Journal of Medical Virology 74:517–520 (2004)

Evaluation of a Peptide-Based Enzyme Immunoassay for Anti-SARS Coronavirus IgG Antibody

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High throughput assays for anti-SARS-CoV IgG antibody detection are need for large-scale epidemiologic studies. The performance of a microplate enzyme immunoassay, DETECT-SARS™, was evaluated for the detection of anti-SARS-CoV IgG antibody. This assay is based on synthetic peptides derived from the nucleocapsid and spike proteins. The results showed that the assay provided a high degree of sensitivity (95.9%) for convalescent serum samples. The level of specificity was close to 90%, and did not show significant variation among different control groups. The high degree of sensitivity together with the high-throughput nature makes it advantageous as a screening assay for studies where handling of a large number of specimens is required. ***J. Med. Virol. 74:517–520, 2004.***

KEY WORDS: coronavirus; enzyme immunoassay; IgG; SARS; serology

INTRODUCTION

A novel infection resulting in severe acute respiratory syndrome (SARS) emerged at the Quangdong province of southern China in November 2002. By March 2003, the infection had spread to Hong Kong, Vietnam, Singapore, Toronto, Taiwan, and to many other cities. A total of 8,098 cases and 774 deaths had been reported to the World Health Organization during the worldwide outbreak, which halted in July 2003 [World Health Organization, 2003a]. The culprit agent is a new coronavirus, SARS-CoV, which is distinct phylogenetically from the three groups of previous known coronaviruses [Drosten et al., 2003; Kuiken et al., 2003; Marra et al., 2003; Peiris et al., 2003b; Poutanen et al., 2003; Rota et al., 2003]. To improve our understanding on this novel devastating human infection, accurate diagnostic tools are indispensable. At present, the availability of high-throughput serological assay for SARS-CoV is still limited. This remains a major hindrance for large-scale epidemiologic study. The evaluation of a commercial assay, DETECT-SARS™ kit (Adaltis, Italia), which is a Conformité Européenne (CE)-marked indirect microplate enzyme immunoassay for the detection of anti-SARS-CoV immunoglobulin (Ig)G antibody from human serum or plasma specimens, is described.

MATERIALS AND METHODS

Study Population and Samples

Non-SARS pneumonia controls. Two hundred and fifty convalescent serum specimens collected from patients admitted to the Prince of Wales Hospital in 2000 for fever or pneumonia were used as non-SARS pneumonia controls. Of these, 50 were from pediatric patients aged 1–16 years (mean: 6.2; SD: 4.5) with 44% girls, 150 were from adults aged 16–64 years (mean: 42.7, SD: 14.8) with 46% female, and 50 were from the elderly aged 65–104 years (mean: 78.5, SD: 8.2) with 42% women.

Non-SARS healthy controls. Two hundred serum specimens that had been collected from medical students in 2000 for pre-varicella vaccination check were used as non-SARS healthy controls. Their ages ranged from 19 to 31 years (mean: 22.6, SD: 1.5) with 55.0% female.

SARS group. The SARS group comprised of 125 SARS patients, aged 21–89 years (mean: 41.1, SD: 16.4)

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Accepted 19 July 2004
DOI 10.1002/jmv.20207
Published online in Wiley InterScience (www.interscience.wiley.com)

with 57.6% female. All these patients presented with an acute onset of fever and progressed to pneumonia, which was otherwise unexplained. They all had seroconversion or fourfold rise in anti-SARS-CoV IgG antibody titre as detected by the immunofluorescence assay as described previously [Chan et al., 2004a]. All these patients fulfilled the United States Centers for Disease Control and Prevention (US CDC) and the World Health Organization (WHO) criteria for SARS [World Health Organization, 2003b; Centers for Disease Control and Prevention, 2004]. Their convalescent blood samples collected at ≥21 days after the onset of illness were retrieved for this study. In addition, 40 had an early blood sample collected between 7 and 14 days after the onset of illness available for this study.

Enzyme-Linked Immunosorbent Assay (EIA)

The DETECT-SARS™ kit incorporates synthetic peptides derived from epitopes of the nucleocapsid and spike proteins of SARS-CoV. In the first phase of development of this kit, the amino acid sequences of the nucleocapsid and spike proteins as predicted from the published sequence of SARS-CoV (GenBank Accession number: NC_004718) were first screened for regions showing high indices of hydrophilicity (Kyle-Doolittle index), antigenicity (Jameson–Wolf index), and surface probability (Emini index). As a result, 20 deduced peptides were synthesized chemically using the 9-fluorenylmethoxycarbonyl (F-MOC) synthesis. These peptides were coated individually on microplates and evaluated for their sensitivity and specificity for detecting anti-SARS-CoV IgG antibodies. A panel consisting of specimens collected from 55 SARS-convalescent patients, 22 patients affected by other respiratory diseases and 200 healthy donors were used to select the most appropriate peptides. Among the 20 peptides tested, one spike peptide close to the N-terminus and two nucleocapsid peptides located respectively at the N- and C-terminus were found to be the best. These three peptides were used in the DETECT-SARS™ kit.

In this study, all the assay procedures were carried out according to the DETECT-SARS™ kit's protocol. Briefly, peptides coated on microplate were allowed to bind with SARS-CoV-specific antibodies present in serum specimens. After a washing step, horseradish peroxidase-labeled goat antibodies specific for human IgG were added. The antigen–antibody complex was then detected by an enzyme substrate added after the second washing step. The results were interpreted according the manufacturer's criteria and were expressed as index values (ratio of absorbance over the cutoff value). Specimens with index values = 1.0 were regarded as "reactive" for anti-SARS-CoV IgG. Index values <0.5 were retested in duplicate. The average index value obtained from the repeat testing was used to classify the status of the specimen. Specimens with an average index remained between 0.5 and 1.0 were regarded as "equivocal."

An in-house indirect immunofluorescence assay for anti-SARS-CoV IgG based on virus-infected cells was used as a reference in this study. This assay has been shown to be highly sensitive and specific. The testing procedure was as described previously [Chan et al., 2004a].

RESULTS

Performance of EIA on Non-SARS Groups

Of the 450 non-SARS specimens, 53 were found to be reactive and 12 were found to be equivocal for anti-SARS-CoV IgG antibody using the DETECT-SARS™ kit (Table I). All these specimens were tested negative by the immunofluorescence assay indicating true-negative specimens. The proportion of specimens showing equivocal results ranged from 0 to 6.0%, and no significant difference was observed among the various control groups ($P = 0.006$ by Chi-square test). The specificity ranged from 78.7 to 90.6%, and no significant difference was observed among the various control groups ($P = 0.119$ by Chi-square test). The absorbance indices of all samples tested are shown in Figure 1. The majority (91%) of the false-positive results were in the low range of indices (from 1.0 to 5.0).

Performance of EIA on SARS Group

The results for the SARS group are shown in Table I. The sensitivity of the DETECT-SARS™ kit for detecting anti-SARS-CoV IgG antibody from convalescent specimens collected ≥21 days after onset of illness was 95.9%. Of the 40 early serum specimens, 15 were tested reactive. The absorbance indices of all specimens tested are shown in Figure 1. Majority of the true-positive

TABLE I. Results of Anti-SARS-IgG for Non-SARS Control Groups

Sample status	No. tested	Reactive	Equivocal (%)	Sensitivity (%)	Specificity (%)
Non-SARS group					
Pediatric pneumonia	50	10	3 (6.0)	—	78.7
Adult pneumonia	150	17	1 (0.6)	—	88.6
Elderly pneumonia	50	8	0	—	84.0
Healthy adults	200	18	8 (4.0)	—	90.6
Overall	450	53	12 (2.7)	—	87.9
SARS group					
Convalescent specimens (≥21 days)	125	116	4 (3.2)	95.9	—
Early specimens (≤14 days)	40	15	1 (2.5)	38.5	—

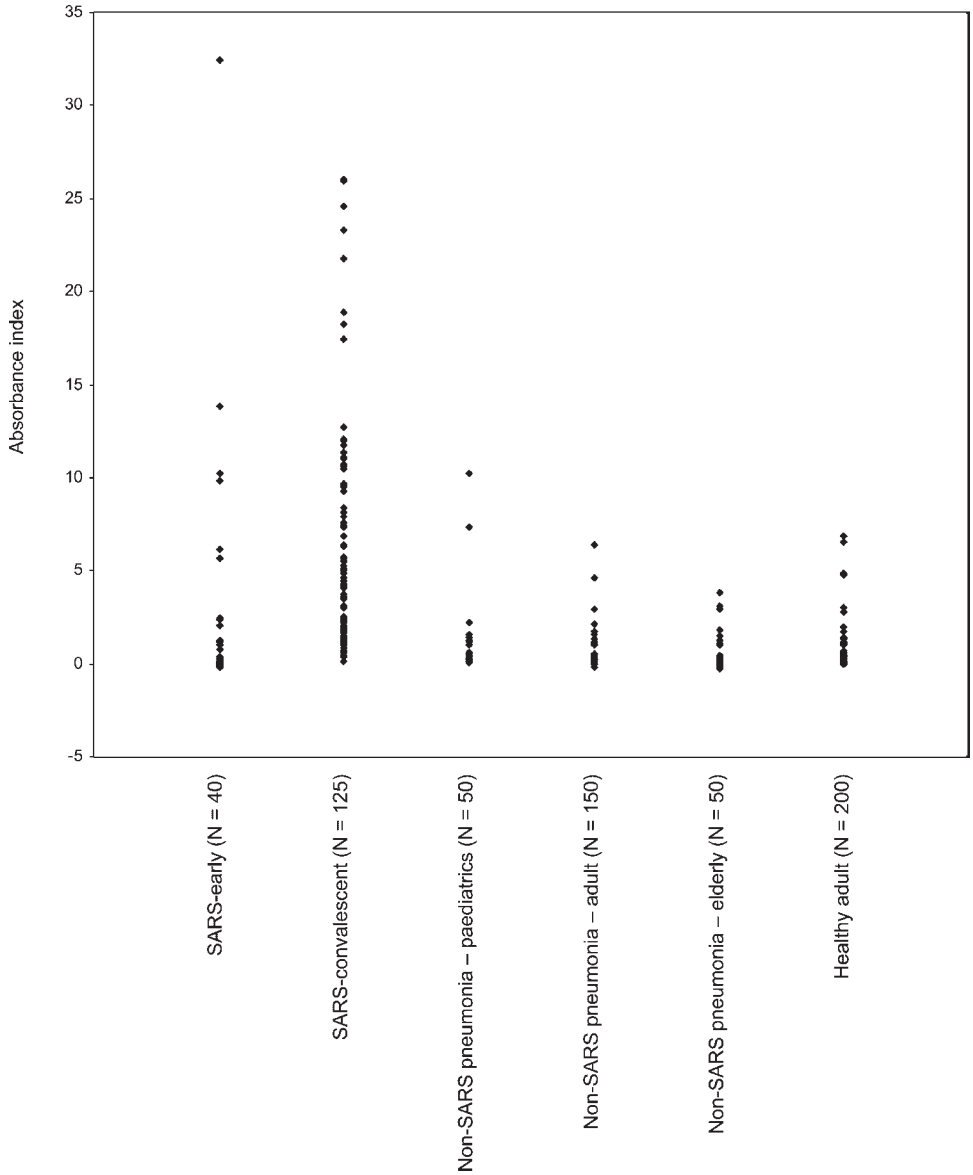

Fig. 1. Absorbance indices for anti-SARS-IgG according to specimen category.

results were in the high range of indices (>5.0). The positive rates according to the time of specimen collection are shown in Figure 2.

DISCUSSION

The results of this study showed that the DETECT-SARS™ kit had a high degree of sensitivity (95.9%) for detecting anti-SARS-IgG antibody from convalescent blood specimens. In the case of specimens collected during the acute phase of disease (day 7–14), the sensitivity of the assay increased from 20% (day 7) to 75% (day 14); it is worth noting that these numbers are similar to those reported for RT-PCR assays applied on respiratory specimens [Peiris et al., 2003a; Chan et al., 2004b; Tang et al., 2004]. The sensitivity of DETECT-SARS™ kit was found to be within the reported range of other

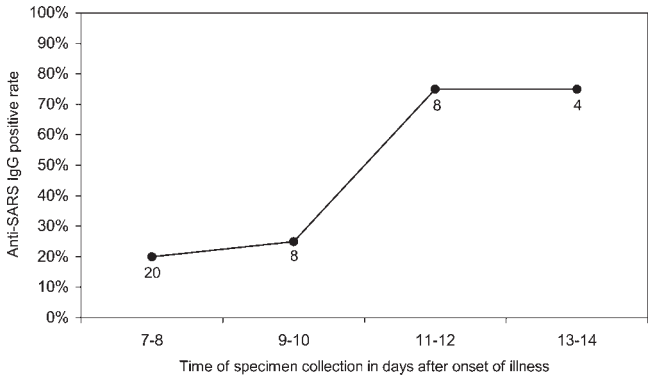

Fig. 2. Anti-SARS-CoV IgG positive rate according to specimen collection time. The number of specimens tested is shown below each point.

enzyme immunoassays that based on purified virions [Chen et al., 2004a], whole viral lysate [Tang et al., 2004], and recombinant proteins [Guan et al., 2004]. The DETECT-SARS^{TM} kit also provided an acceptable specificity close to 90%. There was no significant difference between the reactivity rates of healthy medical students and pneumonia patients, suggesting that nonspecificity was not due to crossreactivity with a pneumonia pathogen. It was found that serum specimens collected from children tended to have a higher chance of showing equivocal or nonspecific reactivity, though it did not reach statistical significance, this observation deserves further investigation. Specimens found reactive by the DETECT-SARS^{TM} kit should be confirmed by another assay with higher specificity.

In comparison with the indirect immunofluorescence assay, the DETECT-SARS^{TM} kit being in a semi-automated microplate format allows a high-throughput performance. This feature together with the high degree of sensitivity makes it an advantage for epidemiological studies where handling of a large number of specimens is required.

Recent studies have shown that the immunodominant epitopes of spike protein to be located between amino acids 441 and 700 [Lu et al., 2004]; and those of nucleocapsid protein are located at amino acids 51–71, 134–208, 249–273, and 349–422 [Chen et al., 2004b]. These data on the immunogenicity of SARS-CoV proteins would be very useful for improving the performance of serological assays.

ACKNOWLEDGMENTS

PKS Chan, JS Tam, JJY Sung received support from the Research Fund for the Control of Infectious Diseases (RFCID) from the Health, Welfare and Food Bureau of the Hong Kong SAR Government.

REFERENCES

Centers for Disease Control and Prevention. 2004. Severe acute respiratory syndrome. http://www.cdc.gov/ncidod/sars/guidance/B/pdf/app1.pdf (assessed on 3 March 2004).

Chan PK, Ng KC, Chan RC, Lam RK, Chow VC, Hui M, Wu A, Lee N, Yap HY, Cheng FW, Sung JJ, Tam JS. 2004a. Immunofluorescence assay for serologic diagnosis of SARS. Emerg Infect Dis 10:530–532.

Chan PK, To WK, Ng KC, Lam RK, Ng TK, Chan RC, Wu A, Yu WC, Lee N, Hui DS, Lai ST, Hon EK, Li CK, Sung JJ, Tam JS. 2004b. Laboratory diagnosis of SARS. Emerg Infect Dis 10:825–831.

Chen X, Zhou B, Li M, Liang X, Wang H, Yang G, Wang H, Le X. 2004a. Serology of severe acute respiratory syndrome: Implications for surveillance and outcome. J Infect Dis 189:1158–1163.

Chen Z, Pei D, Jiang L, Song Y, Wang J, Wang H, Zhou D, Zhai J, Du Z, Li B, Qiu M, Han Y, Guo Z, Yang R. 2004b. Antigenicity analysis of different regions of the severe acute respiraotyr syndrome coronavirus nucleocapsid protein. Clin Chem 50:988–995.

Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RA, Berger A, Burguiere AM, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra JC, Muller S, Rickerts V, Sturmer M, Vieth S, Klenk HD, Osterhaus AD, Schmitz H, Doerr HW. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. New Eng J Med 348:1967–1976.

Guan M, Chen HY, Foo SY, Tan YJ, Goh PY, Wee SH. 2004. Recombinant protein-based enzyme-linked immunosorbent assay and immunochromatographic tests for detection of immunoglobulin G antibodies to severe acute respiratory syndrome (SARS) coronavirus in SARS patients. Clin Diag Lab Immunol 11:287–291.

Kuiken T, Fouchier RA, Schutten M, Rimmelzwaan GF, van Amerongen G, van Riel D, Laman JD, de Jong T, van Doornum G, Lim W, Ling AE, Chan PK, Tam JS, Zambon MC, Gopal R, Drosten C, van der Werf S, Escriou N, Manuguerra JC, Stohr K, Peiris JS, Osterhaus AD. 2003. Newly discovered coronavirus as the primary cause of severe acute respiratory syndrome. Lancet 362:263–270.

Lu L, Manopo I, Leung BP, Chng HH, Ling AE, Chee LL, Ooi EE, Chan SW, Kwong J. 2004. Immunological characterization of the spike protein of the severe acute respiratory syndrome coronavirus. L Clin Microbiol 42:1570–1576.

Marra MA, Jones SJ, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YS, Khattra J, Asano JK, Barber SA, Chan SY, Cloutier A, Coughlin SM, Freeman D, Girn N, Griffith OL, Leach SR, Mayo M, McDonald H, Montgomery SB, Pandoh PK, Petrescu AS, Robertson AG, Schein JE, Siddiqui A, Smailus DE, Stott JM, Yang GS, Plummer F, Andonov A, Artsob H, Bastien N, Bernard K, Booth TF, Bowness D, Czub M, Drebot M, Fernando L, Flick R, Garbutt M, Gray M, Grolla A, Jones S, Feldmann H, Meyers A, Kabani A, Li Y, Normand S, Stroher U, Tipples GA, Tyler S, Vogrig R, Ward D, Watson B, Brunham RC, Krajden M, Petric M, Skowronski DM, Upton C, Roper RL. 2003. The Genome sequence of the SARS-associated coronavirus. Science 300:1399–1404.

Peiris JS, Chu CM, Cheng VC, Chan KS, Hung IF, Poon LL, Law KI, Tang BS, Hon TY, Chan CS, Chan KH, Ng JS, Zheng BJ, Ng WL, Lai RW, Guan Y, Yuen KY, HKU/UCH SARS Study Group. 2003a. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: A prospective study. Lancet 361:1767–1772.

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, Yung RW, Ng TK, Yuen KY, SARS study group. 2003b. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 361:1319–1325.

Poutanen SM, Low DE, Henry B, Finkelstein S, Rose D, Green K, Tellier R, Draker R, Adachi D, Ayers M, Chan AK, Skowronski DM, Salit I, Simor AE, Slutsky AS, Doyle PW, Krajden M, Petric M, Brunham RC, McGeer AJ. 2003. Identification of severe acute respiratory syndrome in Canada. New Engl J Med 348:1995–2005.

Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, Penaranda S, Bankamp B, Maher K, Chen MH, Tong S, Tamin A, Lowe L, Frace M, DeRisi JL, Chen Q, Wang D, Erdman DD, Peret TC, Burns C, Ksiazek TG, Rollin PE, Sanchez A, Liffick S, Holloway B, Limor J, McCaustland K, Olsen-Rasmussen M, Fouchier R, Gunther S, Osterhaus AD, Drosten C, Pallansch MA, Anderson LJ, Bellini WJ. 2003. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science 300: 1394–1399.

Tang P, Louie M, Richardson SE, Smieja M, Simor AE, Jamieson F, Fearon M, Poutanen SM, Mazzulli T, Tellier R, Mahony J, Loeb M, Petrich A, Chernesky M, McGeer A, Low DE, Phillips E, Jones S, Bastien N, Li Y, Dick D, Grolla A, Fernando L, Booth TF, Henry B, Rachlis AR, Matukas LM, Rose DB, Lovinsky R, Walmsley S, Gold WL, Krajden S, Ontario Laboratory Working Group for the Rapid Diagnosis of Emerging Infections. 2004. Interpretation of diagnostic laboratory tests for severe acute respiratory syndrome: The Toronto experience. Can Med Assoc J 170:47–54.

World Health Organization. 2003a. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003 (revised 25 September 2003). http://www.who.int/csr/sars/country/table2003_09_23/en/print.html (assessed on 30 December 2003).

World Health Organization. 2003b. Case definitions for surveillance of severe acute respiratory syndrome (SARS). http://www.who.int/csr/sars/casedefinition/en/print.html (assessed on 14 June 2003).