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SHORT COMMUNICATION

Evaluation of the Dengue NS1 Ag Strip® for Detection of Dengue Virus Antigen in *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Dengue fever is currently one of the most important mosquito-borne diseases that affect humans. With neither vaccines nor treatment available, prevention of the disease relies heavily on surveillance and control of mosquito vectors. In the present study, we have evaluated and showed the potential use of the Dengue NS1 Ag Strip® for the detection of dengue virus (DENV) in *Aedes aegypti*. Initial results showed that the sensitivity of the test kit in detecting DENV in wild-caught mosquitoes is comparable to that of real-time reverse transcriptase–polymerase chain reaction. The detection of naturally infected *Ae. aegypti* with the NS1 rapid test kit in our dengue cluster investigation further illustrates its potential use for surveillance of DENV in wild mosquito populations. The kit can easily be used in a simple field station, and minimal training is required. The results can be obtained in less than an hour. Employment of the kit in the field could help guide mosquito control operations in the prioritization of resources in controlling the transmission of DENV. In this study the potential of the kit for field surveillance of infected dengue vectors, which are epidemiologically important, has been demonstrated.

Key Words: *Aedes aegypti*—Dengue NS1 Ag Strip®—Dengue virus—NS1 protein—Rapid test kit—Real-time RT-PCR.

Introduction

Dengue fever is currently one of the most important mosquito-borne diseases that affect humans in terms of morbidity and mortality. Dengue fever has been reported in over 100 countries and 2.5 billion people live in areas where the disease is endemic (WHO 2009). It is caused by four dengue virus (DENV 1–4), flaviviruses in the family flaviviridae. In Southeast Asia, *Aedes aegypti* is the main vector of DENV and *Aedes albopictus* acts as a secondary vector (Gratz 2004). At present, there is neither effective vaccines nor specific treatment available for dengue; hence, prevention and control of the disease rely heavily on the surveillance and control of vectors to reduce transmission (Bangs et al. 2001). Vector surveillance allows the timely implementation of control measures, such as adulticiding, habitat destruction, source reduction, and community participation, to limit an impending outbreak. Active surveillance of infected mosquitoes may also prove valuable in defining the spatial and temporal risk of acquiring dengue infection (Bangs et al. 2007).

Typically, the detection of DENV in *Aedes* spp. includes isolation and propagation of viruses, reverse transcriptase–polymerase chain reaction (RT-PCR) assays, or enzyme-linked immunosorbent assay or immunofluorescence assay (Kuberski et al. 1977, Chan et al. 1994, Sithiprasasna et al. 1994, Rohani et al. 1997, Chow et al. 1998, Samuel and Tiyagi 2006). However, the need for expensive specialized laboratories and equipment and highly trained staff has made these techniques impractical for regular surveillance of DENV-infected mosquitoes.

Specialized rapid test kits have become available for detecting arboviruses, such as West Nile and Saint Louis encephalitis viruses in mosquito vectors (Nasci et al. 2002, Burkhalter et al. 2006). These kits have the advantage in their ease of use and require minimal laboratory facilities and technical experience. However, to date, none has been validated for DENV detection in its mosquito vectors. Recently, the Dengue NS1 Ag Strip® (BioRad Laboratories), a rapid immunochromatographic assay, has been developed to detect DENV NS1 antigen from acute patient serum samples (Dussart et al. 2008, Chaiyaratana et al. 2009, Pok et al. 2010). NS1 is a structural protein that is produced in both membrane-associated and secreted forms. It is involved in viral replication and has been shown to correlate with viremia (Libraty et al. 2002, Dussart et al. 2006, Thomas et al.

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2010). We report the usefulness of the NS1 rapid test in detecting DENV in laboratory-infected *Ae. aegypti* and in wild-caught mosquito population in Singapore.

Materials and Methods

Mosquito homogenization, NS1 antigen detection, and sample preparation

Individual mosquito was homogenized with Retsch mixer mill MM 200 (Germany) in 250 μL of phosphate-buffered saline. One hundred microliters of the mosquito homogenate was used on Dengue NS1 Ag Strip according to the procedure provided with the kit. To compare with RT-PCR, viral RNA was extracted from 100 μL of the remaining mosquito homogenate using the QIAmp viral RNA mini kit (Qiagen) according to the manufacturer's recommendation and stored at -80°C before its use.

Evaluation of NS1 for DENV detection with laboratory-infected Ae. aegypti

To determine the feasibility of using Dengue NS1 Ag Strip to detect all DENV serotypes antigen in infected mosquitoes, 5- to 7-day-old laboratory-colonized *Ae. aegypti* L. (F_{186}) were fed with an infectious blood meal, consisting of 1:1 washed mini-pig's erythrocyte and freshly passaged DENV 1, 2, 3, and 4 suspension, supplemented with 5 mM ATP as a phagostimulant. Feeding was performed using a Hemotek membrane feeding system (Discovery Workshops) housed in an isolation glove box within an Arthropod Containment Level 3 insectary. Blood meal titer for the infectious meal used in this study was determined by titration to be 7.52, 6.95, 5.52, and 7.52 Log_{10} tissue culture infectious dose_{50}/mL for DENV serotypes 1, 2, 3, and 4, respectively. Mosquitoes were incubated at 30°C and an RH of 80%.

To evaluate the specificity of the NS1 kit for all four serotypes, four sets of eight mosquitoes, each infected with DENV 1, 2, 3, and 4, were sacrificed and homogenized at day-10 postinfectious (pi) blood meal. A dengue group-specific one-step SYBR Green I-based one-step real-time RT-PCR assay was performed as previously described (Lai et al. 2007).

To determine the sensitivity of NS1 Ag Strip, mosquitoes infected with DENV 2 were sacrificed at 1, 2, 3, 4, 7, and 10 days pi blood meal and homogenized. A DENV 2–specific one-step quantitative RT-PCR assay targeting a 177-bp region of the *NS5* gene (Richardson et al. 2006) was performed in a LightCycler 2.0 system using a LightCycler RNA Master SYBR Green Kit I (Roche Diagnostics, GMbH). Real-time quantitative RT-PCR assay conditions follow that of Lai et al. 2007. Amplification of the target gene from each individual mosquitoes was compared against a standard curve (Efficiency = 1.814, error = 0.0461) generated from a 10-fold serial dilutions of *NS5* DENV 2 RNA standard, and LightCycler software version 4.05 was used for data analysis.

Comparison of Dengue NS1 Ag Strip and RT-PCR in detecting DENV in wild-caught mosquitoes

A real-time RT-PCR assay is currently the standard method at the Environmental Health Institute, a public health laboratory, in screening field-collected mosquitoes for the presence of arboviruses. The sensitivity of the Dengue NS1 Ag Strip in detecting DENV antigen in field-caught mosquitoes was thus compared with that of the molecular assay.

Between July and August 2009, 83 in-house developed sticky traps were deployed in 2 apartment blocks located northeast of Singapore, where active DENV transmission was on-going. Sticky traps were checked twice a week for 5 weeks for the presence of mosquitoes. All *Ae. aegypti* caught in the sticky trap were carefully removed and transferred individually into 1.7 mL micro-centrifuge tubes for homogenization for NS1 detection and RT-PCR according to Lai et al. 2007.

Results and Discussion

Specificity of Dengue NS1 Ag Strip to detect all four dengue serotypes

The ability of the Dengue NS1 Ag Strip to detect all DENV serotypes in infected *Ae. aegypti* and to detect DENV NS1 protein from a single infected mosquito at different time points (days pi) was evaluated in the current study. The results obtained by the rapid test kit in detecting DENV 1, 2, 3, and 4 in individual mosquitoes have been found to be congruent to the results obtained by RT-PCR. All eight mosquitoes infected with each serotype were found to be positive for both Dengue NS1 Ag Strip and the molecular method. The rapid test kit did not show any positive results when tested against uninfected mosquitoes. The results show the ability of the rapid test kit in detection all DENV serotypes, and are in agreement with studies conducted with human samples (Dussart et al. 2008, Pok et al. 2010).

Sensitivity of Dengue NS1 Ag Strip in detecting DENV in mosquitoes

Simple dilution of homogenate of infected mosquitoes may not appropriately reflect the biological ratio of viral NS1:viral RNA copy. Sensitivity of the rapid kit was thus determined using DENV 2 orally infected mosquitoes sampled at different time points, which resulted in mosquitoes having varied viral loads (Table 1). All infected mosquitoes were tested positive by the molecular assay; however, two infected mosquitoes sampled at day 2 pi (Table 1) were found to be negative by the rapid test kit. The viral load in these two mosquitoes corresponds to $<2\times10^5$ DENV RNA copy per mosquito, whereas the NS1-positive mosquitoes were all above 4×10^5 DENV RNA copy per mosquito. As illustrated in Table 1, Dengue NS1 Ag Strip assay reported in this study has a detection limit of 10^6 DENV copy per mosquito. Mosquitoes that have ingested low DENV dose and in its early stage of infection (eclipse phase) may escape detection, usually on day 2 pi. Nevertheless, among the 48 infected mosquitoes collected at different time points (1, 2, 3, 4, 7, and 10 pi), the rapid test kit had a sensitivity of 95.8%.

Out of 44 field-caught *Ae. aegypti*, two (4.54%) were found to be infected with DENV by both the real-time RT-PCR assay and the Dengue NS1 Ag Strip tests (Table 2). All field-caught mosquitoes that tested negative by the NS1 rapid test kit were also negative by the real-time molecular detection assays. As a result of this study, NS1 rapid is currently being used to detect DENV in mosquitoes caught from dengue cluster areas in Singapore, as part of vector control assessment. Among 10 clusters, 634 mosquitoes were caught and pooled.

Table 1. Comparison Between Dengue NS1 Ag Strip (BioRad Laboratories) and Real-Time Quantitative Reverse Transcriptase–Polymerase Chain Reaction in Detecting Dengue Virus-2 in Laboratory-Infected Mosquitoes Sampled at Different Time Points

Days pi blood meal	*Mosquito no.*	*Concentration (viral copy number/μL template)*	*Viral copy in each mosquito*	*Dengue NS1 Ag Strip*
1	1	4.40×10^{4}	6.60×10^{6}	+
	2	4.55×10^{4}	6.82×10^{6}	+
	3	9.36×10^{4}	1.40×10^{7}	+
	4	2.02×10^{5}	3.03×10^{7}	+
	5	2.97×10^{4}	4.46×10^{6}	+
	6	7.95×10^{4}	1.19×10^{7}	+
	7	9.33×10^{4}	1.34×10^{7}	+
	8	1.54×10^{4}	2.31×10^{6}	+
2	1	1.15×10^{3}	1.72×10^{5}	−
	2	1.57×10^{5}	2.36×10^{7}	+
	3	3.30×10^{5}	4.95×10^{6}	+
	4	1.59×10^{5}	2.38×10^{6}	+
	5	3.24×10^{4}	4.86×10^{6}	+
	6	5.06×10^{5}	7.59×10^{6}	+
	7	1.27×10^{3}	1.90×10^{5}	−
	8	4.58×10^{4}	6.87×10^{6}	+
3	1	4.05×10^{5}	6.08×10^{7}	+
	2	3.50×10^{5}	5.25×10^{7}	+
	3	3.40×10^{3}	5.10×10^{7}	+
	4	2.48×10^{6}	3.72×10^{7}	+
	5	2.71×10^{6}	4.06×10^{7}	+
	6	9.31×10^{5}	1.40×10^{8}	+
	7	1.21×10^{6}	1.81×10^{7}	+
	8	7.74×10^{5}	1.16×10^{8}	+
4	1	8.36×10^{5}	1.25×10^{8}	+
	2	3.25×10^{6}	4.88×10^{8}	+
	3	1.35×10^{6}	2.02×10^{8}	+
	4	1.68×10^{6}	2.52×10^{8}	+
	5	3.18×10^{3}	4.77×10^{5}	+
	6	1.78×10^{6}	2.67×10^{8}	+
	7	1.30×10^{6}	1.95×10^{8}	+
	8	4.39×10^{6}	6.58×10^{8}	+
7	1	6.77×10^{5}	1.02×10^{8}	+
	2	8.91×10^{5}	1.34×10^{8}	+
	3	4.61×10^{6}	6.92×10^{8}	+
	4	1.85×10^{5}	2.77×10^{7}	+
	5	9.40×10^{5}	1.41×10^{8}	+
	6	9.79×10^{5}	1.47×10^{8}	+
	7	1.17×10^{6}	1.76×10^{8}	+
	8	1.51×10^{6}	2.26×10^{8}	+
10	1	2.93×10^{6}	4.40×10^{8}	+
	2	1.33×10^{6}	1.99×10^{8}	+
	3	3.16×10^{6}	4.74×10^{8}	+
	4	3.45×10^{4}	5.18×10^{6}	+
	5	2.36×10^{6}	3.54×10^{8}	+
	6	9.80×10^{5}	1.47×10^{8}	+
	7	1.49×10^{6}	2.24×10^{8}	+
	8	9.18×10^{5}	1.38×10^{8}	+

pi, postinfectious.

Of the 126 pools, 6 pools were positive for NS1, and confirmed by RT-PCR (Vythilingam et al. unpublished document).

Surveillance for dengue-infected mosquito populations may assist in risk assessment and evaluation of vector control measures. The NS1 rapid test kit can easily be performed with minimal training in a simple field station. The results can be obtained in less than an hour. We have shown the potential of the kit for field surveillance of infected *Aedes* spp. mosquitoes, which are epidemiologically important.

However, it is now well understood that the extrinsic incubation period and the replication rate of DENV in mosquitoes are influenced by the genotypes of the virus, the infectious dose, and the strain of *Ae. aegypti* (Anderson and Rico-Hesse 2007, Pesko et al. 2009). Sensitivity of the rapid test kit will be influenced by the virus–mosquito partnership. Only through more field studies from different countries can we determine the usefulness of the kit in detecting transmission in different epidemiological setting.

Table 2. Number of *Aedes aegypti* Caught Per Week and Number of Mosquitoes Found to be Infected with Dengue Virus by Dengue NS1 Ag Strip (BioRad Laboratories) and Real-Time Reverse Transcriptase–Polymerase Chain Reaction Assay

		No. of positive mosquitoes	
Weeks	*No. of mosquitoes caught/tested*	*Dengue NS1 Ag strip*	*Real-time RT-PCR*
1	17	2	2
2	9	0	0
3	12	0	0
4	6	0	0
5	0	–	–
Total	44	2	2

RT-PCR, reverse transcriptase–polymerase chain reaction.

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Authors' Contributions

C.H.T., P.S.J.W., and M.Z.I.L. were involved in the mosquito–virus infection, and molecular and immunological aspect of the laboratory study, and prepared the article. I.V. led the collection of field mosquitoes. L.C.N. conceptualized and prepared the article.

Disclosure Statement

No competing financial interests exist.

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