Rapid Detection of the Severe Acute Respiratory Syndrome (SARS) Coronavirus by a Loop-Mediated Isothermal Amplification Assay, Leo L.M. Poon,1 Cynthia S.W. Leung,1 Masato Tashiro,2 Kwok Hung Chan,3 Bonnie W.Y. Wong,3 Kwok Yung Yuen,1 Yi Guan,1 and Joseph S.M. Peiris1 (1 Department of Microbiology, Queen Mary Hospital, University of Hong Kong, Hong Kong SAR; 2 Department of Viral Diseases and Vaccine Control, National Institute of Infectious Diseases, Musashi-Murayama, Tokyo, Japan; 3 Department of Microbiology, Queen Mary Hospital, Hong Kong SAR) * address correspondence to

tence of SARS is further highlighted by a recent con-
graphic region
the source of the disease is still circulating in this geo-
ographic region (5). Indeed, the potential risk of reemerg-
ence of SARS is further highlighted by a recent con-
firmed SARS case in January 2004 (6). Therefore, the
establishment of a rapid SARS diagnostic method is a
high priority for control of the disease.

Currently, there are two major diagnostic approaches
for SARS. Detection of antibodies against SARS-CoV is a
sensitive and specific diagnostic approach, but serconver-
sion can be detected only around day 10 of illness (7). In
contrast, PCR-based tests have been shown to be useful
for early SARS diagnosis (8). Quantitative PCR ap-
proaches are a powerful tool for identifying SARS-CoV
early after disease onset (4–9). However, because of
the requirements for sophisticated instrumentation and
expensive reagents, these rapid molecular tests might not
be the method of choice in basic clinical settings in
developing countries or in field situations. It is therefore
critical to develop simple and economical molecular tests
for the above scenarios.

The invention of loop-mediated isothermal amplifi-
cation (LAMP) has opened up a new horizon for molecular
diagnosis (12). This method depends on autocycling
strand displacement DNA synthesis performed by a Bst
DNA polymerase, and a detailed amplification mecha-
nism has been described elsewhere (12). The reaction
relies on recognition of the DNA target by six indepen-
dent sequences, making this kind of assay highly specific.
This method is rapid and has a DNA amplification efficiency equivalent to that of PCR-based methods (12–
14). More importantly, this approach is inexpensive, and
all reactions can be performed in an isothermal environ-
ment. The potential clinical applications of this method
have been demonstrated recently (13). Here we demon-
strate the feasibility of using this technology for detection
of SARS-CoV.

Thirty-one retrospective SARS samples collected be-
tween March 26, 2003, and April 9, 2003, were used in this
study. All SARS patients in this study were confirmed to
be seropositive for SARS-CoV by immunofluorescence assays (2). The age range for these patients was 16–74
years (median, 45 years), and the M:F ratio was 16:15. The
study was approved by our local clinical research ethics
committee. Nasopharyngeal aspirate (NPA) samples were
collected on days 1–15 after disease onset as described
previously (15). NPA samples from patients with other
respiratory diseases (adenovirus, n = 8; respiratory syn-
cytial virus, n = 10; human metapneumovirus, n = 10;
influenza A virus, n = 20; influenza B virus, n = 4; rhinovirus, n = 6) and from healthy individuals (n = 30)
were used as negative controls.

RNA from clinical samples was extracted, and cDNA
was synthesized as described previously (9, 15). In this
study, the ORF1b region of SARS-CoV (nucleotides
17741–17984; accession no.AY274119; see Fig. 1S in
the online Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/
vol50/issue6/) was chosen for SARS diagnosis. DNA
plasmids containing the target sequences were used as
positive controls. To accelerate the amplification reaction,
cDNA for the SARS-CoV ORF1b sequence was amplified
by a modified LAMP reaction (14) in the presence of
six primers: F3 (5’-CTTAGATTGCTACAG-3’); B3c (5’-
AGTCCAGTACATTCTTCT-3’); FIP (5’-AGTGTCTTGT-
TCAGTAGTACCATACAGGTT-3’); BIP (5’-TGTA-
ATGTCAAACGCTTTCCAGCTTTATGACAGTTATTTCT-3’);
Loop B (5’-TCTTTATGACAAAACTGCAAAT-3’); and Loop Fc (5’-
TTTGTGTAATAGCATGATCATACA-3’; see Fig. 1S in
the online Data Supplement). In a typical LAMP reaction,
0.5–1 μL of heat-denatured cDNA was amplified in a
12.5-μL reaction containing 0.4 mM each of the de-
oxynucleotide triphosphates, 1.6 μM each of FIP and BIP,
0.2 μM each of F3 and B3c, 0.8 μM each of Loop F and
Loop Fc, 4 U of Bst DNA polymerase (New England
Biolabs), and 1× Bst polymerase buffer (New England
Biolabs). Reaction mixtures were incubated at 60 °C for
1 h, followed by heat inactivation at 80 °C for 5 min.
Amplified products were analyzed by gel electrophoresis.

In preliminary experiments, reactions were performed
with different copy numbers of the positive control to
determine the detection limit of the assay. Because the
reaction products consist of stem-loop DNA structures
with multiple inverted repeats of the target and cauliflow-
er-like structures with multiple loop-stem-loops (12, 14),
the reaction would produce bands of different sizes in gel
electrophoresis analyses. As shown in Fig. 1, a character-
istic DNA ladder was observed in positive controls (lanes
1 and 26). The detection limit of the assay was 10
copies/reaction (see Fig. 2A in the online Data Supple-
ment), and positive signals were consistently observed in
reactions containing ≥50 copies of the target sequence
(data not shown).

Among 31 SARS samples, the SARS-CoV sequence
could be detected in 20 cases (64%; Fig. 1, lanes 3–14). The
detection rate for SARS-CoV in these samples increased as
the disease progressed (Table 1). In the early stages after
disease onset, 4 of 13 (31%) were positive in the assay. For
samples isolated from day 8 to day 15 after disease onset, positive signals were observed in all of the cases \( (n = 12) \). These results agreed with our previous findings that the viral load in SARS patients peaks at the second week of the disease \( (7) \). Because the targeted sequence contains \( Bgl \) II and \( Xba \) I restriction sites (see Fig. 1S in the online Data Supplement), we also validated the identities of these positive signals by restriction enzyme digestion. All amplified products could be digested by these restriction enzymes as expected (Fig. 2SB in the online Data Supplement and Fig 1). By contrast, no positive signal was observed in healthy individuals \( (n = 30; \text{data not shown}) \), non-SARS patients \( (n = 58; \text{Fig. 1, lanes 17–22}) \), and water controls (Fig. 1, lanes 15 and 27).

In this study, we demonstrated the potential use of LAMP for early SARS diagnosis. Recently we also reported the use of a quantitative PCR method for SARS diagnosis \( (9, 16, 17) \). Compared with quantitative PCR assays, the LAMP assay described in this study has two main shortcomings: (a) the LAMP assay does not allow quantification of SARS-CoV RNA; and (b) the LAMP assay is less sensitive than real-time PCR assays \( (9) \). However, one should note that the detection rates for SARS in the LAMP assay (Table 1) are similar to those with our conventional PCR-based assays \( (18) \). To confirm this observation, we further tested some of these clinical samples with a conventional PCR assay \( (2) \). As shown in Table 1, the detection rate of the LAMP assay was similar to that of the reverse transcription-PCR assay. These results agree with previous findings that the sensitivities of LAMP assays are equivalent to those for conventional PCR-based methods \( (12–14) \).

Our LAMP reaction relies on recognition of viral sequences by six primers, potentially making this kind of assay more specific than conventional PCR assays. Indeed, none of the negative control samples \( (n = 88) \) was positive in our assay. Recently, Parida et al. \( (19) \) reported a real-time closed-tube detection method for West Nile virus in which the amounts of magnesium pyrophosphate precipitates generated in LAMP reactions are measured. This real-time approach for LAMP might further reduce the risk of cross-contamination problems.

The primary goal of this study was to develop a simple and inexpensive test for SARS diagnosis. Unlike the quantitative PCR-based detection approach, the LAMP assay does not require sophisticated instrumentation. Because reactions are performed in an isothermal environment \( (\text{e.g., a water bath}) \), there is no time loss from thermal changes during DNA amplification. The LAMP assay is rapid and does not require expensive reagents or

### Table 1. Detection of SARS CoV by LAMP assay.

| Day after onset | Sample size, n | LAMP assay | PCR* |
|----------------|----------------|------------|------|
| 1–3            | 13             | 4 (31%)    | 5 (38%) |
| 4–7            | 6              | 4 (67%)    | ND*  |
| 8–15           | 12             | 12 (100%)  | 12 (100%) |

*a Reverse transcription-PCR protocol adapted from Peiris et al. \( (2) \).

*b ND, not done.
Instruments. In a SARS outbreak, a diagnostic laboratory might routinely receive hundreds of clinical samples each day for SARS diagnosis. The application of this LAMP test might help to reduce the running cost for SARS diagnosis. From a practical point of view, highly sensitive quantitative reverse transcription-PCR assays should be used to test samples collected from patients within the first week of illness. For samples collected from patients after the first week of disease onset, the LAMP assay might be an inexpensive and accurate alternative for SARS diagnosis.

In conclusion, we report a simple LAMP assay for SARS diagnosis. We believe the inexpensive running costs of the assay make this technology very applicable to laboratories for SARS diagnosis in developing countries. The technique might have great potential to be used in field situations or at the bedside as a preliminary screening test. Regardless of the method used, testing in a suitably accredited laboratory is important, especially during an outbreak, when quality-assured diagnoses are essential. We expect that, with this rapid diagnostic method, prompt identification of this pathogen will facilitate control of the disease and provision of prompt treatment of patients.

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On-Line Quantification of Human Urinary Albumin by a Fluorescence Immunoassay, Sunga Choi,1 Eui Yul Choi,2 Ha Suck Kim,3 and Sang Wook Oh4 (1 Central Research Institute of BodiTech Inc., Chunchon, South Korea; 2 Department of Genetic Engineering, Hallym University, Chunchon, South Korea; 3 Department of Chemistry, Seoul National University, Seoul, South Korea; address correspondence to this author at: Central Research Institute of BodiTech Inc., Chunchon 200-160, South Korea; fax 82-33-258-6899, e-mail sangwoh@empal.com or sangwoh@boditech.co.kr)

Microalbuminuria (MAU), defined as a urinaryalbumin excretion of 30–300 mg/day, indicates a high probability of renal damage and is an accepted predictor for the early diagnosis of nephropathy in diabetic patients (1, 2). In addition, MAU has diagnostic implications in pregnancy as a predictive marker of preeclampsia (3, 4) and may play a role in identifying high risk of developing complications from cardiovascular diseases even in nondiabetic patients (5–7).

Dye-binding assays can measure serum albumin but are too insensitive for MAU testing, making immunochemical assays the most widely used MAU methods (8). These immunoassays include immunoturbidimetry, immunofluorescence, ELISA, RIA, and zone immunoelectrophoresis. Recently, Kessler and coworkers (9, 10) introduced a laser-induced fluorescence system coupled to an automated centrifugal analyzer as a nonimmunologic assay for urinary albumin. Their system was based on the probe Albumin Blue 670/580, which becomes highly fluorescent on binding to albumin.

We report a fluorescence immunochromatography as-