay and highly sensitive quantitative RT-PCR assay was not reported. Here, we have compared the performances of the real-time LAMP assay and a highly sensitive real-time quantitative RT-PCR assay by using retrospective clinical specimens.

In this study, 59 retrospective nasopharyngeal aspirates (NPA) from SARS patients were recruited in this study. All of these patients were confirmed to be SARS patients by serological or PCR tests as described before (16). NPA from patients with unrelated respiratory diseases (adenovirus, $n = 5$; respiratory syncytial virus, $n = 5$; influenza A virus, $n = 5$; influenza B virus, $n = 5$; human coronavirus OC43, $n = 5$; human coronavirus 229E, $n = 1$; human coronavirus NL63, $n = 1$) or healthy individuals ($n = 10$) were recruited as controls. The study was approved by the Clinical Research Ethics Committee. RNA from 560 μl of NPA was extracted and eluted in 30 μl of elution buffer as described previously (20). Two microliters of extracted RNA was subjected to testing by the one-step quantitative RT-PCR (23) and by a Loopamp SARS CoV detection kit (Eiken Chemical, Japan), which was modified from the real-time LAMP assays as described previously (9). The RT-PCR was previously shown to be more sensitive than the conventional RT-PCR assay (20, 23). The performances of these assays and the authenticity of reaction products from these assays were demonstrated in the previous work as mentioned previously (9, 23). Details of the amplification mechanism are described elsewhere (15).

Of 59 SARS samples, 46 (78%) and 42 (71%) samples were positive in the RT-PCR and LAMP assays, respectively (Table 1). The detection rates of these assays were not statistically different from each other (McNemar's test, $P = 0.29$), indicating that these assays have similar performances for SARS CoV detection. None of the controls ($n = 37$) was positive in these

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TABLE 1. Detection of SARS CoV by quantitative PCR and real-time LAMP assays

Quantitative RT-PCR	RT-LAMP		Total
	Positive	Negative	
Positive	40	6[a]	46
Negative	2	11	13
Total	42	17	59

[a] Three of these samples were positive in a repeated LAMP test.

assays. In addition, the SARS samples were divided into different stages of illness for further analysis. For those samples collected after day 3 of disease onset, the detection rates of these assays were similar (Table 2). For samples collected from the first 3 days of disease onset, 14 out of 15 samples were positive in the RT-PCR assay. By contrast, the LAMP assay could detect the SARS-CoV viral sequence in nine of these samples. These results suggested that the RT-PCR test might be more sensitive than the real-time LAMP test. However, the differences of the detection rates between these tests on the early SARS samples was not, but close to, statistically significant (McNemar's test, $P = 0.06$). For those samples that were positive in the RT-PCR, but negative in the real-time LAMP (Table 1; $n = 6$), we retested these samples in the LAMP test again. Three out of six of these samples were positive in the subsequent test. Of the samples that were negative in both real-time LAMP tests, the viral RNA copy numbers in the extracted RNA samples were found to be 3.2, 8.0, and 9.4 copies per μl. Due to an insufficient amount of RNA samples, we could not retest those two samples that were positive for the LAMP but negative for the RT-PCR assays.

Quantitative data generated from quantitative RT-PCR assays were previously reported to be useful for prognosis (4, 10, 14). Of 33 samples which were positive on both assays, the threshold cycle (CT) values from the RT-PCR assay correlated with the threshold time (TT) from the real-time LAMP assay (Fig. 1; Pearson correlation = 0.76, $P < 0.001$). Samples with low viral RNA copies (i.e., with high CT or TT values) trended to deviate from the regression line. This variation might be partly due to sampling errors arising from the stochastic distribution of low-copy-number template molecules (25). Nonetheless, the positive correlation between these assays indicated the quantitative data generated from real-time LAMP might be used for prognosis purposes.

In our analysis, we compared the performance of the real-time LAMP and RT-PCR assays. Both assays have 100% specificity. The sensitivities of the RT-PCR and real-time LAMP tests are 78% and 71%, respectively. Both assays have a positive prediction value of 1. The negative prediction values of the RT-PCR and real-time LAMP assays are 0.74 and 0.68, respectively. We demonstrated that the performance of these assays were comparable. For those early SARS samples isolated from the first 3 days of disease onset, the detection rate of the RT-PCR assay was slightly better than the LAMP assay. Thus, optimized RT-PCR assays would still be the method of choice for early SARS diagnosis. By contrast, for those samples isolated at a later stage of illness, both assays could be used for the detection of SARS CoV. But for situations such as a suspected SARS outbreak in a remote village, running quantitative PCR in field or bedside situations might not be feasible (22). Considering the simplicity of the LAMP approach (13), the real-time LAMP assay might be an attractive alternative for SARS diagnosis in the above scenarios. Regardless of the method used, testing in a suitably accredited laboratory is important for the confirmation of SARS cases, especially during an outbreak, when quality-assured diagnoses are essential (27).

TABLE 2. Performance of quantitative RT-PCR and real-time LAMP assays for SARS diagnosis

Day of onset	No. of samples	No. of samples detected (%) by:	
		Quantitative RT-PCR	RT-LAMP
1–3	15	14 (93)	9 (60)
4–7	37	25 (68)	26 (70)
>7	7	7 (100)	7 (100)
Total	59	46 (78)	42 (71)

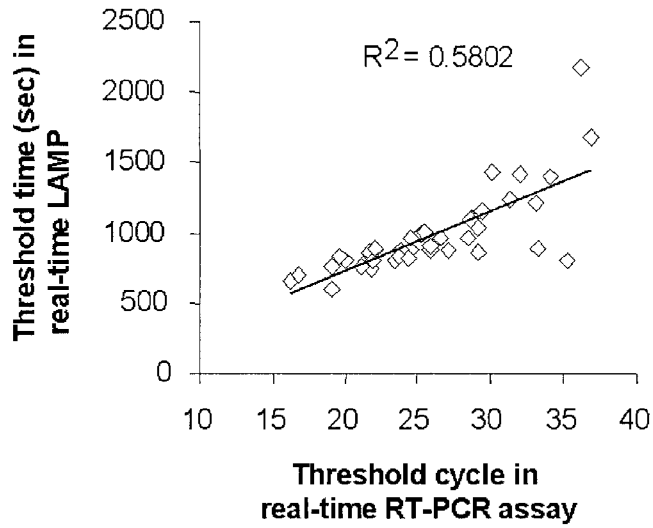

FIG. 1. Correlation between threshold cycle values of the RT-PCR assay and threshold time values of the real-time LAMP assay.

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