



รายงานวิจัยฉบับสมบูรณ์

โครงการ แบบจำลองเชิงคณิตศาสตร์เพื่อยุทธวิธีการวินิจฉัยและ
ควบคุมการระบาดในไข้เลือดออก

โดย ดร.วิรัชดา ปานงาม และคณะ

เดือน ธันวาคม พ.ศ. 2555

สัญญาเลขที่ TRG5380004

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและมหาวิทยาลัยมหิดล

(ความเห็นในรายงานนี้เป็นของผู้วิจัย สกว. และมหาวิทยาลัยมหิดลไม่จำเป็นต้องเห็นด้วยเสมอไป)

กิตติกรรมประกาศ

คณะผู้วิจัยขอขอบคุณ สำนักงานกองทุนสนับสนุนการวิจัย (สกว.), คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล และ มูลนิธิเวลแคม ประเทศอังกฤษ ที่สนับสนุนเงินทุนวิจัย, กลุ่มนักวิจัยและเจ้าหน้าที่จากหน่วยวิจัยโรคเขตร้อนมหิดล-อ็อกซ์ฟอร์ดและภาควิชาสูติวิทยาเขตร้อน คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล ที่ให้ความช่วยเหลือและความร่วมมือตลอดการดำเนินการ รวมถึงคนไข้ในโครงการ ทุกๆท่าน ที่กรุณาให้ข้อมูลในการศึกษาครั้งนี้ ทั้งหมดนี้ทำให้โครงการวิจัยสำเร็จไปได้ด้วยดี

คณะผู้วิจัยหวังว่ารายงานฉบับนี้ คงเป็นประโยชน์สำหรับหน่วยงานที่เกี่ยวข้อง ทั้งภาครัฐและเอกชน ตลอดจนผู้สนใจและนักวิชาการ ที่จะสร้างนำมาพิจารณาเพื่อพัฒนาและปรับปรุงยุทธวิธีการวินิจฉัย และควบคุมการระบาดในไข้เลือดออกของประเทศต่อไป

คณะผู้วิจัย

ธันวาคม 2555

Abstract

Project Code : TRG5380004

Project Title : Mathematical modelling of optimal combinations of dengue diagnosis strategies

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Project Period : 2 years (31st May 2553 to 1st December 2555, with 6 month leave)

Objectives: To use mathematical modelling to assess sensitivity and specificity on current available tests and design optimal diagnosis strategies for dengue for the investigation and containment of outbreaks at community level and the ongoing surveillance of disease burden.

Materials and Methods: Assuming no perfect gold standard technique, we applied Bayesian latent class models (LCMs) to assess the sensitivity of different tests including the reference assay (a combination of Dengue IgM antibody capture ELISA and IgG antibody capture ELISA) and of three rapid diagnostic tests (Panbio NS1 antigen, IgM antibody and IgG antibody rapid immunochromatographic cassette tests). Data from a cohort study of 549 patients suspected of dengue infection presenting at Colombo North Teaching Hospital, Ragama, Sri Lanka, were used to demonstrate this modeling approach.

Results: The model estimated sensitivity and specificity of the reference assay to be 62.0% and 99.6% (as opposed to being a perfect gold standard i.e. sensitivity and specificity of 100%, respectively). Prevalence of dengue infection (24.3%), and sensitivities and specificities of the Panbio NS1 (45.9% and 97.9%), IgM (54.5% and 95.5%) and IgG (62.1% and 84.5%) estimated by Bayesian LCMs were significantly different from those estimated by assuming that the reference assay was perfect.

Conclusions and discussions: The reference assay is an imperfect gold standard. In our setting, the combination of NS1, IgM and IgG rapid diagnostic tests could be used on admission to rule out dengue infection with a high level of accuracy (NPV 95.2%). Further evaluation of rapid diagnostic tests for dengue infection should include the use of appropriate mathematical and statistical models.

Future plan: To include common signs and symptoms as diagnostic tools and assess sensitivity of combinations using modeling approach in order to optimize dengue diagnosis strategies. This will be very useful for any poor settings where dengue test kits may not be available.

Keywords : dengue, Bayesian Latent Class Models (LCMs), diagnosis

บทคัดย่อ

รหัสโครงการ : TRG5380004

ชื่อโครงการ : แบบจำลองเชิงคณิตศาสตร์เพื่อยุทธวิธีการวินิจฉัยและควบคุมการระบาดในไข้เลือดออก

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ระยะเวลาโครงการ : 2 ปี (31 พฤษภาคม 2553 - 1 ธันวาคม 2555, ลาพักงานวิจัย 6 เดือน)

วัตถุประสงค์ : ใช้แบบจำลองทางคณิตศาสตร์เพื่อประเมินความไวและความจำเพาะของชุดตรวจสอบ
ไข้เลือดออกแบบเร่งด่วนที่ใช้ในปัจจุบันและออกแบบการใช้มากกว่าหนึ่งชุดตรวจในการวินิจฉัยที่ดีที่สุดเพื่อ
ตรวจหาการระบาดในระดับชุมชนและการเฝ้าระวังโรคอย่างต่อเนื่อง

วิธีทดลอง : ในกรณีที่ไม่มีวิธีการตรวจสอบโรคไข้เลือดออกที่ให้ผลถูกต้อง 100 เปอร์เซ็นต์ เราใช้
แบบจำลองเชิงคณิตศาสตร์ชนิดกลุ่มแฝง (Bayesian Latent Class Models) เพื่อประเมินความไวของของ
ชุดตรวจไข้เลือดออกแบบเร่งด่วนต่างๆ (Panbio NS1 แอนติเจน, IgM แอนติบอดี และ IgG แอนติบอดี)
รวมทั้งการตรวจสอบที่ใช้เป็นตัวอ้างอิง (การวิเคราะห์จากผลตรวจของโรคไข้เลือดออกจาก การวัด IgM
แอนติบอดี และ IgG แอนติบอดี) ผู้วิจัยได้แสดงการสร้างแบบจำลองเชิงคณิตศาสตร์ชนิดกลุ่มแฝงและการ
วิเคราะห์โดยใช้ข้อมูลจากการศึกษาในผู้ป่วยที่สงสัยว่ามีการติดเชื้อไข้เลือดออกจำนวน 549 คน ใน
โรงพยาบาลนอร์ทโคลัมโบ ประเทศศรีลังกา

ผลการทดลอง : แบบจำลองประมาณความไวและความจำเพาะของชุดตรวจสอบอ้างอิงเป็น 62.0% และ
99.6% ตามลำดับ (เมื่อเทียบกับการสมมุติให้การตรวจสอบอ้างอิงมีความไวและความจำเพาะเป็น 100%)
แบบจำลองเชิงคณิตศาสตร์ประมาณความชุกของการติดเชื้อไวรัสไข้เลือดออกจากข้อมูลตัวอย่างเท่ากับ
24.3% และความไวของ Panbio NS1 (45.9% และ 97.9%), IgM (54.5% และ 95.5%) และ IgG (62.1%
และ 84.5%) ค่าประมาณนี้มีความแตกต่างจากการประมาณโดยการตรวจสอบอ้างอิงอย่างมีนัยสำคัญ

สรุปและวิจารณ์ผลการทดลอง : การตั้งให้ชุดตรวจสอบโรคไข้เลือดออกอ้างอิงมีความไวและความจำเพาะ
100 เปอร์เซ็นต์อาจเป็นการสมมุติค่าที่สูงเกินความเป็นจริง การใช้มากกว่าหนึ่งชุดตรวจในการวินิจฉัยเช่น
การรวมชุดตรวจ NS1, IgM และ IgG สามารถนำมาใช้ในการวินิจฉัยไข้เลือดออกได้อย่างมีประสิทธิภาพสูง
(NPV 95.2%) แบบจำลองทางคณิตศาสตร์และสถิติสามารถเพิ่มประสิทธิภาพในการประเมินชุดตรวจ
ไข้เลือดออกและการออกแบบการใช้มากกว่าหนึ่งชุดตรวจในการวินิจฉัยโรคไข้เลือดออก

ข้อเสนอแนะสำหรับงานวิจัยในอนาคต : การนำอาการที่พบบ่อยมาช่วยในการออกแบบการวินิจฉัยเพื่อ
เพิ่มประสิทธิภาพการตรวจวินิจฉัยโรคไข้เลือดออกซึ่งจะเป็นประโยชน์มากสำหรับพื้นที่ชนบทยากจนที่ไม่มี
ชุดทดสอบไข้เลือดออกใช้

คำหลัก : ไข้เลือดออก แบบจำลองเชิงคณิตศาสตร์ชนิดกลุ่มแฝง การวินิจฉัย

เนื้อหาทางวิจัย

Introduction

Dengue infection is a leading cause of illness and death in the tropics and subtropics. The causative organisms are mosquito-transmitted Dengue viruses, and patients may present with a range of clinical syndromes including viral syndrome, acute undifferentiated febrile illness, dengue fever, dengue hemorrhagic fever and dengue shock syndrome. On presentation, dengue infection often presents with symptoms and signs similar to other acute tropical infectious diseases, and a range of rapid diagnostic tests has been recommended for early diagnosis and patient management [1,2]. There are two main methods for diagnosing dengue infection, namely virus and antibody detection. Virus detection includes viral isolation, polymerase chain reaction (PCR) and detection of nonstructural protein-1 (NS1) antigen. Antibody detection includes haemagglutination inhibition (HAI) tests and enzyme linked immunosorbent assay (ELISA) for detection of dengue IgM and IgG antibodies, usually using paired serum collections and assessing for a quantitative rise in antibody levels. Virus isolation and HAI are considered the gold standard techniques for virus and antibody detections, respectively, but are rarely used since they are time-consuming and laborious [3]. We have repeatedly used the Armed Forces Institute of Medical Sciences (AFRIMS) diagnostic serology methodologies on paired sera as a reference assay to determine the accuracy of alternative diagnostic tests [1,4–7]. We hypothesized that the accuracy of this reference assay is imperfect, and that the accuracy of the alternative diagnostic tests estimated by comparing them with the reference assay might have been underestimated.

Bayesian latent class models (LCMs) have been increasingly used to evaluate the true accuracy of diagnostic tests in prospective cohort studies, as they do not require the assumption that any test is perfect [8–11]. The objective of this study was to use Bayesian LCMs to analyze existing data from a cohort of patients presenting to hospital with suspected dengue infection. We estimated the accuracy of three rapid diagnostic tests (Panbio NS1, IgM and IgG cassette tests), our reference assay for dengue infection, and the combination of all three rapid tests when used at clinical presentation.

Materials and Methods

Study patients and diagnostic tests

The data analyzed in this study was generated during a prospective cohort study of patients suspected of dengue infection. In brief, patients were recruited between June 2006 and June 2007 at Colombo North Teaching Hospital, Ragama, Sri Lanka. Inclusion criteria were the presence of fever ($\geq 38^{\circ}\text{C}$) in patients aged 16 years or more who were suspected to have dengue infection. Blood samples were collected on admission and, where possible, at discharge and at follow-up 2 – 4 weeks later for convalescent-phase specimens. All specimens were stored at -85°C while at the clinical site and transported on dry ice to Bangkok, Thailand, for the test assessments. Reported elsewhere, a case-control study using samples from a subset of 259 of the patients recruited into the cohort was performed to evaluate six commercial point-of-care tests for acute dengue infections by comparing those tests with the reference assay [6].

For the purpose of the current study, all patients enrolled into the cohort were evaluated. In the cohort, every patient was tested with three rapid diagnostic tests including the Panbio first

generation NS1 antigen strip, the Panbio Duo cassette IgM/IgG (Inverness, Australia), and our reference assay. NS1 antigen strip tests were performed on admission samples only. Dengue reference assays were performed at AFRIMS, Bangkok, Thailand as previously described (Figure 1) [7]. In short, AFRIMS tested paired admission and convalescent specimens using dengue (DEN) IgM antibody capture (MAC) ELISAs, IgG antibody capture (GAC) ELISA, and equivalent Japanese encephalitis virus (JEV) MAC and GAC ELISAs [12–16]. An acute primary dengue infection was diagnosed if (1) on the admission sample, the DEN MAC ELISA result was ≥ 40 units (U), the ratio of DEN MAC ELISA to JEV MAC ELISA results was ≥ 1 and the ratio of DEN MAC ELISA to DEN GAC ELISA results was $\geq 1.8:1$, or (2) the DEN MAC ELISA result on the admission sample was < 15 U and in the convalescent-phase specimen ≥ 30 U. An acute secondary dengue infection was diagnosed if (1) on the admission sample, the DEN MAC ELISA result was ≥ 40 U, the ratio of DEN MAC ELISA to JEV MAC ELISA results was ≥ 1 and the ratio of DEN MAC ELISA to DEN GAC ELISA results was $< 1.8:1$, or (2) the DEN MAC ELISA on the admission sample was < 40 U, the ratio of DEN GAC ELISA results on the convalescent-phase specimen compared to the admission specimen was ≥ 2 (2-fold rise) and the DEN GAC ELISA result on the convalescent-phase specimen was ≥ 100 . An acute JEV infection was defined if the DEN MAC ELISA result on the admission sample was ≥ 40 U and the ratio of DEN MAC ELISA to JEV MAC ELISA results was < 1 .

Ethics statement

Ethical approval for the cohort study was obtained from the Faculty of Medicine, University of Kelaniya in Sri Lanka, the Liverpool School of Tropical Medicine in the UK and

the Walter Reed Army Institute of Research in the USA. Written informed consent was obtained from each subject enrolled into the study.

Statistical analysis

AFRIMS serological assay as Gold Standard Model

Three rapid diagnostic test results (NS1 antigen strip, IgM cassette test and IgG cassette test) were analyzed using the reference assay results as the gold standard. Prevalence, sensitivities, specificities, positive and negative predictive values for the three rapid diagnostic tests were calculated with exact 95% confidence intervals using the Stata 11.0 statistical software package (Stata Corp., College Station, Texas).

Bayesian Latent Class Models

Use of LCMs and Bayesian LCMs to determine the accuracy of diagnostic tests when the accuracy of the gold standard is imperfect or unknown has been described in detail elsewhere [17,18]. In brief, Bayesian LCMs do not assume that any test is perfect, but consider that each test could be imperfect in diagnosing the true disease status. The true disease status of the patient population is then defined on the basis of overall prevalence (the probability that a patient with suspected dengue is truly infected with dengue virus). LCMs estimate prevalence and accuracy of each test based on the observed frequency of the possible combinations of test results.

To estimate the accuracy of a diagnostic test by use of LCMs, the best-fitting model, as determined by the presence or absence of correlation between diagnostic tests in the model,

should be used [19]. Possible correlations we evaluated were based on existing knowledge and external evidence. Therefore, correlations amongst serological tests (IgM, IgG and reference tests) were considered. The deviance information criterion (DIC) and Akaike information criterion (AIC) were used to evaluate goodness of model fit and to compare models. A difference in DIC or AIC of more than 10 indicated definite support to the model with the lower value, while a difference of between 5 and 10 was considered substantial, and less than 5 inconclusive. The best-fitted model was used to determine the accuracies of the three diagnostic tests and of their combinations on paired specimens. Then, the accuracies of the diagnostic tests on clinical presentation were determined by using test results for the admission samples only. All models assumed that no prior information (non-informative priors) about the unknown parameters (prevalence, sensitivities and specificities) was available. All parameters and associated 95% credible intervals (CrI) were estimated using WinBUGS 1.4 [20]. Supporting information file 1 and 2 provide full data sets and all of the models used, respectively.

Post-hoc model evaluation

The prediction accuracy of the final Bayesian LCM was evaluated by comparing the prevalence estimated and the final diagnoses made in the cohort study. Final diagnoses were based on microbiology results and physicians' judgment if all microbiology results were negative. Final diagnoses of dengue infection were categorized into four groups, following the dengue classification and case definitions of symptomatic dengue virus infections as described by WHO: (i) undifferentiated fever or viral syndrome, (ii) dengue fever, (iii) dengue hemorrhagic fever and (iv) dengue shock syndrome [21].

Sensitivity analysis

Sensitivity analyses were performed in which patients without convalescent samples were excluded and also in which different prior information were used [22,23].

Results

A total of 617 patients with fever and suspected dengue infection were included in the cohort study. Of these, 68 (11%) patients were excluded from further analysis because the result of at least one diagnostic test on the admission sample was not available. Of 549 patients included in the analysis, the median age was 35 years old (interquartile range [IQR], 25 - 50 years old), and 371 (68%) were male. Convalescent samples were available in 290 (52.8%) patients. Median time between onset of symptoms and collection of admission samples was 5 days (IQR, 3 - 8 days), and median time between onset of symptoms and collection of convalescent samples was 24 days (IQR, 19 - 30 days).

The final diagnoses of the 549 patients were dengue infection (135, 24.6%), chikungunya (102, 18.6%), leptospirosis (33, 6.0%), confirmed infections with no organism identified (38, 6.9%), various bacteraemias (23, 4.2%), confirmed non-infectious diseases causing fever (18, 3.2%), Q fever (17, 3.1%), rickettsial infections (14, 2.6%), tuberculosis (5, 0.9%), urinary tract infection (5, 0.9%), mumps (2, 0.4%), varicella (1, 0.2%), viral hepatitis (1, 0.2%), malaria (1, 0.2%), hantavirus infection (1, 0.2%) and undiagnosed febrile illnesses (184, 33.5%). The total number of diagnoses exceeds 549 as 31 cases had more than one diagnosis. Ten (1.8%) patients died in hospital due to leptospirosis (4, 40%), cerebrovascular accidents (3, 30%), liver

failure (2, 20%) and leukaemia (1, 10%). Of 135 patients diagnosed with dengue infection, 131 had dengue fever, 4 had dengue hemorrhagic fever, and none had dengue shock syndrome.

Of 549 patients, 84 (15.3%) had positive results on the reference assay for dengue infection (26 primary infections and 58 secondary infections). None were positive for acute JEV infection. The NS1 antigen strip test, IgM cassette test and IgG cassette test were positive in 69, 91 and 147 patients, respectively. Only 13 patients (2.4%) were positive for the reference assay and all three rapid diagnostic tests.

Reference assay as a perfect gold standard

We first assumed that the reference assay was a perfect gold standard (100% sensitivity and 100% specificity), and used this assumption to calculate sensitivities, specificities, PPVs and NPVs of the three rapid diagnostic tests and their combinations (Table 1). The estimated prevalence of dengue infection in the study population was 15.3%. The NS1 antigen test, IgM cassette test and IgG cassette test all had low sensitivity (54.8%, 50.0% and 61.9%, respectively) with variable specificity (95.1%, 89.5%, and 79.6%, respectively).

Bayesian LCM

Bayesian LCMs were then applied to obtain an unbiased estimate of the accuracy of each diagnostic test. The models included all four diagnostic tests, including NS1 antigen test, IgM cassette test, IgG cassette test and our reference assay. First, we defined the best fitting Bayesian LCM by determining the presence of correlations between all three serological tests (IgM cassette test, IgG cassette test and our reference assay). Of the five plausible models (Table 2), the difference in DIC and AIC between the best fitting model (model 2) and the other four

models were inconclusive (differences were less than 5). Supporting information file 3 shows the prevalence and accuracy of diagnostic tests estimated by all five models. In short, there was no substantial difference between all five models. Model 2, which had the lowest DIC and AIC, and included the correlation between IgM and IgG cassette tests, was selected as the best-fitted model.

Using this model, the prevalence of dengue infection in the study population was estimated to be 24.3% (95% CrI 19.1% – 30.0%). The Bayesian LCM indicated that the reference assay had very high specificity (99.6, 95% CrI 97.9% – 100%), but low sensitivity (62.0%, 95% CrI 49.5% – 75.9%). Sensitivities and specificities of the Panbio NS1 (45.9% and 97.9%), IgM (54.5% and 95.5%) and IgG (62.1% and 84.5%) estimated by Bayesian LCM were significantly different from those estimated by assuming that the reference assay was perfect. Sensitivity and specificity for a combination of NS1 and IgM rapid tests, where a sample was defined as positive if either test was positive, were 78.9% and 93.7%, respectively. Sensitivity and specificity for a combination of NS1, IgM and IgG rapid tests, where a sample was defined as positive if any test was positive, were 91.7% and 79.8%, respectively (Table 1).

To determine the accuracy of the rapid serological tests on clinical presentation, only test results of IgM and IgG cassette test on admission samples were considered using the best-fitted Bayesian LCM. The sensitivity of IgM cassette test and IgG cassette test on clinical presentation was 39.7% and 42.6%, respectively. Sensitivity and specificity of a combination of NS1 and IgM were 72.8% and 94.7%, respectively, with positive predictive value (PPV) and negative predictive value (NPV) of 81.5% and 91.6%, respectively. Sensitivity and specificity of a combination of NS1, IgM and IgG rapid tests were 87.0% and 82.8%, respectively, with PPV and NPV of 62.0% and 95.2% respectively.

Post-hoc model validation

According to the final diagnoses, 24.6% of the patients were classified as dengue infection (dengue fever or dengue hemorrhagic fever). This indicated that the estimated prevalence of dengue infection in the study population using Bayesian LCMs (24.3%) was credible.

Sensitivity analysis

Sensitivity analysis was performed in which 259 of 549 (47.2%) patients without convalescent samples were excluded. By use of the best-fitted Bayesian LCM, the sensitivities of our reference assay, IgM cassette test and IgG cassette test were estimated to be 76.3% (95% CrI 59.2%-90.4%), 60.8% (95% CrI 49.5%-71.7%) and 68.3% (95% CrI 57.0%-78.3%), respectively, for patients suspected of dengue infection who had a convalescent sample. Specificity of those tests was not substantially different from the previous estimate, although all CrIs were wider as a consequence of the reduced sample sizes. There was no substantial change when different prior information was used (Supporting information file 3).

Discussion

The key findings of this study are that the true sensitivity of our reference assay (AFRIMS MAC and GAC ELISA on paired serum) estimated by Bayesian LCM was very low (62.0%). The reduction in sensitivity of our reference assay from 100% assumed by the gold standard model to 62% as estimated by the Bayesian LCM model is due to the difference in the estimation methods. While the gold standard model assumed that our reference assay is perfect (sensitivity = 100%), Bayesian LCM estimated the true sensitivity of our reference assay using

the results of every diagnostic test included in the model. Bayesian LCM also gave an estimated prevalence of 24.3% in patients who were suspected of dengue infection, compared with 15.3% based on our reference assay alone. This higher estimated prevalence is credible, since 24.6% patients had final diagnosis of dengue fever or dengue hemorrhagic fever based on the WHO definition and the exclusion of other diseases.

There are several potential explanations as to why our reference assay had such a low sensitivity in our setting. In common with other research and reflecting real life, we also failed to obtain a convalescent serum specimen from 47.2% of patients, either because they died, they refused to be bled on discharge, or they were lost to follow-up. The results from our sensitivity analysis show that sensitivity of our reference assay was 76.3% (95% CrI 59.2%-90.4%) in the ideal situation, in which convalescent samples were obtained from all patients. This increase in sensitivity is consistent with existing knowledge; however, this also suggests that a number of patients with dengue infection had a false-negative test result by our reference assay even if a convalescent-phase sample was available. Other possible explanations for the low sensitivity of our reference assay are that patients with dengue infection have variation in their immune response, insufficient time between paired serum collections, and that the cutoff level of DEN MAC and GAC ELISA used might not be optimal to detect some patients with true dengue infection [11].

Evaluation of diagnostic tests when the accuracy of the gold standard is unknown is an active area of biostatistical research, as the use of an imperfect gold standard to evaluate the accuracy of alternative tests is flawed and leads to biased results [18,24]. Our study has shown that our reference assay represents a flawed reference standard against which to compare alternative diagnostic tests for dengue infection in a prospective study, and in this study we have

demonstrated the usefulness of statistical models under such circumstances. For example, when compared with our reference assay, the IgM cassette test had a specificity of 89.5% (95% CI 86.3-92.1), representing a mediocre diagnostic test. When recalculated using Bayesian LCMs, the specificity of the IgM cassette test was 95.5% (95% CrI 92.0-98.3), representing a test with a high degree of specificity. The range of 95% CrI for specificity of the IgM cassette test estimated by the Bayesian LCM barely overlaps the range of 95% CI estimated by the gold standard model. When assessing the diagnostic utility of these rapid tests, use of estimates derived using Bayesian LCMs is preferable as they are unbiased by the false assumption that our reference assay is perfect.

Considering the true prevalence of dengue infection in a cohort population, Bayesian LCMs can be used to calculate unbiased estimates of PPV and NPV to determine the clinical usefulness of each diagnostic test and combinations of those tests. A combination of NS1, IgM and IgG on admission samples had an NPV of 95.2%, suggesting that negativity of all three tests could be used to rule out dengue infection with a high degree of accuracy in our setting. In addition, a combination of NS1 and IgM on the admission sample had a PPV of 81.5%, suggesting that positivity of either NS1 or IgM could be used to diagnose dengue infection with high level of confidence. This is consistent with many previous studies describing potential combinations of two or three tests in clinical setting [6,16,25–27]. It can be seen that the gold standard model minimally overestimates NPV for the combination of NS1, IgM and IgG performed on the admission samples compared to the Bayesian LCM (97.8 [95% CI 95.7 – 99.0] vs. 95.2 [95% CrI 91.9 – 97.5]), and markedly underestimates PPV for the combination of NS1 and IgM performed on the admission samples compared to the Bayesian LCM (47.1 [95% CI 37.8 - 56.4] vs. 81.5 [95% CrI 69.8-91.6]). Again, estimates by Bayesian LCM should be used

because it does not falsely assume that our reference assay is perfect. Note that median duration of symptoms between onset of symptoms and collection of on-admission sample in our study was 5 days.

The data set inconclusively supported a positive correlation between the two serological tests detecting IgM and IgG immune response in patients with dengue, a finding that could be interpreted as meaning that both IgM and IgG cassette tests are more likely to be positive if the immune response is high, and to be negative if it is low. A positive correlation was not found between the IgM cassette test and the reference assay and between the IgG cassette test and the reference assay, even though our reference assays detect the response of both antibodies to dengue infection. Possible explanations are that the technology and antigens used for the IgM and IgG cassette tests were different from those used for the DEN MAC and GAC ELISA, respectively. This is supported by the simple tabulation and Kappa statistics that demonstrated poor agreement between the IgM cassette test and the reference assay (Kappa value=0.38) and between the IgG cassette test and the reference assay (Kappa value=0.32).

This study has several limitations. Using basic Bayesian LCMs to estimate the sensitivity and specificity of each test in a population does not allow us to determine the effect on these parameters of symptom duration, antimicrobials received prior to presentation, and timing of convalescent samples at the level of individual patients. These effects could be evaluated in advanced Bayesian LCMs [28]. Evaluation of other diagnostic tests, including viral isolation and HAI, was not done as those tests were not performed in our cohort study. PCR was performed in only a subset of patients who had our reference assay positive to determine serotype identity or who provided admission samples only [29]; therefore, PCR could not be assessed using Bayesian LCM in this study. It should be noted that the rapid diagnostic tests evaluated in this study are

earlier versions of rapid tests for NS1, IgM and IgG. Currently available versions of these rapid diagnostic tests were only evaluated in the case-control data set of our cohort study; therefore, these newer tests could not be assessed using Bayesian LCM.

We conclude that our reference assay, a combination of AFRIMS DEN MAC and GAC ELISA on paired serum, has lower than expected sensitivity as it does not take dengue virus detection into consideration and hence is an imperfect gold standard against which to compare alternative diagnostic tests. Bayesian LCMs could be used to evaluate the accuracy of alternative diagnostic tests when the accuracy of the gold standard is unknown or is imperfect. On clinical presentation, a combination of the NS1, IgM and IgG cassette tests could be used as a set of rapid diagnostic tests for diagnosing dengue infection with a high level of accuracy.

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Table 1. Prevalence and sensitivities, specificities, and positive and negative predictive values (PPV's and NPV's) of diagnostic tests using the reference assay as gold standard and for final Bayesian latent class models

Parameters	Reference assay as gold standard ^a	Final Bayesian latent class model ^b	Final Bayesian latent class model (using admission sample only) ^b
Prevalence	15.3 (12.4 – 18.6)	24.3 (19.1 – 30.0)	NA
Reference assay			
Sensitivity	100	62.0 (49.5 – 75.9)	NA
Specificity	100	99.6 (97.9 – 100)	NA
PPV	100	97.8 (89.7 – 99.9)	NA
NPV	100	89.1 (83.1 – 94.1)	NA
Panbio NS1 antigen test			
Sensitivity	54.8 (43.5 – 65.7)	45.9 (36.0 – 56.4)	NA
Specificity	95.1 (92.7 – 96.8)	97.9 (95.5 – 99.7)	NA
PPV	66.7 (54.3 – 77.6)	87.3 (74.0 – 98.1)	NA
NPV	92.1 (89.3 – 94.3)	84.9 (79.4 – 89.6)	NA
Panbio Duo cassette IgM			
Sensitivity	50.0 (38.9 – 61.1)	54.5 (45.4 – 63.8)	39.7 (35.2 – 44.1)
Specificity	89.5 (86.3 – 92.1)	95.5 (92.0 – 98.3)	96.6 (94.6 – 98.5)
PPV	46.2 (35.6 – 56.9)	79.5 (63.5 – 92.3)	79.2 (64.2 – 91.9)
NPV	90.8 (87.8 – 93.3)	86.8 (81.7 – 90.6)	83.3 (78.5 – 87.3)

Panbio Duo cassette IgG			
Sensitivity	61.9 (50.7 – 72.3)	62.1 (52.8 – 71.4)	42.6 (38.1 – 47.1)
Specificity	79.6 (75.6 – 83.1)	84.5 (80.1 – 88.5)	87.2 (85.4 – 89.1)
PPV	35.4 (27.7 – 43.7)	56.2 (44.6 – 67.6)	51.7 (41.3 – 61.6)
NPV	92.0 (88.9 – 94.5)	87.5 (82.2 – 91.6)	82.6 (77.5 – 86.8)
Panbio NS1 + IgM ^c			
Sensitivity	79.8 (69.6 – 87.7)	78.9 (72.4 – 84.8)	72.8 (66.5 – 78.7)
Specificity	86.2 (82.8 – 89.2)	93.7 (90.7 – 96.8)	94.7 (91.9 – 97.5)
PPV	51.1 (42.3 – 60.0)	80.1 (68.2 – 90.6)	81.5 (69.8 – 91.6)
NPV	95.9 (93.6 – 97.6)	93.3 (89.5 – 95.9)	91.6 (87.5 – 94.5)
Panbio NS1 + IgM + IgG ^c			
Sensitivity	92.9 (85.1 – 97.3)	91.7 (86.2 – 96.1)	87.0 (81.2 – 92.0)
Specificity	72.5 (68.2 – 76.5)	79.8 (76.6 – 83.3)	82.8 (79.9 – 86.0)
PPV	37.9 (31.2 – 44.9)	59.3 (48.9 – 69.0)	62.0 (51.8 – 71.4)
NPV	98.3 (96.2 – 99.4)	96.8 (93.9 – 98.7)	95.2 (91.9 – 97.5)

^a Prevalence and accuracy of each test was estimated by the observed proportion classified by considering that the reference assay was perfect (100% sensitivity and 100% specificity). 95% confidence intervals (CI) were obtained in Stata 11.1.

^b Prevalence and accuracy of each test was estimated by Bayesian latent class models by considering that the reference assay could be imperfect. Posterior estimates and 95% credible intervals (CrI) of each parameter were obtained in WinBUGs from 10,000 iterations of each of two chains starting from different initial values following a burn-in period of 5,000 iterations.

^c A combination considers that positivity of either test is positive for dengue infection.

NA = Not applicable

Table 2. Description and model selection criteria

Model	Correlation^a	Scientific Background	DIC^b	AIC^c
0	None	It is possible that all (NS1, IgM, IgG and the reference assay) are not correlated.	113.9	110.3
1	IgM and the reference assay	IgM and the reference assay are based on IgM response. Both tests are more likely to be positive if the amount of IgM in blood in an infected subject is high, and to be negative if the amount of IgM in blood in the infected subject is low.	113.0	111.1
2	IgM and IgG	IgM and IgG are based on antibody response. Both tests are more likely to be positive if antibody response in an infected subject is high, and to be negative if antibody response in the infected subject is low.	108.5	106.7
3	IgG and the reference assay	IgG and the reference assay are based on IgG response. Both tests are more likely to be positive if the amount of IgG in blood in an infected subject is high, and to be negative if the amount of IgG in blood in the infected subject is low.	112.6	110.9
4	IgM, IgG and the reference assay	IgM, IgG and the reference assay are based on antibody response. All three tests are more likely to be positive if antibody response in an infected subject is high, and to be negative if antibody response in the infected subject is low.	NA	110.7

^a All correlations were in infected subjects.

^b **DIC** (deviance information criteria) is a generalization of AIC in a Bayesian setting. DIC was not applicable (NA) in model 4, which assumed a correlation between more than two tests.

^c **AIC** (akaike information criteria) were used to evaluate goodness of model fit and to compare models.

A difference in DIC or AIC of more than 10 indicated definite support to the model with the lower value, while a difference of between 5 and 10 was considered substantial, and less than 5 inconclusive.

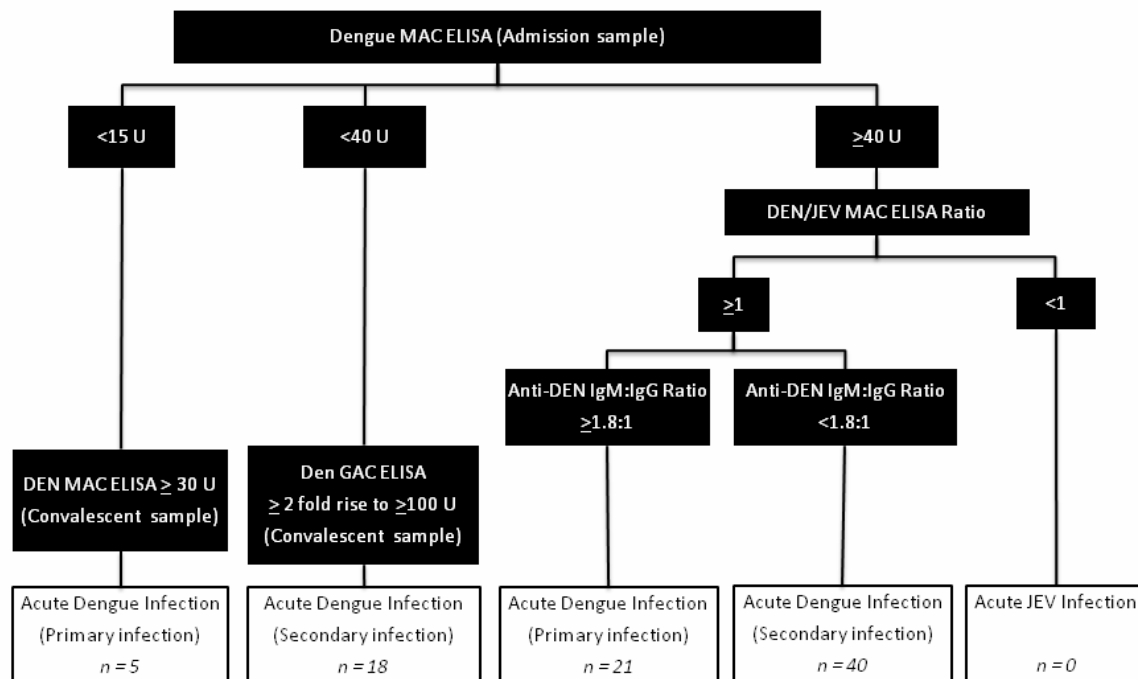


Figure 1. Flowchart showing the AFRIMS diagnostic algorithm for dengue infection

Supporting information legends (ดูรายละเอียดในภาคผนวก)

Supporting information file1

Text S1. Ragama fever study dataset

Supporting information file 2

Text S2.1. WinBUGS code for Model 0

Text S2.2. WinBUGS code for Model 1 (Bernoulli model with random effect)

Text S2.3. WinBUGS code for Model 2 (Bernoulli model with random effect)

Text S2.4. WinBUGS code for Model 3 (Bernoulli model with random effect)

Text S2.5. WinBUGS code for Model 4 (Bernoulli model with random effect)

Supporting information file3

Table S3.1. Prevalence, sensitivities and specificities (95% credible interval) for different diagnostic tests of dengue using Bayesian latent class and random-effects models in full data set (N=549)

Table S3.2. Prevalence, sensitivities and specificities for different diagnostic tests of dengue using Bayesian latent class and random-effects models in full data set (N=549) and in a subset of patients who have convalescent samples (N=290)

Table S3.3. Prevalence, sensitivities and specificities for different diagnostic tests of dengue using Bayesian latent class and random-effects models with non-informative, sceptical and enthusiastic priors

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ (ระบุชื่อผู้แต่ง ชื่อเรื่อง ชื่อวารสาร ปี เล่มที่ เลขที่ และหน้า)

ชื่อเรื่อง Estimating the true accuracy of diagnostic tests for Dengue infection using Bayesian latent class models

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2. การนำผลงานวิจัยไปใช้ประโยชน์
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งานวิจัยนี้ร่วมกับงานวิจัยอื่นๆ ได้แก่ Blacksell 2011, Hu 2011, Wang 2010 และ Fry 2011 เป็นต้น มีบทสรุปที่สนับสนุนการรวมชุดตรวจสองชนิดขึ้นไปไว้ในบรรจุภัณฑ์เดียวกัน และเลือกหาการรวมชุดตรวจที่ดีที่สุด ตัวอย่างการรวมชุดตรวจสองชนิดขึ้นไปในบรรจุภัณฑ์เดียวที่มีจำหน่ายตามท้องตลาดแล้วได้แก่ Panbio Dengue Duo Cassette ผลิตโดย Inverness, BIOLINE Dengue Duo NS1 antigen & IgG & IgM Combo Device ผลิตโดย Standard Diagnostics เป็นต้น

- เชิงสาธารณะ (มีเครือข่ายความร่วมมือ/สร้างกระแสความสนใจในวงกว้าง)

มีการสร้างกระแสความสนใจของการนำแบบจำลองเชิงคณิตศาสตร์ชนิดกลุ่มแฝง (Bayesian Latent Class Models) ไปประเมินความไวและความจำเพาะของการตรวจวินิจฉัยไข้ไทฟอยด์ ซึ่งเข้าข่ายเดียวกับไข้เลือดออกที่ไม่มีวิธีการตรวจสอบโรคที่ให้ผลถูกต้องที่เหมาะสมจะนำมาใช้เป็นที่อ้างอิงได้ งานวิจัยอยู่ในระหว่างการดำเนินการและกำลังเขียนต้นร่าง

ชื่อเรื่อง Evaluation of a Typhoid IgM flow assay for the diagnosis of typhoid fever in Cambodian children using a Bayesian modeling approach assuming an imperfect gold standard

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ไม่มี
- 3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

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ESTIMATING THE TRUE ACCURACY OF DIAGNOSTIC TESTS FOR DENGUE INFECTION USING BAYESIAN LATENT CLASS MODELS

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Number of pages 27; tables 2; figures 1; supplementary files 3

(ดูรายละเอียดในเนื้อหาของงานวิจัย)

Supporting information file 1

Text S1 Ragama fever study dataset

The data used in Bayesian latent class models were structured as a matrix of test result profile of each individual subject (549 records). The value of 1 represents a positive and 0 represents a negative test result. Each column of matrix “y” represents Panbio NS1 antigen, Panbio IgM antibody, Panbio IgG antibody, reference assay, a combination between Panbio NS1 and IgM and a combination between Panbio NS1 IgM and IgG. Each row represents the result profile for each patient. Variable ‘sample.size’ represents the number of patients in the study, ‘no.test’ represents the the diagnostic tests being used in the likelihood (Panbio NS1 antigen, Panbio IgM antibody, Panbio IgG antibody and Reference assay), ‘permu’ represents all possible test result profiles. This is used to calculate the posterior predicted frequency of each profile.

```
# data
list(
  sample.size=549,
  no.test=4,
  permu=16,
  y=structure(.Data=c(0,0,0,0,0,0,
0,0,0,0,0,0,
0,0,0,0,0,0,
1,1,1,1,1,1,
1,1,1,0,1,1,
1,0,0,0,1,1,
1,1,0,1,1,1,
0,0,0,0,0,0,
0,0,0,0,0,0,
0,0,0,0,0,0,
(remaining data was omitted)
```

```
0,0,0,0,0,0,
0,0,0,0,0,0,
0,0,0,0,0,0,
0,0,0,0,0,0,
0,0,0,0,0,0,
0,0,0,0,0,0,
1,1,0,1,1,1,
0,1,1,0,1,1,
0,1,0,0,1,1,
1,1,0,1,1,1),.Dim=c(549,6)) )
```

Supporting information file 2

Text S2.1 WinBUGS code for Model 0

A Bernoullie model – conditional independence model (result of each diagnostic test on a given patient was independent conditional on true disease status of the patient)

```
#Model 0
#NS1: j = 1
#IgM: j = 2
#IgG: j = 3
#Reference assay: j = 4
#Combination of NS1 and IgM (either positive is positive): j = 5
#Combination of NS1, IgM and Ig G (any positive is positive): j = 6
#No random effect
model{
# Likelihood
for (i in 1:sample.size){
  disease[i]~dbern(prev)
for (j in 1:no.test){
  y[i,j]~dbern(p[i,j])
  ypred[i,j]~dbern(p[i,j])
  logit(p[i,j])<-disease[i]*alpha[j]+(1-disease[i])*beta[j]
  accuracy[i,j]<-equals(disease[i],y[i,j])
}
}
# Combination
for (i in 1:sample.size){
for (j in 5:6){
  positive.in.disease[i,j]<-disease[i]*y[i,j]
  positive.in.nodisease[i,j]<-(1-disease[i])*y[i,j]
  accuracy[i,j]<-equals(disease[i],y[i,j])
}
```

```

    }
  }
# Prior
prev~dbeta(1,1)
for (j in 1:no.test) {
  s[j]~dbeta(1,1)
  alpha[j]<-logit(s[j])
  x[j]~dbeta(1,1)|(0.6,)
  beta[j]<--logit(x[j])
}
# Prediction
for (i in 1:sample.size) {
  for (k in 1:permu) {
    for (j in 1:no.test) {
      arraymatched.predicted[i,k,j]<-equals(ypred[i,j],profile[k,j])
      arraymatched.observed[i,k,j]<-equals(y[i,j],profile[k,j])
    }
    nmatched.predicted[i,k]<-sum(arraymatched.predicted[i,k,])
    matchedprofile.predicted[i,k]<-equals(nmatched.predicted[i,k],no.test)
    nmatched.observed[i,k]<-sum(arraymatched.observed[i,k,])
    matchedprofile.observed[i,k]<-equals(nmatched.observed[i,k],no.test)
  }
}
for(k in 1:permu){
  freq.predicted[k]<-sum(matchedprofile.predicted[,k])
  freq.observed[k]<-sum(matchedprofile.observed[,k])
}
# Combination
for (j in 5:6){
  s[j]<-sum(positive.in.disease[,j]) / sum(disease[])
  x[j]<-1-sum(positive.in.nodisease[,j])/(sample.size-sum(disease[]))
}

```

```

# PPV and NPV
for (j in 1:6){
  ppv[j]<- s[j]*prev / (s[j]*prev + (1-x[j])*(1-prev))
  npv[j]<- x[j]*(1-prev) / (x[j]*(1-prev) + (1-s[j])*(prev))
  acc[j]<- sum(accuracy[,j]) / sample.size
}

# Bayesian p value
for (k in 1:permu) {
  pvalue[k]<-step(freq.predicted[k]-freq.observed[k])
}

}

```

Text S2.2 WinBUGS code for Model 1 (Bernoulli model with random effect)

Bernoulli model – conditional dependence between IgM and Reference assay (results of IgM and the reference assay of infected patients were correlated)

Model 1 (Random effect)

Random effect variable represented different IgM level in each infected subject

#Model 1

#NS1: j = 1

#IgM: j = 2

#IgG: j = 3

#Reference assay: j = 4

#Combination of NS1 and IgM (either positive is positive): j = 5

#Combination of NS1, IgM and Ig G (any positive is positive): j = 6

#Random effect, IgM, represents variation in IgM in diseased subject

model{

Likelihood

for (i in 1:sample.size){

 disease[i]~dbern(prev)

 re[i] ~ dnorm(0,1)

for (j in 1:no.test){

 y[i,j]~dbern(p[i,j])

 ypred[i,j]~dbern(p[i,j])

 accuracy[i,j]<-equals(disease[i],y[i,j])

 }

#NS1

 logit(p[i,1])<-disease[i]*alpha[1]+(1-disease[i])*beta[1]

#IgM

 logit(p[i,2])<-disease[i]*alpha[2]+(1-disease[i])*beta[2]+disease[i]*IgM*re[i]

 logit(positive.in.disease[i,2])<-disease[i]*alpha[2]+disease[i]*IgM*re[i]+(1-disease[i])*(-1000)

```

#lgG
logit(p[i,3])<-disease[i]*alpha[3]+(1-disease[i])*beta[3]
#Reference assay
logit(p[i,4])<-disease[i]*alpha[4]+(1-disease[i])*beta[4]+disease[i]*lgM*re[i]
logit(positive.in.disease[i,4])<-disease[i]*alpha[4]+disease[i]*lgM*re[i]+(1-disease[i])*(-1000)
}
# Combination
for (i in 1:sample.size){
for (j in 5:6){
positive.in.disease[i,j]<-disease[i]*y[i,j]
positive.in.nodisease[i,j]<-(1-disease[i])*y[i,j]
accuracy[i,j]<-equals(disease[i],y[i,j])
}
}
# Prior
prev~dbeta(1,1)
lgM~dnorm(0,0.1)|0,.)
for (j in 1:no.test) {
s[j]~dbeta(1,1)
alpha[j]<-logit(s[j])
x[j]~dbeta(1,1)|0.4,)
beta[j]<--logit(x[j])
}
se[1] <- s[1]
se[2] <- sum(positive.in.disease[,2])/sum(disease[])
se[3] <- s[3]
se[4] <- sum(positive.in.disease[,4])/sum(disease[])
# Prediction (similar to Text S2.1)
# Predicted and observed frequency (similar to Text S2.1)
# Combination
for (j in 5:6){
s[j]<-sum(positive.in.disease[,j]) / sum(disease[])

```

```

        x[j]<-1-sum(positive.in.nodisease[,j])/(sample.size-sum(disease[]))
        se[j] <- s[j]
    }
# PPV and NPV
for (j in 1:6){
    ppv[j]<- se[j]*prev / (se[j]*prev + (1-x[j])*(1-prev))
    npv[j]<- x[j]*(1-prev) / (x[j]*(1-prev) + (1-se[j])*(prev))
    acc[j]<- sum(accuracy[,j]) / sample.size
}
# Bayesian p value (similar to Text S2.1)
}

```

Text S2.3 WinBUGS code for Model 2 (Bernoulli model with random effect)

Bernoulli model – conditional dependence between IgM and IgG (results of IgM and IgG of infected patients were correlated)

Model 2 (Random effect)

Random effect variable represented different IgM and IgG antibodies level in each infected subject

#Model 2

#NS1: j = 1

#IgM: j = 2

#IgG: j = 3

#Reference assay: j = 4

#Combination of NS1 and IgM (either positive is positive): j = 5

#Combination of NS1, IgM and Ig G (any positive is positive): j = 6

#Random effect, IgMG, represents variation in IgM and IgG in diseased subject

model{

Likelihood

for (i in 1:sample.size){

 disease[i]~dbern(prev)

 re[i] ~ dnorm(0,1)

for (j in 1:no.test){

 y[i,j]~dbern(p[i,j])

 ypred[i,j]~dbern(p[i,j])

 accuracy[i,j]<-equals(disease[i],y[i,j])

 }

#NS1

 logit(p[i,1])<-disease[i]*alpha[1]+(1-disease[i])*beta[1]

#IgM

 logit(p[i,2])<-disease[i]*alpha[2]+(1-disease[i])*beta[2]+disease[i]*IgMG*re[i]

```

logit(positive.in.disease[i,2])<-disease[i]*alpha[2]+disease[i]*lgMG*re[i]+(1-disease[i])*(-1000)
#lgG
logit(p[i,3])<-disease[i]*alpha[3]+(1-disease[i])*beta[3]+disease[i]*lgMG*re[i]
logit(positive.in.disease[i,3])<-disease[i]*alpha[3]+disease[i]*lgMG*re[i]+(1-disease[i])*(-1000)
#Reference assay
logit(p[i,4])<-disease[i]*alpha[4]+(1-disease[i])*beta[4]
}
# Combination
for (i in 1:sample.size){
for (j in 5:6){
positive.in.disease[i,j]<-disease[i]*y[i,j]
positive.in.nodisease[i,j]<-(1-disease[i])*y[i,j]
accuracy[i,j]<-equals(disease[i],y[i,j])
}
}
# Prior
prev~dbeta(1,1)
lgMG~dnorm(0,0.1)l(0,)
for (j in 1:no.test) {
s[j]~dbeta(1,1)
alpha[j]<-logit(s[j])
x[j]~dbeta(1,1)l(0.4,)
beta[j]<--logit(x[j])
}
se[1] <- s[1]
se[2] <- sum(positive.in.disease[,2])/sum(disease[])
se[3] <- sum(positive.in.disease[,3])/sum(disease[])
se[4] <- s[4]

# Prediction (similar to Text S2.1)
# Predicted and observed frequency (similar to Text S2.1)
# Sensitivity and specificity of the combinations (similar to Text S2.2)

```

```
# PPV and NPV (similar to Text S2.2)
# Bayesian p value (similar to Text S2.1)
```

```
}
```

Text S2.4 WinBUGS code for Model 3 (Bernoulli model with random effect)

```
# Bernoulli model – conditional dependence between IgG and the reference assay (results of IgG
and the reference assay of infected patients were correlated)
```

```
# Model 3 (Random effect)
```

```
# Random effect variable represented different IgG level in each infected subject
```

```
#Model 3
```

```
#Bernoulli model - conditional dependence, re, between IgG and the reference assay
```

```
#NS1: j = 1
```

```
#IgM: j = 2
```

```
#IgG: j = 3
```

```
#Reference assay: j = 4
```

```
#Combination of NS1 and IgM (either positive is positive): j = 5
```

```
#Combination of NS1, IgM and Ig G (any positive is positive): j = 6
```

```
#Random effect, IgG, represents variation in IgG in diseased subject
```

```
model{
```

```
# Likelihood
```

```
for (i in 1:sample.size){
```

```
    disease[i]~dbern(prev)
```

```
    re[i] ~ dnorm(0,1)
```

```
for (j in 1:no.test){
```

```
    y[i,j]~dbern(p[i,j])
```

```
    ypred[i,j]~dbern(p[i,j])
```

```
    accuracy[i,j]<-equals(disease[i],ypred[i,j])
```

```
    }
```

```
#NS1
```

```
    logit(p[i,1])<-disease[i]*alpha[1]+(1-disease[i])*beta[1]
```

```

#IgM
logit(p[i,2])<-disease[i]*alpha[2]+(1-disease[i])*beta[2]
#IgG
logit(p[i,3])<-disease[i]*alpha[3]+(1-disease[i])*beta[3]+disease[i]*lgG*re[i]
logit(positive.in.disease[i,3])<-disease[i]*alpha[3]+disease[i]*lgG*re[i]+(1-disease[i])*(-1000)
#Reference assay
logit(p[i,4])<-disease[i]*alpha[4]+(1-disease[i])*beta[4]+disease[i]*lgG*re[i]
logit(positive.in.disease[i,4])<-disease[i]*alpha[4]+disease[i]*lgG*re[i]+(1-disease[i])*(-1000)
}
# Combination
for (i in 1:sample.size){
for (j in 5:6){
positive.in.disease[i,j]<-disease[i]*y[i,j]
positive.in.nodisease[i,j]<-(1-disease[i])*y[i,j]
accuracy[i,j]<-equals(disease[i],y[i,j])
}
}
# Prior
prev~dbeta(1,1)
lgG~dnorm(0,0.1)l(0,)
for (j in 1:no.test) {
s[j]~dbeta(1,1)
alpha[j]<-logit(s[j])
x[j]~dbeta(1,1)l(0.4,)
beta[j]<--logit(x[j])
}
se[1] <- s[1]
se[2] <- s[2]
se[3] <- sum(positive.in.disease[,3])/sum(disease[])
se[4] <- sum(positive.in.disease[,4])/sum(disease[])

# Prediction (similar to Text S2.1)

```

```
# Predicted and observed frequency (similar to Text S2.1)
# Sensitivity and specificity of the combinations (similar to Text S2.2)
# PPV and NPV (similar to Text S2.2)
# Bayesian p value (similar to Text S2.1)

}
```

Text S2.5 WinBUGS code for Model 4 (Bernoulli model with random effect)

Bernoulli model – conditional dependence between all serological tests (results of IgM, IgG and the Reference assay of infected patients were correlated)

Model 4 (Random effect)

Random effect variable represented different antibodies level in each infected subject

#Model 4

#NS1: j = 1

#IgM: j = 2

#IgG: j = 3

#Reference assay: j = 4

#Combination of NS1 and IgM (either positive is positive): j = 5

#Combination of NS1, IgM and Ig G (any positive is positive): j = 6

#Random effect, Ab, represents variation in antibodies level (IgM, IgG and reference assay) in diseased subject

model{

Likelihood

for (i in 1:sample.size){

 disease[i]~dbern(prev)

 re[i] ~ dnorm(0,1)

#NS1

 y[i,1]~dbern(p[i,1])

 ypred[i,1]~dbern(p[i,1])

 logit(p[i,1])<-disease[i]*alpha[1]+(1-disease[i])*beta[1]

 accuracy[i,1]<-equals(disease[i],y[i,1])

for (j in 2:no.test){

 y[i,j]~dbern(p[i,j])

 ypred[i,j]~dbern(p[i,j])

 logit(p[i,j])<-disease[i]*alpha[j]+(1-disease[i])*beta[j]+disease[i]*Ab*re[i]

```

    logit(positive.in.disease[i,j])<-disease[i]*alpha[j]+disease[i]*Ab*re[i]+(1-disease[i])*(-1000)
    accuracy[i,j]<-equals(disease[i],y[i,j])
  }
}

# Combination
for (i in 1:sample.size){
  for (j in 5:6){
    positive.in.disease[i,j]<-disease[i]*y[i,j]
    positive.in.nodisease[i,j]<-(1-disease[i])*y[i,j]
    accuracy[i,j]<-equals(disease[i],y[i,j])
  }
}

# Prior
prev~dbeta(1,1)
Ab~dnorm(0,0.1)l(0,)
for (j in 1:no.test) {
  s[j]~dbeta(1,1)
  alpha[j]<-logit(s[j])
  x[j]~dbeta(1,1)l(0.4,)
  beta[j]<--logit(x[j])
}

se[1] <- s[1]
for (j in 2:no.test){
  se[j] <-sum(positive.in.disease[,j])/sum(disease[])
}

# Prediction (similar to Text S2.1)
# Predicted and observed frequency (similar to Text S2.1)
# Sensitivity and specificity of the combinations (similar to Text S2.2)
# PPV and NPV (similar to Text S2.2)
# Bayesian p value (similar to Text S2.1)
}

```

Supporting information file 3

Table S3.1 Prevalence, sensitivities and specificities (95% credible interval) for different diagnostic tests of dengue using Bayesian latent class and random-effects models in full data set (N=549)

Parameters	Model 0 ^a	Model 1 ^b	Model 2 ^c	Model 3 ^d	Model 4 ^e
Prevalence	23.8 (18.4 – 29.8)	23.9 (18.3 – 30.2)	24.3 (19.1 – 30.0)	24.1 (18.4 – 30.3)	24.6 (18.7 – 31.6)
Reference assay					
Sensitivity	63.0 (50.1 – 77.9)	62.5 (49.4 – 77.6)	62.0 (49.5 – 75.9)	62.1 (49.1 – 77.4)	60.9 (47.5 – 76.9)
Specificity	99.4 (97.7 – 100)	99.4 (97.7 – 100)	99.6 (97.9 – 100)	99.4 (97.7 – 100)	99.5 (97.7 – 100)
PPV	97.3 (88.5 – 99.9)	97.3 (88.7 – 99.9)	97.8 (89.7 – 99.9)	97.2 (88.8 – 100)	97.4 (88.8 – 99.9)
NPV	89.6 (83.4 – 94.9)	89.5 (82.7 – 94.9)	89.1 (83.1 – 94.1)	89.2 (82.6 – 94.8)	88.7 (81.2 – 94.5)
Panbio NS1 antigen test					
Sensitivity	45.4 (35.3 – 56.9)	45.5 (35.2 – 57.3)	45.9 (36.0 – 56.4)	45.3 (35.3 – 57.4)	45.6 (35.1 – 57.8)
Specificity	97.5 (95.3 – 99.3)	97.6 (95.2 – 99.3)	97.9 (95.5 – 99.7)	97.6 (95.4 – 99.5)	98.0 (95.6 – 99.8)
PPV	84.7 (72.0 – 95.6)	85.4 (72.2 – 96.0)	87.3 (74.0 – 98.1)	85.8 (72.6 – 97.0)	88.2 (73.9 – 99.1)
NPV	85.2 (79.3 – 90.3)	85.1 (79.1 – 90.4)	84.9 (79.4 – 89.6)	85.0 (78.8 – 90.4)	84.7 (77.8 – 90.2)
Panbio Duo cassette IgM					
Sensitivity	55.9 (45.9 – 66.1)	55.1 (45.3 – 65.2)	54.5 (45.4 – 63.8)	55.5 (45.3 – 65.3)	53.4 (42.7 – 64.2)
Specificity	95.6 (91.8 – 98.7)	95.5 (91.6 – 98.6)	95.5 (92.0 – 98.3)	95.7 (91.6 – 99.0)	95.4 (91.3 – 98.6)
PPV	79.7 (61.8 – 94.1)	79.3 (60.9 – 93.9)	79.5 (63.5 – 92.3)	80.3 (60.9 – 95.5)	79.1 (60.7 – 94.1)
NPV	87.5 (82.4 – 91.4)	87.2 (82.0 – 91.3)	86.8 (81.7 – 90.6)	87.2 (82.0 – 91.3)	86.4 (80.1 – 90.9)
Panbio Duo cassette IgG					
Sensitivity	63.7 (54.0 – 73.4)	63.6 (53.8 – 72.9)	62.1 (52.8 – 71.4)	62.6 (52.6 – 72.2)	61.6 (50.7 – 71.9)
Specificity	84.7 (80.2 – 88.7)	84.7 (80.2 – 89.1)	84.5 (80.1 – 88.5)	84.5 (80.0 – 88.7)	84.5 (80.0 – 88.8)
PPV	56.5 (43.7 – 68.4)	56.8 (43.3 – 69.7)	56.2 (44.6 – 67.6)	56.2 (42.9 – 68.3)	56.4 (43.0 – 69.1)
NPV	88.3 (83.2 – 92.3)	88.2 (82.9 – 92.2)	87.5 (82.2 – 91.6)	87.8 (82.2 – 92.0)	87.1 (80.6 – 91.8)
Panbio NS1 + IgM^f					
Sensitivity	79.6 (72.9 – 85.4)	79.0 (71.8 – 85.1)	78.9 (72.4 – 84.8)	79.4 (72.6 – 85.3)	78.2 (69.6 – 84.8)
Specificity	93.4 (90.1 – 96.8)	93.4 (90.0 – 96.7)	93.7 (90.7 – 96.8)	93.7 (90.0 – 97.4)	93.7 (90.2 – 97.4)
PPV	79.1 (66.0 – 90.4)	79.0 (65.6 – 90.5)	80.1 (68.2 – 90.6)	80.0 (65.9 – 92.4)	80.2 (66.6 – 92.4)
NPV	93.7 (89.8 – 96.2)	93.4 (89.2 – 96.2)	93.3 (89.5 – 95.9)	93.5 (90.0 – 96.1)	93.0 (87.9 – 96.0)
Panbio NS1 + IgM + IgG^f					
Sensitivity	93.2 (88.8 – 97.2)	93.0 (88.2 – 97.0)	91.7 (86.2 – 96.1)	92.7 (87.8 – 96.9)	91.8 (84.2 – 96.6)

Specificity	79.8 (76.1 – 83.8)	79.9 (76.0 – 84.1)	79.8 (76.6 – 83.3)	79.9 (76.0 – 84.1)	80.0 (76.2 – 84.4)
PPV	59.0 (47.8 – 69.7)	59.3 (47.4 – 70.5)	59.3 (48.9 – 69.0)	59.4 (47.7 – 70.7)	59.9 (48.0 – 71.8)
NPV	97.4 (95.1 – 99.1)	97.4 (94.8 – 99.0)	96.8 (93.9 – 98.7)	97.2 (94.5 – 99.0)	96.8 (92.5 – 98.8)

- ^a The model assumes that NS1, IgM, IgG and the reference assay are not correlated.
- ^b The model assumes that IgM and the reference assay are correlated based on IgM response i.e. both tests are more likely to be positive if the amount of IgM in blood in an infected subject is high, and to be negative if the amount of IgM in blood in the infected subject is low.
- ^c The model assumes that IgM and IgG tests are correlated based on antibody response i.e. both tests are more likely to be positive if antibody response in an infected subject is high, and to be negative if antibody response in the infected subject is low.
- ^d The model assumes that IgG and the reference assay are correlated based on IgG response i.e. both tests are more likely to be positive if the amount of IgG in blood in an infected subject is high, and to be negative if the amount of IgG in blood in the infected subject is low.
- ^e IgM, IgG and the reference assay are correlated based on antibody response i.e. all three tests are more likely to be positive if antibody response in an infected subject is high, and to be negative if antibody response in the infected subject is low.
- ^f A combination considers that positivity of either test is positive for dengue infection.

Table S3.2 Prevalence, sensitivities and specificities for different diagnostic tests of dengue using Bayesian latent class and random-effects models in full data set (N=549) and in a subset of patients who have convalescent samples (N=290)

Parameters	Final Bayesian model in full data set (95% credible interval)	Final Bayesian model in patients with convalescent samples (95% credible interval)
Prevalence	24.3 (19.1 – 30.0)	25.9 (19.8 – 33.7)
Reference assay		
Sensitivity	62.0 (49.5 – 75.9)	76.3 (59.2 – 90.4)
Specificity	99.6 (97.9 – 100)	98.9 (95.9 – 100)
PPV	97.8 (89.7 – 99.9)	96.1 (85.1 – 99.8)
NPV	89.1 (83.1 – 94.1)	92.3 (84.0 – 97.3)
Panbio NS1 antigen test		
Sensitivity	45.9 (36.0 – 56.4)	58.2 (43.6 – 72.8)
Specificity	97.9 (95.5 – 99.7)	99.5 (97.5 – 100)
PPV	87.3 (74.0 – 98.1)	97.7 (88.3 – 100)
NPV	84.9 (79.4 – 89.6)	87.3 (78.9 – 92.9)
Panbio Duo cassette IgM		
Sensitivity	54.5 (45.4 – 63.8)	60.8 (49.5 – 71.7)
Specificity	95.5 (92.0 – 98.3)	89.0 (82.9 – 94.8)
PPV	79.5 (63.5 – 92.3)	65.7 (48.5 – 84.6)
NPV	86.8 (81.7 – 90.6)	86.7 (80.9 – 91.2)
Panbio Duo cassette IgG		
Sensitivity	62.1 (52.8 – 71.4)	68.3 (57.0 – 78.3)
Specificity	84.5 (80.1 – 88.5)	75.4 (68.7 – 82.1)
PPV	56.2 (44.6 – 67.6)	49.2 (36.3 – 64.1)
NPV	87.5 (82.2 – 91.6)	87.2 (81.1 – 91.9)
Panbio NS1 + IgM^a		
Sensitivity	78.9 (72.4 – 84.8)	87.7 (81.3 – 94.0)
Specificity	93.7 (90.7 – 96.8)	89.0 (85.7 – 94.1)
PPV	80.1 (68.2 – 90.6)	73.7 (62.2 – 87.2)
NPV	93.3 (89.5 – 95.9)	95.4 (91.4 – 98.1)
Panbio NS1 + IgM + IgG^a		
Sensitivity	91.7 (86.2 – 96.1)	94.5 (89.9 – 98.7)
Specificity	79.8 (76.6 – 83.3)	70.5 (67.5 – 75.7)
PPV	59.3 (48.9 – 69.0)	52.9 (42.7 – 65.3)
NPV	96.8 (93.9 – 98.7)	97.4 (94.3 – 99.5)

^aA combination considers that positivity of either test is positive for dengue infection.

Table S3.3 Prevalence, sensitivities and specificities for different diagnostic tests of dengue using Bayesian latent class and random-effects models with non-informative, sceptical and enthusiastic priors

Parameters	Bayesian model with non-informative priors (95% credible interval) ^a	Bayesian model with sceptical priors (95% credible interval) ^b	Bayesian model with enthusiastic priors (95% credible interval) ^c
Prevalence	24.3 (19.1 – 30.0)	23.2 (18.0 – 28.6)	24.2 (18.9 – 30.0)
Reference assay			
Sensitivity	62.0 (49.5 – 75.9)	63.8 (51.6 – 77.3)	63.7 (51.3 – 77.4)
Specificity	99.6 (97.9 – 100)	99.0 (97.1 – 99.8)	99.8 (98.4 – 100)
PPV	97.8 (89.7 – 99.9)	95.0 (85.7 – 99.2)	99.1 (92.3 – 100)
NPV	89.1 (83.1 – 94.1)	90.1 (84.6 – 94.8)	89.6 (83.7 – 94.6)
Panbio NS1 antigen test			
Sensitivity	45.9 (36.0 – 56.4)	46.9 (36.6 – 58.1)	47.5 (37.8 – 57.9)
Specificity	97.9 (95.5 – 99.7)	97.3 (95.0 – 99.1)	98.1 (95.8 – 99.9)
PPV	87.3 (74.0 – 98.1)	84.1 (70.4 – 94.9)	88.6 (75.5 – 99.7)
NPV	84.9 (79.4 – 89.6)	85.9 (80.1 – 90.5)	85.4 (80.1 – 90.1)
Panbio Duo cassette IgM			
Sensitivity	54.5 (45.4 – 63.8)	55.2 (46.1 – 64.8)	55.6 (46.5 – 64.7)
Specificity	95.5 (92.0 – 98.3)	94.8 (91.2 – 97.6)	95.5 (92.0 – 98.4)
PPV	79.5 (63.5 – 92.3)	76.1 (60.0 – 89.3)	80.0 (64.1 – 92.9)
NPV	86.8 (81.7 – 90.6)	87.6 (83.0 – 91.4)	87.1 (82.4 – 91.0)
Panbio Duo cassette IgG			
Sensitivity	62.1 (52.8 – 71.4)	62.9 (53.9 – 72.0)	63.0 (53.8 – 71.9)
Specificity	84.5 (80.1 – 88.5)	84.0 (79.6 – 87.9)	84.6 (80.3 – 88.4)
PPV	56.2 (44.6 – 67.6)	54.2 (42.3 – 65.4)	56.5 (45.3 – 67.1)

NPV	87.5 (82.2 – 91.6)	88.3 (83.5 – 92.2)	87.8 (82.6 – 91.8)
Panbio NS1 + IgM ^d			
Sensitivity	78.9 (72.4 – 84.8)	80.0 (73.4 – 85.9)	79.4 (73.4 – 84.5)
Specificity	93.7 (90.7 – 96.8)	93.0 (90.0 – 95.8)	93.8 (90.6 – 97.0)
PPV	80.1 (68.2 – 90.6)	77.5 (65.3 – 87.4)	80.2 (67.8 – 91.3)
NPV	93.3 (89.5 – 95.9)	93.9 (90.5 – 96.4)	93.5 (90.0 – 95.8)
Panbio NS1 + IgM + IgG ^d			
Sensitivity	91.7 (86.2 – 96.1)	92.5 (87.3 – 96.9)	91.9 (87.2 – 95.8)
Specificity	79.8 (76.6 – 83.3)	79.0 (75.9 – 82.2)	79.7 (76.5 – 83.3)
PPV	59.3 (48.9 – 69.0)	57.1 (46.8 – 66.4)	59.0 (48.8 – 69.0)
NPV	96.8 (93.9 – 98.7)	97.3 (94.6 – 99.0)	96.9 (94.3 – 98.5)

^a Beta(1,1) was selected as non-informative priors and used for sensitivities and specificities of every test. This prior assumed that no prior information (non-informative priors) about the unknown parameters (prevalence, sensitivities and specificities) was available.

^b Beta(2,2) was selected as sceptical priors and used for sensitivities and specificities of every test. This prior strongly believed that sensitivity and specificity of each test is around 50%, and there is less than 2% chance that sensitivity and specificity of each test is more than 95% or less than 5%.

^c Beta(3.5,0.5) was selected as enthusiastic priors and used for sensitivities and specificities of every test. This prior strongly believed that sensitivity and specificity of each test is close to 93%, and there is less than 5% chance that sensitivity and specificity of each test is than 50%.

^d A combination considers that positivity of either test is positive for dengue infection.

Poster presented at the Oxford Network Meeting, Vientiane, Laos in February 2554

(see Poster.pdf for detail)

Background

There are two main methods for diagnosing dengue infection, namely virus and antibody detection. The Armed Forces Institute of Medical Sciences (AFRIMS) diagnostic serology methodologies (Figure 1) have been used repeatedly on paired sera as a reference assay to determine accuracy of alternative diagnostic tests [1]. We hypothesized that the accuracy of this reference assay is imperfect, and that the accuracy of the alternative diagnostic tests estimated by comparing them with the reference assay might have been underestimated.

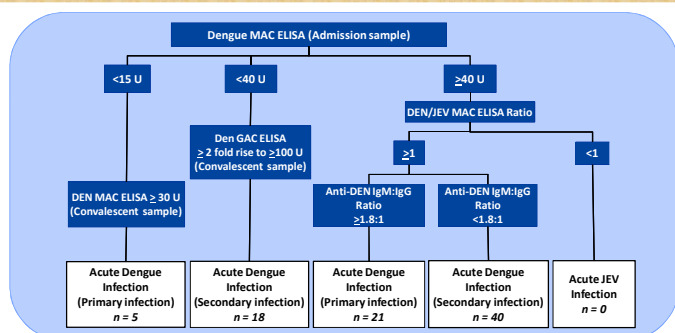


Figure 1: Flowchart showing the AFRIMS diagnostic algorithm for dengue infection

Objective

To use Bayesian Latent Class Model (LCM) to estimate the accuracy of three rapid diagnostic tests (Panbio NS1, IgM and IgG cassette tests), of the reference assay for dengue infection, and of those when used in combination and on clinical presentation.

Data and Methods

Data from a prospective cohort study of patients suspected of dengue during Ragama Fever Study conducted in Sri Lanka between 2006 and 2007 were used. Bayesian approach was taken to assess the accuracy of these commercial test kits compared with the traditional technique of using a gold standard. The Bayesian LCM of conditional independence and with appropriate conditional dependency, when correlations between tests can be explained scientifically, were explored.

Bayesian LCM does not assume that any test is perfect, but considers that each test could be imperfect in diagnosing the true disease status. The true disease status of the patient population is then defined on the basis of overall prevalence (the probability that a patient with suspected dengue is truly infected with dengue virus). LCMs estimate prevalence and accuracy of each test based on the observed frequency of the possible combinations of test results. [2,3]

Calculation:

$$P(T = 1) = \pi \text{ Sens} + (1 - \pi)(1 - \text{Spec})$$

$$P(T = 0) = \pi (1 - \text{Sens}) + (1 - \pi)\text{Spec}$$

where π is disease prevalence estimate

Given $Y = 0$ or 1

$$P(T = Y) = \pi \text{ Sens}^Y (1 - \text{Sens})^{1-Y} + (1 - \pi) \text{ Spec}^{1-Y} (1 - \text{Spec})^Y$$

For J tests:

$$P(T_1 = Y_1, T_2 = Y_2, \dots, T_J = Y_J) = \pi \prod_{j=1}^J \text{Sens}_j^{Y_j} (1 - \text{Sens}_j)^{1-Y_j} + (1 - \pi) \prod_{j=1}^J \text{Spec}_j^{1-Y_j} (1 - \text{Spec}_j)^{Y_j}$$

Profile	Frequency
1111	11
1110	2
1101	3
...	...
0000	62
TOTAL	100

Acknowledgement

We would like to thank the staff at Mahidol-Oxford Tropical Medicine Research unit for all support. The study is funded by Thailand Research Fund and Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Results

The final diagnoses of the 549 patients were dengue infection (135, 24.6%), chikungunya (102, 18.6%), leptospirosis (33, 6.0%) and others (279, 50.8%). Of 135 patients diagnosed with dengue infection, 131 were dengue fever, 4 were dengue hemorrhagic fever, and none were dengue shock syndrome.

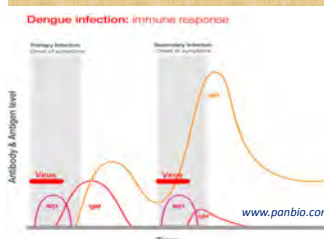
84 (15.3%) had positive results on the reference assay for dengue infection (26 primary infection and 58 secondary infection). None were positive for acute JEV infection. NS1 antigenic strip test, IgM cassette test and IgG cassette test were positive in 69, 91 and 147 patients, respectively. Only 13 patients (2.4%) were positive for the reference assay and all three rapid diagnostic tests. The main results are shown in Table 1.

Parameters	AFRIMS as gold standard Mean (95% CI)	Bayesian LCM Posterior estimate (95% CrI)	Bayesian LCM Using admission sample only Posterior estimate (95% CrI)
Prevalence	15.3 (12.4 – 18.6)	24.3 (19.1 – 30.0)	
AFRIMS reference assay			
Sensitivity	100	62.0 (49.5 – 75.9)	NA
Specificity	100	99.6 (97.9 – 100)	NA
Panbio NS1 antigen test			
Sensitivity	54.8 (43.5 – 65.7)	45.9 (36.0 – 56.4)	NA
Specificity	95.1 (92.7 – 96.8)	97.9 (95.5 – 99.7)	NA
Panbio Duo cassette IgM			
Sensitivity	50.0 (38.9 – 61.1)	54.5 (45.4 – 63.8)	39.7 (35.2 – 44.1)
Specificity	89.5 (86.3 – 92.1)	95.5 (92.0 – 98.3)	96.6 (94.6 – 98.5)
Panbio Duo cassette IgG			
Sensitivity	61.9 (50.7 – 72.3)	62.1 (52.8 – 71.4)	42.6 (38.1 – 47.1)
Specificity	79.6 (75.6 – 83.1)	84.5 (80.1 – 88.5)	87.2 (85.4 – 89.1)
Panbio NS1 + IgM			
Sensitivity	79.8 (69.6 – 87.7)	78.9 (72.4 – 84.8)	72.8 (66.5 – 78.7)
Specificity	86.2 (82.8 – 89.2)	93.7 (90.7 – 96.8)	94.7 (91.9 – 97.5)
PPV	51.1 (42.3 – 60.0)	80.1 (68.2 – 90.6)	81.5 (69.8 – 91.6)
NPV	95.9 (93.6 – 97.6)	93.3 (89.5 – 95.9)	91.6 (87.5 – 94.5)
Panbio NS1 + IgM + IgG			
Sensitivity	92.9 (85.1 – 97.3)	91.7 (86.2 – 96.1)	87.0 (81.2 – 92.0)
Specificity	72.5 (68.2 – 76.5)	79.8 (76.6 – 83.3)	82.8 (79.9 – 86.0)
PPV	37.9 (31.2 – 44.9)	59.3 (48.9 – 69.0)	62.0 (51.8 – 71.4)
NPV	98.3 (96.2 – 99.4)	96.8 (93.9 – 98.7)	95.2 (91.9 – 97.5)

Table 1: Diagnostic accuracy, sensitivity and specificity, estimated using a gold standard technique and Bayesian approach

Conclusion

- The true sensitivity of the reference assay (AFRIMS MAC and GAC ELISA on paired serum) estimated by Bayesian LCM was very low (62.0%).
- An estimated prevalence of 24.3% in patients who were suspected dengue infection, compared with 15.3% based on the reference assay alone.
- A combination of NS1, IgM and IgG on admission sample had NPV of 95.2%, suggesting that negativity of all three tests could be used to rule out dengue infection with high degree of accuracy.
- A combination of NS1 and IgM on admission sample had PPV of 81.5%, suggesting that positivity of either NS1 or IgM could be used to diagnose dengue infection with high level of confidence.



Work plan:

- To incorporate days of fever (which can affect sensitivity of test) into the model.
- To optimize diagnostic algorithm for predicting severity (primary/secondary infection)
- To add clinical symptoms such as fever or rash into the diagnostic algorithm since laboratory diagnostic tools may not be practical in some rural settings.

References

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