Application of the Dengue Virus NS1 Antigen Rapid Test for On-Site Detection of Imported Dengue Cases at Airports

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We used the dengue virus NS1 antigen (Ag) rapid test for on-site detection of imported dengue cases at airports. Among 22 positive cases of dengue identified from 850 patients with a fever suspected to have dengue, 17 were NS1 Ag test positive. These findings demonstrate the usefulness of the NS1 Ag rapid test in screening imported dengue cases at airports.

Rapid and accurate detection of dengue virus (DENV) infection from acute-phase viremic blood samples from patients with a fever contributes greatly to patient management in hospitals and control measures in public health. In addition, rapid detection of imported dengue cases at airports can help to reduce the annual local outbreaks in a country where dengue is not endemic, such as Taiwan. We have previously reported on screening for fever at airports in Taiwan as part of active surveillance for a panel of notifiable infectious diseases, such as dengue, gastroenteritis caused by enteric bacteria, malaria, and chikungunya (10, 12). A total of 298 imported dengue cases were identified at airports in Taiwan from July 2003 to August 2008 using real-time one-step reverse transcription-PCR (RT-PCR) and envelope/membrane-specific capture immunoglobulin M (IgM) and IgG enzyme-linked immunosorbent assays (ELISAs) (8, 9, 11).

For molecular diagnosis, a real-time one-step RT-PCR was performed using two sets of consensus primers, one primer set targeting a region of the nonstructural protein 5 (NS5) genes to detect all flaviviruses and the other primer set targeting a region of the capsid gene to detect all DENV serotypes. Positive samples were then confirmed by DENV serotyping using four sets of serotype-specific primers targeting the capsid gene to differentiate the DENV serotypes (8, 11). For serological diagnosis, envelope/membrane-specific capture IgM and IgG ELISAs were used to detect and differentiate primary and secondary DENV infections in acute-phase and convalescent-phase serum samples (9, 11). Differentiation of primary and secondary DENV infections was defined by the ratio of the IgM-to-IgG readings, \( \geq 1.2 \) or \( < 1.2 \), respectively. In addition, a simple and sensitive nonstructural protein 1 (NS1) serotype-specific IgG ELISA was used to differentiate the immunological status of individuals into naïve, primary, or secondary DENV infections using acute-phase, convalescent-phase, or postinfection serum samples (3, 9, 11). A unique feature of this assay is that differentiation of primary and secondary DENV infections can be made when DENV-specific IgG antibody has not been produced in the early acute-phase serum samples.

Most of these imported dengue cases were viremic when arriving at airports. It would be desirable to reduce the time gap between clinical and laboratory diagnoses to prevent the local transmission of the imported DENVs. Recent advances on the development of DENV NS1 antigen (Ag) assay offer promising new perspectives on the rapid diagnosis of dengue, although limited sensitivity was reported in patients with acute febrile illness in an area where dengue is endemic (1, 4, 6, 7). A comparison of the sensitivities of two commercial assays to detect DENV NS1 protein using acute-phase serum samples collected 1 to 5 days postonset of illness (DPO) from Puerto Rico suggested that the sensitivities of these test kits need to be improved, especially for patients with secondary infections and a specific DENV serotype (DENV serotype 4 [DENV-4]), whereas the specificities of these kits are excellent (2). More recently, a commercial DENV NS1 Ag strip rapid test kit (Bio-Rad Laboratories, Marnes La Coquette, France) with a lateral-flow immunochromatography format detecting NS1 antigen within 15 to 30 min has become available. An evaluation study based on a panel of 222 serum samples from confirmed dengue cases collected from French Guiana showed 81.0 to 84.8% sensitivity for four different DENV serotypes and 100% specificity, suggesting its potential use as a first-line test in the field (5). Our interest in using the NS1 antigen rapid test kit for on-site detection of imported dengue cases at airports prompted us to conduct an evaluation. We started with a retrospective study to evaluate the sensitivity and specificity of the rapid test kit using a panel of 112 RT-PCR-positive acute-phase serum samples collected from imported dengue cases of 87 primary infections and 25 secondary infections. The DENVs in these serum samples represented a wide variety of DENV strains circulating in Asian countries from 2005 to 2007. The results of tests showed sensitivities of 80.5% and 48.0% for primary and secondary infections, respectively. Among pa-
TABLE 1. Summary of test results and data on 22 imported dengue cases identified by screening individuals for fever at airports in Taiwan

| Case no. | Return date (mo/day) in 2008a | DPO | Countryb | Airport in Taiwan | NS1 Ag rapid test resultc | RT-PCR resultd | IgM/IgG ELISA resulte | Infectionf |
|----------|-------------------------------|-----|----------|------------------|--------------------------|----------------|---------------------|------------|
| 1        | 6/28                          | 2   | Vietnam  | Taoyuan          | +++                       | D1             | –/–                 | Primary    |
| 2        | 7/16                          | 2   | Vietnam  | Taoyuan          | +++                       | D1             | –/–                 | Primary    |
| 3        | 7/22                          | 3   | Thailand | Taoyuan          | +++                       | D1             | –/–                 | Primary    |
| 4        | 7/27                          | 2   | India    | Taoyuan          | +++                       | D1             | –/–                 | Primary    |
| 5        | 7/30                          | 2   | Thailand | Taoyuan          | –                          | D2             | –/–                 | Secondary  |
| 69       | 7/31                          | 6   | Thailand | Taoyuan          | +                          | –/–             | –/–                 | Primary    |
| 7        | 8/01                          | 6   | Vietnam  | Taoyuan          | +++                       | –              | –/–                 | Primary    |
| 8        | 8/04                          | 1   | Thailand | Taoyuan          | +++                       | D1             | –/–                 | Secondary  |
| 9        | 8/10                          | 2   | Thailand | Taoyuan          | +++                       | D1             | –/–                 | Primary    |
| 10       | 8/16                          | 4   | Thailand | Kaohsiung        | +                          | D3             | –/–                 | Primary    |
| 11       | 8/17                          | 2   | Cambodia | Taoyuan          | +++                       | D3             | –/–                 | Primary    |
| 12       | 8/23                          | 7   | Thailand | Taoyuan          | +++                       | –              | +/+/+               | Primary    |
| 13h      | 8/23                          | 1   | Philippines | Kaohsiung       | +                          | –/–             | –/–                 | Primary    |
| 14       | 8/25                          | 1   | Philippines | Taoyuan          | +                          | D3             | –/–                 | Secondary  |
| 15       | 8/25                          | 1   | Indonesia| Taoyuan          | –                          | D1             | –/–                 | Primary    |
| 16       | 8/26                          | 1   | Malaysia | Taoyuan          | +++                       | D1             | –/–                 | Primary    |
| 17       | 8/26                          | 3   | Philippines | Kaohsiung       | +                          | D3             | –/–                 | Primary    |
| 18       | 8/30                          | 4   | Thailand | Taoyuan          | +++                       | –              | +/–                 | Primary    |
| 19       | 9/03                          | 1   | Cambodia | Taoyuan          | –                          | D2             | –/–                 | Primary    |
| 20       | 9/05                          | 2   | Bangladesh| Taoyuan          | +                          | D3             | –/–                 | Primary    |
| 21       | 9/10                          | 4   | Vietnam  | Taoyuan          | –                          | D1             | –/–                 | Primary    |
| 22       | 9/12                          | 4   | Malaysia | Taoyuan          | –                          | D2             | –/–                 | Primary    |

a Date the individual returned to Taiwan or entered Taiwan.

b Country the individual visited or the native country of the individual.

c NS1 Ag rapid test results are shown as follows: +++, strongly positive; ++, moderately positive; +, positive; –, negative.

d RT-PCR results are shown as follows: D1 to D3, dengue virus serotypes 1 to 3, respectively; -, no dengue virus detected.

e IgM and IgG ELISA results are shown as follows: –, negative; +, positive; ++, strongly positive. The IgM ELISA result is shown before the slash, and the IgG ELISA result is shown after the slash.

f Differentiation of primary and secondary DENV infections was based on combined analysis of acute-phase and/or convalescent-phase serum samples by envelope/membrane-specific capture IgM and IgG ELISAs and NS1 serotype-specific IgG ELISA (9).

h IgM seroconversion on day 17 after the onset of illness.

i IgM seroconversion on day 18 after the onset of illness.

Patients with primary infection, DENV-1 was the most sensitive to be detected (97.5% [39/40]), followed by DENV-3 (75% [15/20]), DENV-4 (67% [6/9]), and DENV-2 (56% [10/18]). Further dynamic study using early convalescent-phase serum samples showed positive detection of NS1 antigens up to 9 DPO for primary infection, in contrast to a dramatic decrease in assay sensitivity from 1 to 5 DPO for secondary infection. The specificity of this kit was excellent (100%) when tested on 50 negative acute-phase serum samples.

As dengue is not endemic in Taiwan, most people are naïve to DENV infection. The relative high sensitivity in detecting patients with primary infections and the overall high specificity of the Bio-Rad NS1 Ag strip test kit make it well suited for the detection of imported dengue cases at airports. A pilot study examining the application of the Bio-Rad NS1 Ag strip test for on-site detection of imported dengue cases at airports was started 18 June 2008. By 12 September 2008, we had screened 850 patients with fevers suspected to have dengue. Table 1 shows a summary of data and test results for 22 individuals with acute-phase serum samples that tested positive by combination analysis of RT-PCR, NS1 Ag rapid test, and capture IgM and IgG ELISAs. All of these imported cases had returned from Asian countries, including Thailand (8 cases), Vietnam (4 cases), the Philippines (3 cases), Malaysia (2 cases), Cambodia (2 cases), Indonesia (1 case), India (1 case), and Bangladesh (1 case). Among the 22 positive serum samples, 17 (77.3%) were NS1 Ag test positive, 17 (77.3%) were RT-PCR positive, and 3 were IgM positive. For the five NS1 Ag test-negative serum samples, three cases were travelers returning from Thailand, Cambodia, and Malaysia who were infected with DENV-2, and the other two cases were travelers returning from Indonesia and Vietnam who were infected with DENV-1. A combined analysis of acute-phase and/or convalescent-phase serum samples by envelope/membrane-specific capture IgM and IgG ELISAs and NS1 serotype-specific IgG ELISA showed 19 cases of primary infection (all from Taiwan) and 3 cases of secondary infection (all foreigners; one from Thailand [case 8], and one from the Philippines [case 14], respectively) (9).

These findings demonstrate the usefulness of the NS1 Ag rapid test kit in detecting imported dengue cases at airports. After the successful evaluation, the DENV NS1 rapid test is now routinely applied for on-site detection of imported dengue cases at airports in Taiwan. The Bio-Rad Ag strip rapid test offers several advantages over current routine assays (ELISA and RT-PCR), such as rapidity, simplicity, high sensitivity with a longer detection time (1 to 9 DPO) for primary infection, and excellent specificity (100%). Most important, the patients can be quickly diagnosed as positive and advised to go to hospitals for medical treatment due to the high positive predictive value. No emergency control measures, such as insecticide spray, will be needed. Future improvement of the test sensitivity to detect low levels of NS1 antigens in the acute-phase serum samples from individuals with secondary infections and various DENV
strains of all four serotypes circulating in different geographic areas will make it more reliable when used as a first-line test in the diagnosis of dengue in hospitals and at airports. It is anticipated that if the manufacturer can provide antibodies with increased sensitivity for the detection of all four DENV NS1 antigens, an already valuable test may be improved.

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REFERENCES

1. Alcon, S., A. Talarmin, M. Debruyne, A. Falconar, V. Deubel, and M. Flament. 2002. Enzyme-linked immunosorbent assay specific to dengue virus type 1 nonstructural protein NS1 reveals circulation of the antigen in the blood during the acute phase of disease in patients experiencing primary or secondary infections. J. Clin. Microbiol. 40:376–381.
2. Bessoff, K., M. Delorey, W. Sun, and E. Hunsperger. 2008. Comparison of two commercially available dengue virus (DENV) NS1 capture enzyme-linked immunosorbent assays using a single clinical sample for diagnosis of acute DENV infection. Clin. Vaccine Immunol. 15:140–148.
3. Chang, S. F., J. H. Huang, L. K. Chen, C. L. Su, T. L. Liao, L. J. Chien, T. H. Lin, C. J. Su, and P. Y. Shu. 2008. Retrospective serological study on sequential dengue virus serotypes 1 to 4 epidemics in Tainan City, Taiwan, 1994 to 2000. J. Microbiol. Immunol. Infect. 41:377–385.
4. Chuansumrit, A., W. Chaiyaratana, V. Pongthanapisith, K. Tanraratchakit, S. Lertwonggrath, and S. Yoksan. 2008. The use of dengue nonstructural protein 1 antigen for the early diagnosis during the febrile stage in patients with dengue infection. Pediatr. Infect. Dis. J. 27:43–48.
5. Dussart, P., L. Petit, B. Labeau, L. Bremond, A. Leduc, D. Mona, S. Mathieu, and L. Baril. 2008. Evaluation of two new commercial tests for the diagnosis of acute dengue virus infection using NS1 antigen detection in human serum. PLoS Negl. Trop. Dis. 2:e280.
6. Kumarasamy, V., A. H. Wahab, S. K. Chua, Z. Hassan, Y. K. Chem, M. Mohamad, and K. B. Chua. 2007. Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. J. Virol. Methods 140:75–79.
7. Lapphra, K., A. Sangcharanswai, K. Chokephubulkit, S. Tiengrim, W. Piriyakarnsukal, T. Chakorn, S. Yoksan, L. Wattanamongkol, and V. Thamlikitkul. 2008. Evaluation of an NS1 antigen detection for diagnosis of acute dengue infection in patients with acute febrile illness. Diagn. Microbiol. Infect. Dis. 60:387–391.
8. Shu, P. Y., S. F. Chang, Y. C. Kuo, Y. Y. Yueh, L. J. Chien, C. L. Sue, T. H. Lin, and J. H. Huang. 2003. Development of group- and serotype-specific one-step SYBR green I real-time reverse transcription-PCR for dengue virus. J. Clin. Microbiol. 41:2408–2416.
9. Shu, P. Y., L. K. Chen, S. F. Chang, Y. Y. Yueh, L. Chow, L. J. Chien, C. Chin, T. H. Lin, and J. H. Huang. 2003. Comparison of capture immunoglobulin M (IgM) and IgG enzyme-linked immunosorbent assay (ELISA) and nonstructural protein NS1 serotype-specific IgG ELISA for differentiation of primary and secondary dengue virus infections. Clin. Diagn. Lab. Immunol. 10:622–630.
10. Shu, P. Y., L. J. Chien, S. F. Chang, C. L. Su, Y. C. Kuo, T. L. Liao, M. S. Ho, T. H. Lin, and J. H. Huang. 2005. Fever screening at airports and imported dengue. Emerg. Infect. Dis. 11:460–462.
11. Shu, P. Y., and J. H. Huang. 2004. Current advances in dengue diagnosis. Clin. Diagn. Lab. Immunol. 11:642–650.
12. Shu, P. Y., C. F. Yang, C. L. Su, C. Y. Chen, S. F. Chang, K. H. Tsai, C. H. Cheng, and J. H. Huang. 2008. Two imported chikungunya cases, Taiwan. Emerg. Infect. Dis. 14:1326–1327.