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

Paul K.S. Chan,1,2* W.K. To,2 Esther Y.M. Liu,2 T.K. Ng,2 John S. Tam,1,2 Joseph J.Y. Sung,1 Jean-Michel Lacroix,4 and Michel Houde4

1Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China
2Department of Microbiology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China
3Department of Pathology, Princess Margaret Hospital, Kowloon, Shatin, New Territories, Hong Kong SAR, China
4Adaltis Development, Inc., Montreal, Quebec, Canada

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-SARSTM, 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.

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

INTRODUCTION

A novel infection resulting in severe acute respiratory syndrome (SARS) emerged at the Guangdong 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; Kuijken 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-SARSTM kit (Adaltis, Italia), which is a Conformité Européenne (CE)-marked indirect microplate enzyme immunoassay for the detection of anti-SARS-CoV immunoglobulin (IgG 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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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–Wolff 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-SARSTM 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

| 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           | —              |
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

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

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™ 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™ kit should be confirmed by another assay with higher specificity.

In comparison with the indirect immunofluorescence assay, the DETECT-SARS™ 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.

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