Introduction

Up to 390 million dengue cases are thought to occur each year, and approximately 2.5 billion people are at risk for infection worldwide, with no vaccine or antiviral approved to reduce disease burden [1,2]. Accurate and affordable diagnostic tests are a crucial component of combating this debilitating mosquito-borne infection. Such assays would permit early diagnosis of dengue and thus improve clinical management of patients. Dengue is caused by any of four serotypes of dengue virus (DENV-1, 2, 3 and 4), a single-stranded, positive sense enveloped RNA virus that belongs to the genus Flavivirus [3,4]. During outbreaks, the number of people reporting to clinics with severe dengue can overwhelm the public health systems of many urban centers. Differential diagnosis based on symptoms is challenging due to dengue’s non-specific symptoms such as fever, aches and fatigue that often overlap with other endemic infections. Dengue-associated mortality can be reduced from 20–30% in severe cases to less than 1% with appropriate fluid replacement and supportive care, which is greatly facilitated by early diagnosis [5–7]. A positive laboratory test often alerts physicians to closely monitor platelet levels and other disease specific warning symptoms associated with severe disease. From a public health perspective, identification of dengue can geographically focus countermeasures such as targeted vector control.

While early and appropriate management of dengue is correlated with better outcome [8], no single laboratory test can be used to accurately diagnose disease over the course of illness. Traditional laboratory techniques for dengue diagnosis include detection of RNA using reverse transcription polymerase chain reaction (RT-PCR) or viral isolation followed by indirect immunofluorescence assay (IFA); both methods are effective during the first five days of illness and tend to decrease in...
sensitivity as viremia wanes over time [9–11]. However, RT-PCR requires specialized reagents and trained personnel, while virus isolation can take days or weeks to complete. The most widely used method for diagnosing dengue is an enzyme-linked immunosorbent assay (ELISA) which measures anti-DENV IgM or IgG antibodies in patient serum. These antibodies are not reliably detectable until 3–4 days post symptom onset (PSO) [12] and requires the collection of a second blood sample 14–21 days after the first visit for a definitive diagnosis. Serological diagnosis does not therefore inform immediate treatment decisions during acute illness. Since dengue patients often present within 3 days PSO (unpublished observation), false negative results with antibody-based assays remain a concern. Numerous successful molecular assays have been developed that detect DENV nucleic acid within the acute phase of illness [13–17]; however, these RT-PCR or isothermal molecular detection systems have yet to transition from the laboratory to a point-of-care format.

There are now a number of assays in development or on the market for diagnosing dengue during the acute stage of infection [18,19]. In 2000, the first ELISA capable of detecting DENV non-structural protein-1 (NS1) was developed. NS1 is found in both membrane and soluble forms and is highly conserved [20]. A soluble hexameric form of NS1 is released during DENV infection and accumulates in high concentrations (up to 50 μg/ml) in human serum [21,22]. Importantly, NS1 is detectable early during the acute phase (Day 0 to 6 PSO) of both primary and secondary DENV infections [21,23]. Together, the magnitude and timing of NS1 levels in human clinical specimens makes it an attractive target for diagnostic assay development [20,22,24]. Recent work has demonstrated that an NS1 antigen-based assay used in conjunction with a serological diagnostic marker (e.g., anti-DENV IgM) can enhance the sensitivity and specificity of dengue diagnosis through all stages of the disease [25–28]. Furthermore, quantitative detection of NS1 may help predict the risk associated with DENV infection, as high NS1 levels have been found to correlate with dengue hemorrhagic fever (DHF) [21,23,29,30]. Recently, NS1 tests have also been reported to be effective for detecting DENV in vector populations [31,32]. These tests can therefore improve both clinical management and vector surveillance.

The objective of this study was to compare seven commercially available DENV NS1 tests, four RDTs and three ELISAs, utilizing serum samples from confirmed DENV-infected and uninfected febrile patients collected in an endemic setting in Peru. While other groups have evaluated some of these assays in the past [26,27,33–37], this study also includes new dengue NS1 tests developed by In Bios, Inc. This is also the first assessment of dengue NS1 products utilizing DENV strains circulating in Peru. Overall, these results add to the growing body of literature about NS1 test performance and may aid public health decision-makers in selecting tests for specific applications.

Methods

Human Use Statement

The procedures applied in this study were done in accordance with the ethical standards of the Naval Medical Research Center (NMRC; Silver Spring, MD) Institutional Review Board and with the Helsinki Declaration of 1975, as revised in 1983. Study protocols were approved by the NMRC and Naval Medical Research Unit No. 6 (NAMRU-6, Lima, Peru) Institutional Review Boards (NMRCD.2000.0006 and NMRCD.2001.0002) in compliance with all applicable federal regulations governing the protection of human subjects. Study protocols were also reviewed by public health authorities in Peru (Instituto Nacional de Salud). Written consent was obtained from subjects 18 years of age and older. For younger participants written consent was obtained from a parent or legal guardian, and written assent was obtained from the participant.

Clinical Samples

Evaluation of the NS1 assays was conducted at NMRC using serum samples from a Surveillance and Etiology of Acute Febrile Illnesses in Peru (Study protocol NMRCD.2000.0006) at regional sites in Piura, Tumbes, Madre de Dios, and Iquitos. Surveillance and Etiology of Acute Febrile Illnesses in Ecuador and Honduras was performed under study protocol NMRCD.2001.0002, with samples from Honduras collected at the Instituto Hondureño de Seguridad Social, Tegucigalpa. Patients with an acute febrile illness were enrolled when reporting fever (≥38°C oral, tympanic, or rectal; ≥37.5°C axillary) for five days or less, accompanied with headache, muscle, ocular and/or joint pain, to public, military or private health facilities around regional sites. Symptoms and demographic information were collected.

DENV Reference Testing

Virus Isolation.

Virus isolation was attempted for all acute samples, and DENV was identified using serotype-specific IFAs. Briefly, African green monkey Vero (37°C) and Aedes albopictus mosquito C6/36 (28°C) cell cultures were each inoculated with diluted serum. Upon observation of cytopathic effect (CPE), or ten days post-inoculation if no CPE was observed, cells were removed from the flask and prepared for microscopic examination by standard indirect IFA. This was followed by the addition of fluorescein-conjugated goat anti-mouse IgG. DENV serotypes were identified using serotype-specific monoclonal antibodies (DENV-1: 15F3, DENV-2: 3H5; DENV-3: 5D4; DENV-4: 1H10).

Serology.

DENV IgM and IgG titers were determined by ELISA, as previously described [38,39]. Viral antigens for the ELISAs were produced at the NAMRU-6-Lima laboratory from pooled supernatants of infected Vero cell cultures using DENV-1 West Pac 74, DENV-2 S16803, DENV-3 CH53489, and DENV-4 TVP-360. Prior to homogenization, antigen preparations were inactivated using 3 mM binary ethylenimine. Any acute sample for which IgG levels were below the cut-off for positivity or for which IgM/IgG ratio was >0.5 was defined as a primary infection. High levels of IgG in acute samples defined secondary infections. As part of ongoing surveillance protocols, the serum samples were also routinely tested by IFA and IgM capture ELISA for evidence of recent infection by a panel of zoonotic and vector-borne pathogens, includingalphaviruses, orthobunyaviruses, and arenaviruses [40]. Non-DENV pathogens tested for included St. Louis encephalitis, yellow fever, West Nile, Venezuelan equine encephalitis, Eastern equine encephalitis, mayaro, oropouche, Q-fever, and typhus and rickettsia group of rickettsias. Precedently identified reactive sera were used as positive controls, and DENV-uninfected human serum was used as a negative control. Samples exceeding the reference cut-off value, calculated as the mean of seven antibody-negative samples (normal human serum) plus three standard deviations, were considered antibody positive. Positive samples were subsequently re-tested at four-fold serial dilutions (1:100, 1:400, 1:1600, and 1:6400).

Commercial tests for DENV NS1 antigen detection

Seven commercially available dengue NS1 kits were evaluated, including four RDTs and three in ELISA format. These kits were: “Dengue NS1 Ag STRIP” and “Platelia Dengue NS1 Ag ELISA”
Characteristics of the study population and test devices

Initially, 250 acute serum samples from febrile individuals were obtained for this study (Figure 1A). Serum samples were characterized for DENV infection using traditional laboratory techniques that have been described previously [40]. We utilized 200 samples categorized as DENV-positive based on virus isolation and 41 samples categorized as DENV-negative based on testing by virus isolation and serology. Nine samples initially categorized as DENV-negative based on absence of virus isolation were later found to be positive for DENV IgM. These samples were excluded from further analysis, as ELISA results alone were determined to be specific enough to confirm acute DENV infection. None of the isolation-negative samples used for this study had IgM titers against any other flavivirus infections tested (see methods for testing panel), with the exception of one sample that had IgM titers suggestive of prior St. Louis encephalitis virus exposure. Due to volume restrictions, not all samples were tested on all products.

Confirmed dengue cases and other febrile illnesses (OFI) were both evenly distributed between male and female; 51% male and 54% male respectively (Figure 1). The age distribution was unimodal, with a median age of 30 years, and an age range from five to 81 years. The median day of sample collection PSO was Day 2 (range 0–14). Greater than 80% of our samples were collected between Days 1–4 PSO (Figure 1B). DENV-infected samples included all four DENV serotypes (Figure 1).

A comparison of several functional attributes of each product is presented in Table 1. The indications for use ranged from serum only (Panbio, InBios) to EDTA-treated whole blood, serum, and plasma (SD). Only the serum claim was evaluated in this study. As expected, the NS1 ELISA kits required several additional steps when compared to RDTs and approximately 2–3 hours of assay time. RDTs required 15–30 minutes. The volume of sample required for the SD RDT (three drops corresponding to about 105 µl) was higher than other RDTs which required 50 µl. Only the SD and InBios RDTs can be stored at room temperature while the others need to be refrigerated at 2–8°C.

Test device performance

Among RDTs, Bio-Rad demonstrated the highest overall sensitivity of 79.1% (95% C.I. 71.8–85.2%), followed by InBios (76.5%; 64.6–85.9%), SD (72.4%; 64.5–79.3%) and Panbio (71.9%; 64.1–78.9%; Table 2). The specificity for each test was as follows: Bio-Rad (100%; 95% C.I. 91.1–100.0%), InBios (97.3%; 86.2–99.9%), SD (100%; 91.1–100%) and Panbio (95.0%; 83.1–99.4%). The loss in overall sensitivity was due to the very low sensitivity of all tests to DENV-4 (Table 2), with only 58.1% (Bio-Rad), 53.5% (SD), 44.2% (Panbio) and 42.1% (InBios) sensitivity. The sensitivity was highest to DENV-1 for all four RDTs, ranging from 91.4 to 95.2%, while sensitivity to DENV-2 and DENV-3 ranged from 69.2 to 87.3%.

We found the following overall sensitivity for each ELISA: InBios 95.9% (95% C.I. 86.0–99.5%), Bio-Rad 89.4% (82.6–94.3%) and Panbio 85.6% (78.9–90.9%; Table 2). Sensitivity exceeded 90% for DENV-1 for all three ELISA kits. DENV-4 sensitivity varied the most, ranging from 100% (InBios) to 75.0% (Bio-Rad) and 66.7% (Panbio). The overall sensitivity for ELISA kits was higher than that for the RDTs. Specifically, the Bio-Rad ELISA was significantly more sensitive than all RDT’s (p = 0.02, p = 0.02, p < 0.001, and p < 0.001 compared to the Bio-Rad, InBios, Panbio, and SD RDTs, respectively), while the Panbio ELISA was significantly more sensitive than the Panbio and SD RDT’s (p = 0.004 and p = 0.006, respectively). The corresponding overall specificities for each ELISA kit were: InBios 100.0% (95% C.I. 90.3–100%), Bio-Rad 97.3% (86.2–99.9%), and Panbio 95.0% (83.1–99.4%), which were similar to the RDTs.

Very few false positive results were obtained from OFI samples for any of the kits; the resulting specificity for all test articles was determined to be between 95–100%. Only three of the OFI samples were reactive to one or more test articles. No specific etiology was identified for these samples. One sample collected on day 1 PSO was reactive to both the Panbio RDT and ELISA, and one sample from day 5 PSO was positive using both the Panbio and Bio-Rad ELISA; the remaining sample, from day 7 PSO, was reactive to the Panbio RDT but was not reactive to any other test article.

The highest sensitivity of the NS1 assays was generally found between days 2–4 PSO. The Bio-Rad and Panbio RDTs demonstrated peak sensitivity on day 3 PSO (84.4%), while SD and InBios RDTs peaked on day 4 (78.3% and 83.3%, respectively; Figure 2A). The Bio-Rad and Panbio ELISAs also displayed peak sensitivity on day 4 PSO. Most RDTs generally showed decreasing sensitivity for every day removed from the peak. Among DENV positive samples, 81 samples were characterized as a primary infection and 90 samples were characterized as secondary infection (Figure 2B). On average, all tests were 10.5% (range: 2% for InBios ELISA to 26% for InBios RDT) more sensitive for detecting primary dengue infections compared with secondary infections, as has been reported by others [34,44]. Overall, the ELISAs displayed better sensitivity than RDTs at all time points.
Discussion

RDTs that can be performed near the patient’s point-of-care are being adopted worldwide for their utility in initial diagnosis. Simple assays capable of providing an answer within 15–30 minutes of sampling are highly desirable, especially in resource-limited settings. The performance of the RDTs will need to be weighed in context with other attributes that may be important to the end user (Table 1) including local market price, sample matrix that can be used, volume of sample necessary, storage temperature and shelf life. We found that ELISA kits had superior sensitivity when compared to RDTs. Because of their superior performance, ELISAs would be the recommended diagnostic choice when laboratories with trained personnel and equipment are available.

Depending on the prevalence of dengue and other febrile diseases, the positive and negative predictive values of the devices tested will vary. However, given the high specificity observed for both RDTs and ELISAs, the positive predictive value (PPV) of these devices is expected to be greater than 85% in most endemic countries, where dengue accounts for over 30% of febrile disease (PPV ranging from 86% for the Panbio RDT to 100% for the Bio-Rad RDT, SD RDT, and InBios ELISA). Thus individuals testing positive are unlikely to require further confirmatory testing. We found few RDTs or ELISAs reacting to the OFI samples, but an explicit cross-reactivity panel was not performed; false positives as a result of a cross-reactive antigen can adversely affect the PPV. RDTs had lower sensitivity than ELISAs, consequently the negative predictive value of an NS1 ELISA is likely to be superior to that of RDTs. Individuals testing negative on an RDT but still presenting with high clinical suspicion of dengue could be re-tested using laboratory assays, which may include a combination of NS1 ELISA, RT-PCR, and MAC-ELISA. Even so, RDTs can have considerable utility by significantly reducing the amount of confirmatory testing required.

Three factors appeared to correlate with the likelihood that a given sample will produce a false negative result. The first factor was the day PSO. Most tests achieved maximum sensitivity on days 2–4 PSO. This is likely correlated with temporal changes in NS1 antigen levels in patient sera [33]. Previous studies have shown that NS1 antigenemia fluctuates throughout disease with detectable levels occurring with the start of illness. NS1 levels have been shown to peak around day 4–5 PSO during primary infections, but wane earlier in secondary infections [23]. The second factor was the infection status: we found overall lower sensitivity in secondary infections for all test articles. This phenomenon has been previously observed and may be due to antibodies against DENV NS1 in the patient sample forming antigen-antibody complexes, thereby reducing access to the target epitopes for the test articles [34,45]. The third factor potentially contributing to false negative results was the infecting DENV serotype. This factor was most pronounced in the RDTs, where DENV-4 sensitivity averaged only 50%. Other groups have reported different sensitivities for NS1 diagnostic tests for DENV-2 and DENV-4 [36,46], using both clinical samples and tissue culture-derived virus. A number of reasons may exist for this: (i) large antigenic distance between circulating DENV-4 strains (from Peru in this case) and the antibodies used in the commercial assays leading to poor binding, or (ii) lower overall viremia and NS1 antigen levels in DENV-4 infections making it a less abundant target [34,47].

Certain limitations of this study relate to the types of samples used. The study was performed using retrospective samples, and the study results would have been even more directly applicable had it been performed prospectively during the course of routine dengue surveillance activities. Using a panel of well-characterized samples eliminates borderline or weak positive samples which would likely be included if the evaluation were prospective. Additionally, the same samples should ideally be evaluated on all assays, however volume restrictions precluded this direct comparison. As a result of these limitations, the idealized performance experienced in a laboratory setting may not be reproduced under field conditions. Another limitation of these results is that the performance of test devices can be influenced by several study specific variables: the reference methodology chosen, the type of samples collected, the PSO day, and the circulating serotypes and strains represented in a given evaluation panel. The Bio-Rad, SD, and to a lesser extent, Panbio NS1 tests have all been evaluated by...
### Table 1. Characteristics of dengue NS1 diagnostics.

| Assay type | Rapid diagnostic tests | ELISA format assays |
|------------|-------------------------|---------------------|
| Manufacturer | BioRad | InBios | Panbio | BioRad | InBios | Panbio |
| **Blood matrices** | Plasma, sera | Sera | Sera | EDTA-treated blood, plasma, sera | Sera | Sera |
| **Assay time (Minutes)** | 15–30 | 30 | 15 | 15–20 | 140 | 111 |
| **Volume necessary** | 50 µL | 50 µL | 105 µL | 50 µL | 50 µL | 75 µL |
| **Format** | Dipstick | Dipstick | Dipstick | Cassette | 96-well | 96-well |
| **Extra materials required** | Tubes, pipette | Pipette, tubes | Pipette | No | Pipette, incubator, plate reader | Pipette, incubator, plate reader |
| **Storage** | 2–8 °C | Room Temp. | 2–8 °C | Room Temp. | 2–8 °C | 2–8 °C |

Matrix, assay time, required volume, required additional equipment and storage temperature for each diagnostic test.

**Table 2. Device sensitivity and specificity: Numbers of samples tested for RDTs and ELISAs, to show serotype-specific as well as overall sensitivity and specificity.**

| Assay type | Rapid diagnostic test | ELISA format assays |
|------------|-------------------------|---------------------|
| Manufacturer | BioRad | InBios | Panbio | BioRad | InBios | Panbio |
| **DENV1** | 95.2% (40/42) | 91.4% (32/35) | 92.9% (39/42) | 92.9% (39/42) | 100.0% (45/45) | 93.8% (30/32) |
| **DENV2** | 76.9% (20/26) | 83.3% (5/6) | 80.8% (21/26) | 69.2% (18/26) | 80.0% (12/15) | 100.0% (6/6) |
| **DENV3** | 85.7% (36/42) | 87.5% (7/8) | 73.8% (31/42) | 73.2% (30/41) | 96.3% (26/27) | 100.0% (5/5) |
| **DENV4** | 58.1% (25/43) | 42.1% (8/19) | 44.2% (19/43) | 53.5% (23/43) | 75.0% (27/36) | 100.0% (6/6) |
| **Overall sensitivity** | 79.1% (121/153) | 76.5% (52/68) | 71.9% (110/153) | 72.4% (110/152) | 89.4% (110/123) | 95.9% (47/49) |
| **Overall specificity** | 100.0% (0/40) | 97.4% (1/38) | 95% (2/40) | 100.0% (0/40) | 97.4% (1/38) | 100.0% (0/36) |

**Note:** Matrix, assay time, required volume, required additional equipment and storage temperature for each diagnostic test.

**doi:** 10.1371/journal.pone.0113411.t001

**doi:** 10.1371/journal.pone.0113411.t002
multiple groups and our results are in broad agreement with previously published retrospective studies evaluating these test articles [48,49]. To our knowledge, this is the first published report describing the performance of InBios NS1 assays. This is also the first time Panbio Rapid NS1 tests have been evaluated using circulating DENV samples from South America. The sensitivity for NS1 tests reported in the literature can vary based on study design and the reference method used, from 58–99% for RDTs and 37–93% for ELISAs. This complicates side-by-side comparison of our data with previously reported results. Our evaluation did reveal sensitivities higher than the median values reported which may be due to our use of virus isolation instead of qRT-PCR as the reference method. Because qRT-PCR can be more sensitive than virus isolation, our positive specimens may have possessed higher viremia, resulting in better overall sensitivity. Future work will need to focus on prospective evaluation of NS1 tests in clinical settings, located in varied geographic locations representing a broad variety of circulating DENV strains.

Supporting Information

Table S1 Raw data.

(=XLSX)

Acknowledgments

We thank Ms. Carolina Guevara and Mr. Roger Castillo at NAMRU-6 for providing clinical samples and reference data for this study. We thank Mr. Christophe Salanon from Bio-Rad, Ms. Andrea Valks from Panbio, Dr. Syamal Raychaudhuri from InBios, and Mr. Carl Stubbs from Focus Diagnostics for generously sharing their dengue NS1 assays for evaluation. Dr. Peifang Sun and Mr. Theron Gilliland Jr. helped review the manuscript.
Disclaimer: The views expressed in this article are those of the authors, and do not necessarily reflect the official policy or position of the Department of the Navy, the Department of Defense, or the US Government.

Copyright Statement: Dr. Allison Dauner and Dr. Subhamoy Pal are employed by the Henry M. Jackson Foundation for the Advancement of Military Medicine, and are funded to do this work by the US Government. Dr. Jonathan Jue is a Lieutenant Colonel, US Army. While performing the work described in this article, the authors acted as military service members or employees of the US Government. This work was prepared as part of their official duties. Title 17 U.S.C. § 103 provides that ‘Copyright protection under this title is not available for any work of the United States Government.’ Title 17 U.S.C. § 101 defines a U.S. Government work as a work prepared by military service members or employees of the U.S. Government as part of those persons’ official duties.

Author Contributions
Conceived and designed the experiments: SP AD BF SW. Performed the experiments: AD IM. Analyzed the data: SP AD BF. Contributed reagents/materials/analysis tools: BF PG AM EH TK. Wrote the paper: SP AD BF EH.

References
1. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, et al. (2013) The global distribution and burden of dengue. Nature 496: 540–547.
2. WHO. Strengthening implementation of the global strategy for dengue fever and dengue hemorrhagic fever, prevention and control. Geneva, Switzerland: World Health Organization. 1999 p.
3. Trent DW, Manske CL, Fox GE, Chu MC, Kliks SC, et al. (2005) The molecular epidemiology of Dengue viruses, genetic variation and microevolution. In: Kurstik E, editor. Applied Virology Research. Virus Variation and epidemiology. Plenum, NY: Plenum Press.
4. Rico-Hesse R, Cline (2000) Microevolution and virulence of dengue viruses. Adv Virus Res 59: 315–341.
5. Kalayanarooj S (1999) Standardized clinical management: evidence of reduction of dengue hemorrhagic fever case fatality rate in Thailand. Dengue Bull 23: 10–17.
6. Lan NT, Hung NT, Ha DQ, Phuong BT, Lien LH, et al. (1998) Treatment of plasma viremia and soluble nonstructural protein 1 concentrations in dengue: differential effects according to serotype and immune status. J Infect Dis 203: 1292–1300.
7. Wu SJ, Pal S, Ekanayake S, Greenwald D, Lara S, et al. (2008) A dry-format ELISA assay with pentax dengue IgM particle agglutination assay to evaluate quantitative RT-PCR method for sensitive detection of dengue virus in serum. J Virol Methods 140: 102–107.
8. WHO (2009) Dengue guidelines for diagnosis, treatment, prevention and control. Geneva 27, Switzerland: World Health Organization.
9. Teles FR, Prazeres DM, Lima-Filho JL (2005) Trends in dengue diagnosis. Rev Soc Bras Med Trop 38: 415–422.
10. Kao CL, King CC, Chao DY, Wu HL, Chang GJ (2005) Laboratory diagnosis of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase-PCR. J Clin Microbiol 36: 2634–2639.
11. Lan NT, Hung NT, Ha DQ, Phuong BT, Lien LB, et al. (1998) Treatment of plasma viremia and soluble nonstructural protein 1 concentrations in dengue: differential effects according to serotype and immune status. J Infect Dis 203: 1292–1300.
12. Simons CP, Chau TH, Tuan NM, Hoang DM, et al. (2007) Maternal antibody and viral factors in the pathogenesis of dengue virus in human pregnancy. J Virol Methods 145: 46–51.
13. Lee WP, ZZH, Hoang DT, Thanh NT, Tran LT, et al. (2008) Diagnostic accuracy of commercial NS1-based diagnostic tests for early dengue virus infection. Clin Diagn Lab Immunol 11: 642–650.
14. Voge NV, Sanchez-Vargas I, Blair CD, Eisen L, Beatty BJ (2013) Detection of dengue virus NS1 antigen in Ae. aegypti using a commercially available kit. Am J Trop Med Hyg 88: 260–266.
15. Pan CC, Tsai CC, Yang SP, Wang TL, Chen CH, et al. (2010) Evaluation of a commercial dengue NS1 antigen-capture ELISA for laboratory diagnosis of acute dengue virus infection. J Virol Methods 165: 121–125.
16. Hang VT, Nguyet NM, Trung DT, Tricou V, Yoksan S, et al. (2009) Diagnostic accuracy of Dengue NS1 Tests. PLoS Negl Trop Dis 3: 211.
17. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, et al. (2007) An antigen-capture ELISA for detection of dengue virus antigen in Aedes aegypti (Diptera: Culicidae). Vector Borne Zoonotic Dis 11: 789–792.
18. Voge NV, Sanchez-Vargas I, Blair CD, Eisen L, Beatty BJ (2013) Detection of dengue virus NS1 antigen in Ae. aegypti using a commercially available kit. Am J Trop Med Hyg 88: 260–266.
19. Lan NT, Hung NT, Ha DQ, Phuong BT, Lien LH, et al. (1998) Treatment of plasma viremia and soluble nonstructural protein 1 concentrations in dengue: differential effects according to serotype and immune status. J Infect Dis 203: 1292–1300.
20. Alston S, Talarmin A, Debryneau M, Falconar A, Dreydel V, et al. (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.
21. Teles FR, Prazeres DM, Lima-Filho JL (2005) Trends in dengue diagnosis. Rev Soc Bras Med Trop 38: 415–422.
22. Arboviral etiologies of acute febrile illnesses in Western South America, where dengue and Japanese encephalitis co-circulate. Am J Trop Med Hyg 40: 418–427.
23. Avirutnan P, Punyadee N, Noisakran S, Komoltri C, Thiemmeca S, et al. (2006) Evaluation of Dengue NS1 Tests for detection of dengue virus antigen in Aedes aegypti (Diptera: Culicidae). Vector Borne Zoonotic Dis 11: 789–792.
24. Innis BL, Nisalak A, Nimmannitya S, Kusalerdchariya S, Chongswasdi V, et al. (2007) An antigen-capture ELISA for detection of dengue virus antigen in Aedes aegypti (Diptera: Culicidae). Vector Borne Zoonotic Dis 11: 789–792.
25. Hang VT, Nguyet NM, Trung DT, Tricou V, Yoksan S, et al. (2009) Diagnostic accuracy of Dengue NS1 Tests. PLoS Negl Trop Dis 3: 211.
specificity and relationship to viraemia and antibody responses. PLoS Negl Trop Dis 3: e360.

46. Bessoff K, Delorey M, Sun W, Hunsperger E (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: 1513–1518.

47. Thai KT, Phuong HL, Thanh Nga TT, Giao PT, Hung le Q, et al. (2010) Clinical, epidemiological and virological features of Dengue virus infections in Vietnamese patients presenting to primary care facilities with acute undifferentiated fever. J Infect 60: 229–237.

48. Blacksell SD (2012) Commercial dengue rapid diagnostic tests for point-of-care application: recent evaluations and future needs? J Biomed Biotechnol 2012: 151967.

49. Chappuis F, Alirol E, d’Acremont V, Bottieau E, Yansouni CP (2013) Rapid diagnostic tests for non-malarial febrile illness in the tropics. Clin Microbiol Infect 19: 422–431.