SARS-CoV Infection Was from at Least Two Origins in the Taiwan Area

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Key Words
Multiplex RT-PCR · SARS, genomic sequence analysis · SARS, Taiwan · SARS-CoV genome · SARS-CoV, infection origin · Severe acute respiratory syndrome

Abstract
Objective: Severe acute respiratory syndrome (SARS) is caused by a new coronavirus. Genomic sequence analysis will provide the molecular epidemiology and help to develop vaccines. Methods: We developed a rapid method to amplify and sequence the whole SARS-CoV genome from clinical specimens. The technique employed one-step multiplex RT-PCR to amplify the whole SARS-CoV genome, and then nested PCR was performed to amplify a 2-kb region separately. The PCR products were sequenced. Results: We sequenced the genomes of SARS-CoV from 3 clinical specimens obtained in Taiwan. The sequences were similar to those reported by other groups, except that 17 single nucleotide variations and two 2-nucleotide deletions, and a 1-nucleotide deletion were found. All the variations in the clinical specimens did not alter the amino acid sequence. Of these 17 sequenced variants, two loci (positions 26203 and 27812) were segregated together as a specific genotype – T:T or C:C. Phylogenetic analysis showed two major clusters of SARS patients in Taiwan. Conclusion: We developed a very economical and rapid method to sequence the whole genome of SARS-CoV, which can avoid cultural influence. From our results, SARS patients in Taiwan may be infected from two different origins.

Introduction
An atypical pneumonia with high contagiousness first appeared in the Guangdong Province of the People's Republic of China in November 2002, which infected 792 cases and caused 31 deaths. This disease spread to Hong Kong first, and caused an outbreak in many countries including Vietnam, Canada, Singapore, Taiwan and other countries. Almost 9,000 individuals have been infected and over 900 have died from the disease since February 2003 [1]. This new and deadly syndrome was first brought to the attention of the World Health Organization (WHO) by Dr. Carlo Urbani, and was named as severe acute respiratory syndrome (SARS) [2]. The pathogenesis of SARS is believed to be a novel coronavirus (SARS-CoV), which was first found by Peiris et al. [3] in Hong Kong and
was confirmed by other groups [4–8]. The analysis of genomic sequences showed that the virus is different from previously known coronaviruses [9–11], and serological studies also confirmed that the virus has not been found in humans [12].

The Coronaviridae family is a diverse group of large, enveloped and positive-stranded RNA viruses, and these viruses may cause respiratory and enteric infections in humans and animals. The previously known human coronaviruses are frequent causes of the common cold. Life-threatening pneumonia caused by coronaviruses is uncommon. Generally speaking, the genome of coronavirus is the largest (about 30 kb in length) found in any RNA viruses, which encodes 23 putative proteins, including 4 major structure proteins: nucleocapsid (N), spike (S), membrane (M) and envelope (E) proteins. The N, S and M mature proteins contribute to generating the host immune response [13, 14]. Similarly, the genome of SARS-CoV is a 29729-nucleotide with polyadenylated RNA and about 41% GC content. The genomic organization of SARS-CoV is similar to that of a typical coronavirus, containing the characteristic gene order (5′-replicase, spike, envelope, membrane, and nucleocapsid-3′) and short untranslated regions at both termini [9–11].

The RNA viruses have high rates in genetic mutations, resulting in evolution of new viral strains and escaping from host defenses [15]. It is very important to understand the mutation rate of the SARS virus spreading through the population for the development of effective vaccines. Meanwhile, several studies have shown that adaptation of a virus to non-natural host cells induces genetic changes in viral genome, and these culture-mediated mutations may produce bias and affect evolutionary studies [16–20]. In order to avoid these causations, we sequenced the entire SARS-CoV directly using clinical specimens. Using these approaches, we were able to analyze the origin of SARS-CoV infection more accurately.

### Materials and Methods

#### Patients

We collected clinical specimens including nasal and pharyngeal swabs, blood and stool from 3 SARS patients. All patients fitted the WHO definition of being probable SARS cases: fever of 38 °C or higher, respiratory symptoms, hypoxia, chest radiograph changes suggestive of pneumonia, and history of close contact with SARS patients. The clinical features of 3 cases are shown in table 1.

#### Methods

Total RNA was extracted from the nasopharyngeal samples of the patients using a commercial kit (QIAamp Viral RNA Mini Kits, Qiagen Inc., Calif., USA). For RT-PCR analysis of SARS-CoV, the upstream primer 5′-CTAACATGCTTAGGATAATGG-3′ and the downstream primer 5′-CAGGTAAGCGTAAAACTCATC-3′ were used to amplify part of polymerase gene, the methods used were identical to Ksiazek et al. [7].

In order to have enough PCR products of SARS-CoV genome for sequencing, we utilized a multiplex one-step RT-PCR method to amplify the entire genome followed by nested PCR to separately amplify each region of the genome. The strategy is shown in figure 1. The PCR products were then purified and sequenced. The whole SARS-CoV genome was amplified in two reactions (the primers used are shown in table 2) using a multiplex one-step RT-PCR method; the reaction T1 amplified 8 regions of the genome, which are nucleo-

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**Table 1. The clinical features of 3 SARS patients**

|                  | Case 1 | Case 2 | Case 3 |
|------------------|--------|--------|--------|
| Age, years       | 56     | 44     | 26     |
| Sex              | M      | M      | F      |
| Temperature on admission, °C | 38.5   | >38    | >38    |
| WBC/μl           | 6,760  | 5,360  | 4,120  |
| Lymphocytes, %   | 27     | 19.4   | 28.2   |
| Platelets/μl     | 126 × 10³ | 125 × 10³ | 190 × 10³ |
| LDH, IU/l        | 197    | 116    | 266    |
| GOT, IU/l        | 66     | 116    | 266    |
| GPT, IU/l        | 70     | 197    | 188    |
| Chest radiograph | Accentuation of bronchovascular marking | Pneumonia left lower lobe | Pneumonia right lower lobe |
| Co-morbidities   | α-thal-1 | DM, chronic hepatitis | Nil |
| Outcome          | Death  | Survived | Survived |
Fig. 1. The strategy of sequencing analysis of SARS-CoV from clinical specimens. We used two separate multiplex RT-PCR (one contains 8 pairs of primers (T1), and the other contains 7 pairs of primers (T2)) to amplify the whole SARS-CoV genome. The PCR products of T1 were subjected to nested PCR amplification using primer pairs t1, t3, t5, t7, t9, t11, t13, and t15 separately, and T2 products were

Table 2. The primers used for multiplex RT-PCR

| Reaction | Sequences | Locations (nt) |
|----------|-----------|----------------|
| Reaction T1 | 5'-GCCAACCAACCTCGATCTCTTG-3' (F) | 29–50 |
| | 5'-CCTTGAGAAATCTCAACTCTGC-3' (R) | 2,194–2,173 |
| | 5'-TGTCTGTCAGGCTCCTGCG-3' (F) | 3,852–3,871 |
| | 5'-ATGTGACAGATAGCTCTCGT-3' (R) | 6,070–6,051 |
| | 5'-GGCAGAATCTGCTTTGTAAAG-3' (F) | 7,841–7,860 |
| | 5'-ACAGGTGTACCTTTTGACATGC-3' (R) | 10,030–10,031 |
| | 5'-GTACAGTCTCTAATATGCTGAGC-3' (F) | 11,767–11,788 |
| | 5'-AGTGTGTTCGAGATCATCAG-3' (R) | 14,164–14,145 |
| | 5'-GTGTCACTGGCTATTGACGTG-3' (F) | 15,948–15,967 |
| | 5'-AGTGGTTTTGCGAGATCAGC-3' (R) | 18,119–18,100 |
| | 5'-GCTATCGTGAAGCAGCTTGC-3' (F) | 19,836–19,856 |
| | 5'-GTTATCGAGCATTTCCTCGC-3' (R) | 22,177–22,157 |
| | 5'-CGATTTCGGTGATTTCGTAC-3' (F) | 23,771–23,790 |
| | 5'-AACTCAGGTTCCCAGTACCG-3' (R) | 25,864–25,845 |
| | 5'-CACATGGGGATAGCACTACT-3' (F) | 27,551–27,570 |
| | 5'-CACATGGGGATAGCACTACT-3' (R) | 29,700–29,681 |

| Reaction T2 | 5'-TCCTGATTTGCAAAGAGCAGC-3' (F) | 1,941–1,962 |
| | 5'-TGCACACTCATAGAAGGATGC-3' (R) | 4,058–4,039 |
| | 5'-GCTTACTACAGAGCAAGGCC-3' (F) | 5,908–5,927 |
| | 5'-GAGCTGTGAAGAATGCTTGCC-3' (R) | 8,044–8,025 |
| | 5'-GCTATCGTGAAGCAGCTTGC-3' (F) | 9,869–9,888 |
| | 5'-GTATGTCGACCTTTGCTCCGC-3' (R) | 12,006–11,987 |
| | 5'-CGATTTCGGTGATTTCGTAC-3' (F) | 14,021–14,040 |
| | 5'-AACACTGCCGTCTTCCAGACCG-3' (R) | 16,129–16,110 |
| | 5'-CACACAATCCTGAAACTACG-3' (F) | 17,804–17,824 |
| | 5'-CATTGACGTAGCTTGTGCTC-3' (R) | 20,040–20,021 |
| | 5'-GCATTAAAATCTGCTCAGGAGC-3' (F) | 21,956–21,977 |
| | 5'-CTAGGCCTCGCCATATTGCCC-3' (R) | 23,961–23,942 |
| | 5'-GTTTGGCGTTTGACATGCAGAC-3' (F) | 25,647–25,666 |
| | 5'-CAATGAAAGATTTCTAGTGC-3' (R) | 27,794–27,774 |

nt = Nucleotide; F = forward primer; R = reverse primer.
amplified in a different reaction using primer pair t2, t4, t6, t8, t10, t12 and t14, respectively. The primers of t1, t2, t3, t4,...t15 are shown in table 3.

The multiplex one-step RT-PCR was performed using a commercial kit and the procedure used was recommended by the manufacturer (Qiagen One-Step RT-PCR Kit, Qiagen Inc.). Briefly, 50 µl of reaction solution contains 1–2 µl of RNA solution from the clinical extract, 0.6 µl of enzyme mix, 6 units Rnase inhibitor. Sample mixtures were placed in a thermal cycler with a temperature at 45°C for 30 min, and then incubated at 95°C for 15 min before PCR amplification. The PCR amplification consisted of 3 steps including denaturation at 94°C for 10 s, annealing at 50 or 54°C for 1 min, and extension at 68°C for 2.5 min. The 3 steps were repeated for 40 cycles. The nested PCR was performed as follows: 50 µl of reaction solution contains 1 µl of multiplex PCR product, 0.2 µM of each nested PCR primer, 0.2 µM of each dNTP, 1 × Taq buffer, and 1 unit Taq polymerase. The PCR condition included denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 2 min, and these 3 steps were repeated for 35 cycles. The PCR products were resolved on agarose gels, and the proper PCR fragments were excised from gels and then purified using a commercial kit (Qiagel II Gel Extraction Kit, Qiagen Inc.). DNA sequencing was performed using the di-deoxy chain termination method as described in Big Dye™ Terminator cycle sequencer and using an ABI 310 machine (Applied Biosystems Inc., Calif., USA). The primers used for sequencing will be provided on request. Phylogenetic analysis of the SARS-CoV genomes was carried out by ClustalW (http://www.ebi.ac.uk/clustalw/index.html).

### Results

RT-PCR analytic strategy for SARS-CoV is shown in figure 1. The PCR product of SARS-CoV is a fragment of 368 bp using specific primers (fig. 2). Among 87 cases of SARS suspects examined, 3 were positive for the SARS-CoV. These 3 cases were further used for sequencing analysis of entire genomes.

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**Table 3. The SARS-CoV primers used for nested-PCR**

| Name | Sequences Location (nt) |
|------|-------------------------|
| t1   | 5'-GTGATAGCTTTCTCTAACCAG-3' (F) | 50–69 |
| t2   | 5'-TCCATTTAAGAGTGAAGCC-3' (R) | 2,155–2,136 |
| t3   | 5'-AGTACTTTCTCCAAGTGTCG-3' (R) | 1,992–2,001 |
| t4   | 5'-GAAGCTGCGGAGTTCCTATTG-3' (F) | 3,903–3,922 |
| t5   | 5'-GAGCCTCGAAGACAGGACG-3' (R) | 7,875–7,894 |
| t6   | 5'-GACCTGGCGAGACAGGACG-3' (R) | 9,990–9,971 |
| t7   | 5'-AACATAGCTTTCTCAGGACG-3' (R) | 9,913–9,932 |
| t8   | 5'-AGGTAGCTTTCTCAGGACG-3' (R) | 11,933–11,914 |
| t9   | 5'-AAGTGGGAGGAGTGAAGCC-3' (R) | 11,793–11,802 |
| t10  | 5'-AACATAGCTTTCTCAGGACG-3' (R) | 14,051–14,060 |
| t11  | 5'-CAACATAGCTTTCTCAGGACG-3' (R) | 16,070–16,051 |
| t12  | 5'-CATCCTAATCAGGAGTACG-3' (F) | 19,874–19,894 |
| t13  | 5'-CTGTAGGAGGAGTGAAGCC-3' (R) | 22,126–22,107 |
| t14  | 5'-CTATGTATAGCCTTGTTCC-3' (F) | 21,980–21,999 |
| t15  | 5'-CTATGTATAGCCTTGTTCC-3' (F) | 23,940–23,921 |
| t16  | 5'-CGTCTTCTTTATGGGAGTGAAGCC-3' (F) | 23,881–23,901 |
| t17  | 5'-TGAGCTCGGAGGAGTGAAGCC-3' (F) | 25,790–25,771 |
| t18  | 5'-GCCACTTTCTCAGGACG-3' (R) | 26,695–25,715 |
| t19  | 5'-TGAGCTCGGAGGAGTGAAGCC-3' (R) | 27,750–27,731 |
| t20  | 5'-TGAGCTCGGAGGAGTGAAGCC-3' (R) | 27,579–27,598 |
| t21  | 5'-TAGGGCTCCTCTCATAGGACG-3' (R) | 29,662–29,643 |

nt = Nucleotide; F = forward primer; R = reverse primer.

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Fig. 2. The results of RT-PCR analysis of SARS-CoV are shown. The positive case had a PCR fragment of 368-bp. Lane 1 is a positive control; lane 2 is a negative control; lanes 3–5 are the 3 positive cases collected in Taichung, Taiwan; lanes 6 and 7 are negative cases. M = 100-bp ladder markers.

Table 4. The sequence variants of SARS-CoV in Taiwan area

| Origin      | Location (nt)   |
|-------------|-----------------|
| TWC2        | T   A   C   C   T   C   C   A   T   A   C   C   C   G   C   A   A   T   T   T   C   C |
| TWC3        | C   A   C   C   T   C   C   A   T   A   C   C   C   G   C   A   A   T   T   T   C   C |
| TWY         | C   A   C   C   T   C   T   A   T   A   C   C   T   G   C   A   A   T   T   T   T   C |
| TWS         | C   A   C   C   T   C   C   A   T   A   C   T   C   T   G   C   A   A   A   T   T   T   T   C |
| TWK         | C   A   C   C   T   C   C   A   T   A   C   T   T   G   C   A   A   T   T   T   T   T   T |
| TWJ         | C   A   C   C   T   C   C   A   C   –   C   T   T   G   C   A   –   –   T   T   T   C |
| TWJ         | T   A   C   C   T   C   C   A   T   A   C   C   C   G   C   A   A   T   T   T   T   C |
| TC1         | C   A   C   C   T   C   C   A   T   A   C   C   C   G   C   A   A   T   T   T   C   C |
| TC2         | C   A   C   C   T   C   T   C   T   C   A   T   A   C   T   G   C   A   A   A   T   T   T   T |
| TC3         | C   A   C   C   T   C   C   A   T   A   C   C   C   T   G   C   A   A   C   T   T   T   T |
| TWC        | C   A   T   C   C   C   G   T   A   C   C   C   C   T   T   A   A   T   –   –   C   C |
| TW1         | C   G   T   C   C   C   A   A   T   A   C   C   C   C   T   C   A   A   T   T   T   C   C |

TC1 (clinical specimen) and TWC (cultural specimen) is the first case in Taiwan area; TC2 and TC3 from mid-Taiwan area; TW1, TWC2, TWC3, TWY, TWS, TWK, TWJ and TWH from northern part of Taiwan.

Table 5. Comparison of the sequence variants between cultural isolates and clinical specimens in Taiwan area

| Samples     | Location (nt)   |
|-------------|-----------------|
|             | 1,782 3,165 3,852 8,160 11,493 13,098 16,325 26,203 26,477* 26,600* 27,812 |
| Clinical specimen  | |
| TC1         | C   A   C   C   T   C   C   A   C   G   C   C |
| TC2         | C   A   C   C   T   T   T   T   A   T   G   C   T |
| TC3         | C   A   C   C   T   C   C   A   T   G   C   T |
| TWC3        | C   A   C   C   T   C   C   A   C   G   C   C |

Cultural isolates

| TWC*        | C   A   T   C   C   C   G   C   T   T   C |
| TWC2        | T   A   C   C   T   C   C   A   C   G   C   C |
| TW1         | C   G   T   C   C   C   A   C   T   C   C |

* The base substitution causes amino acid change. nt 26,477 G→T (Cys 27 Phe, M protein); nt 26,600 C→T (Ala 681/Val1, M protein). + A two-base deletion (TT) at 27,808–27,809 was not shown.
The whole SARS-CoV genome was amplified by multiplex RT-PCR in two separate reactions, followed by 15 different nested PCRs to amplify a 2-kb fragment in each reaction that cover the whole SARS-CoV genome (fig. 3). These 15 nested PCR products were subjected for direct sequencing. The results were submitted to GenBank, and the accession numbers are AY348314 for TC3, AY338175 for TC2 and AY338174 for TC1.

The genomic sequences of these clinical specimens were similar to those reported from other groups. We compared those genomic and found there were 20 differences, which are 17 single nucleotide variations, two 2-nucleotide deletions and a 1-nucleotide deletion (table 4). We further compared the complete genomic sequences of four clinical specimens with the complete genomic sequences of three cultural isolates in Taiwan. The differences are shown in table 5. In total, there were 11 single nucleotide sequence variations, and one deletion of two nucleotides in the non-coding region. Of the 11 base substitutions, 9 did not alter the original amino acid coding, and the other 2 changed the amino acid sequence. The two missense mutations were all located at the M protein coding area, which were Cys 27 Phe for nt 26477 G→T, and Ala 68 Val for nt 26600 C→T, and appeared only in the cultural isolates. In addition, the two-base deletion mutations were found in the cultural isolates. Of the 11 variants in these samples, two loci (positions 26,203 and 27,812) were identified, and they were segregated together as a specific genotype – T:T or C:C (table 6). The C:C genotype was linked to infection originated at the Amoy Garden in Hong Kong; the T:T genotype was linked to infection acquired from Hoping Hospital in Taipei, Taiwan.

The phylogenetic analysis is shown in figure 4. We analyzed the cases associated with the Hotel M of Hong Kong and the cases in Taiwan. Two major clusters were observed after the cases were analyzed.
Discussion

We developed a rapid method to sequence and analyze the whole genome of SARS-CoV from clinical specimens. It has been reported that some pathogenic microbes undergo adaptation in response to laboratory cultivation. Host-mediated mutations in several viruses have been documented, such as HIV, Japanese encephalitis, hepatitis A, Sendai and influenza A viruses [17–20]. Molecular evolution studies using such sequences may be at risk of the data containing laboratory factors [16]; the analytic data that do not represent random samples of natural pathogen populations or the sampling design is unknown. In this study, we can directly detect the whole genome of SARS-CoV from clinical samples, so that it will avoid the bias mentioned above. Although TC1 (a clinical specimen) and TWC (an isolate) were from the same patient, phylogenetic analysis revealed that they were located at different positions of the phylogenetic tree. Our results confirmed that the sequences of a cultural isolate may be different from those of a clinical specimen although they are derived from the same origin. We analyzed the sequences of the variant observed in cultural isolates and clinical specimens which were published in GenBank. Interestingly, we found that the nt 3,852 C, 11,493 T and 26,477 G appeared in all the clinical specimens, but only in two of 23 cultural isolates. The nt 3,852 T, 11,493 C and 26,477 T were found in all the cultural isolates except...
TWC2 and in none of clinical specimens (table 5). From these discrepancies, these mutations may be most likely due to cultural adaptation, but we are unable to completely exclude the possibility of the shifting of two original different viruses during culturing process.

In clinical specimens or cultural isolates, two variants segregated tightly, nt 26,203 C with 27,812 C (C:C) and 26,203 T with 27,812 T (T:T). The C:C type appeared in TC1 and TWC3, and all the isolates in other infection areas of the world. The T:T type appeared only in the Taiwan area. From these results, the SARS infection in Taiwan could be from two origins. One is from either the Amoy Garden or Hotel M in Hong Kong, the other has yet to be identified.

There were 4 sequence variations (nt 8,160, 13,098, 26,203, 27,812) in clinical specimens. All of the variations did not change the amino acid coding, indicating that the SARS-CoV maintains a stable structure, and it may favor the development of SARS-CoV vaccine. In the cultural isolates, we found 2 missense mutations that were all located at the M protein coding region. These mutations may be necessary for cultural adaptation. They may also appear in the clinical specimens, caused by other factors such as immunological adaptation. The M protein of SARS-CoV may play an important role in the immune response of infected patients.

Multiplex RT-PCR methods have been used to study the expression of more than ten genes simultaneously [21], to detect pathogens or subtypes [22, 23], and to verify multiple chromosome translocations [24]. In order to increase the specificity, a nested PCR [22] or a probe [25], enzyme hybridization methods [26], or microarray [27] have been used. In this study, we used multiplex RT-PCR to amplify the whole SARS genome in two separate reactions, and the PCR products were further amplified using 15 pairs of nested primers so that the products covered the whole genome in 15 separate tubes. The 15 PCR products were then sequenced. A similar approach has been used to analyze the HN gene of human parainfluenza virus [28], however, only a small portion of human parainfluenza genomes was analyzed. In contrast to this report, we analyze the whole genome of the biggest RNA virus. Although multiple RT-PCR to amplify the whole SARS genome can be used as a detection method, it is difficult to evaluate the quantitative change in the clinical specimens. So, we first amplified the Pol region of SARS virus to detect the virus and evaluate the quantitative changes of SARS virus, and then used multiplex RT-PCR and direct sequencing to analyze the whole genome.

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**References**

1. WHO: Summary table of SARS cases by country, November 1, 2002–August 7 2003. http://www.who.int/csr/sars/country/2003-07-11/en/
2. Reilly B, Van Herp M, Servand D, Dentico N: SARS and Carlo Urbani. N Engl J Med 2003;348:1951–1952.
3. Peiris JS, Lai ST, Poon LL, Guan Y, Yam LY, Lim W, Nicholls J, Yee WK, Yan WW, Cheung MT, Cheng VC, Chan KH, Tsang DN, Ying RW, Ng TK, Yuen KY; SARS study group: Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003;361:1319–1325.
4. Lee N, Hui D, Wu A, Chan P, Cameron P, Joynt GM, Akuja A, Yung MY, Leung CB, To KF, Lui SF, Szeto CC, Chung S, Sung JF: A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med 2003;348:1986–1994.
5. Tsang KW, Ho PL, Ooi GC, Yee WK, Wang T, Chan-Yeung M, Lam WK, Seto WH, Yam LY, Cheung TM, Wong PC, Lam B, Ip MS, Chan J, Yuen KY, Lai KN: A cluster of cases of severe acute respiratory syndrome in Hong Kong. N Engl J Med 2003;348:1977–1985.
6. Poutanen SM, Low DE, Henry B, Finkelstein S, Rose D, Green K, Tellier R, Draker R, Ada-ichi D, Ayers M, Chan AK, Skowronski DM, Salit I, Simon AE, Slutsky AS, Doyle PW, Krajden M, Petric M, Brunham RC, McGeer AJ: National Microbiology Laboratory, Canada; Canadian Severe Acute Respiratory Syndrome Study Team: Identification of severe acute respiratory syndrome in Canada. N Engl J Med 2003;348:1995–2005.
7. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, Tong S, Urbani C, Cooper JA, Lim W, Rollin PE, Dowell SF, Ling AE, Humphrey CD, Shih WJ, Guarnier J, Paddock CD, Rota P, Fields B, DeRisi J, Yang YJ, Cox N, Hughes JM, LeDuc JW, Bellini WJ, Anderson LJ: SARS Working Group: A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003;348:1953–1966.
8. Drosten C, Guntner S, Preiser W, van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RA, Berger A, Burguereau AM, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra JC, Muller S, Rickers V, Sturmer M, Vieth S, Klenk HD, Osterhaus AD, Schmitz H, Doerr HW: Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 2003;348:1967–1976.
