# Sensitivity and specificity of DPP® Fever Panel II Asia in the diagnosis of malaria, dengue and melioidosis

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## 1.4 Keywords

Rapid diagnostic test, accuracy, sensitivity, specificity, melioidosis, dengue, malaria, chikungunya, zika virus infection

**NOTE:**

**Aim – JMM (journal of medical microbiology) short communication (1000-3000 words; quite flexible) or Full article (3000 words but can go with a bit less)**

## 2. Abstract

**Introduction.** Rapid diagnostic tests that can facilitate the diagnosis of a panel of tropical infectious diseases are critically needed. The 'DPP® Fever Panel II Asia' is a multiplex lateral flow immunoassay comprising of antigen and IgM panels for the diagnosis of a broad range of pathogens that commonly cause febrile illness in Southeast Asia.

**Aim.** To evaluate sensitivity and specificity of DPP® Fever Panel II for malaria, dengue and melioidosis.

**Methodology.** We conducted a cohort-based case-control study. Both cases and controls were derived from a prospective observational study of patients presenting with community-acquired infections and sepsis in northeast Thailand (Ubon-sepsis). We included 143 and 98 patients diagnosed with malaria or dengue based on a positive polymerase chain reaction assay and 177 patients with melioidosis based on a clinical specimen culture positive for *Burkholderia pseudomallei*. Controls included 200 patients who were blood culture positive for *Staphylococcus aureus*, *Escherichia coli* or *Klebsiella pneumoniae*, and cases of the other diseases. Serum samples collected from all patients within 24 hours of admission were stored and tested using the DPP® Fever Panel II Asia antigen and IgM multiplex assays. We selected cut-off values for each individual test corresponding to a specificity of ≥95%. When assessing diagnostic tests in combination, results were considered positive if either individual test was positive.

**Results.** Within the DPP® Fever Panel II antigen assay, a combination of pLDH and HRPII had a sensitivity of 91% (130/143 malaria case patients) and a specificity of 97% (458/471 non-malaria control patients). The combination of Dengue NS1 antigen test and Dengue antibody test had a sensitivity of 60% (60/98 malaria case patients) and a specificity of 91% (472/516 non-dengue control patients). The *B. pseudomallei* CPS Antigen test had a sensitivity of 27% (47/177 melioidosis case patients) and a specificity of 97% (424/437 non-melioidosis control patients).

**Conclusion.** DPP® Fever Panel II Asia has an accuracy for the diagnosis of malaria, dengue and melioidosis comparable to other established disease specific RDTs.

## 3. Introduction

Malaria, dengue and melioidosis are common tropical infectious diseases in Southeast Asia [1, 2]. Malaria is a mosquito-borne disease, caused by *Plasmodium* parasites. In 2020, the World Health Organization (WHO) reported that there were 229 million malaria cases worldwide and 409,000 cases died [3]. Dengue is a mosquito-borne viral disease, caused by Dengue virus. A modelling study estimated 104 million dengue cases per year worldwide and 40,467 cases died [4]. Melioidosis, an infectious disease caused by the Gram-negative bacterium *Burkholderia pseudomallei* [2]. A modelling study estimated 165,000 melioidosis cases per year worldwide and 89,000 died [5]. Naturally acquired melioidosis results from exposure through skin inoculation, inhalation or ingestion of *B. pseudomallei*, which is commonly present in soils and surface water in tropical countries [2, 5]. Culture positivity for *B. pseudomallei* from any clinical sample is a definitive diagnosis for melioidosis since the organism is never part of the normal human flora (i.e. 100% specificity) [2]. Malaria [6], dengue [7] and melioidosis patients [8] commonly present with acute febrile illness or sepsis, which is a syndrome defined by a dysregulated host response to infection resulting in significant organ dysfunction and death. Sepsis can be caused by a variety of agents, including bacteria, fungi, and viruses [9]. Malaria, dengue and melioidosis require different specific treatment. Rapid diagnostic tests that can diagnose a panel of common tropical infectious diseases with high specificity among patients presenting with acute febrile illness or sepsis in tropics are critically needed.

Rapid diagnostic tests with low specificity have limited utility for the clinical decision making in many clinical settings [10]. This is recently highlighted by the use of serological RDTs for COVID-19; particularly at the onset of clinical symptoms [10, 11]. Misuse of diagnostic tests with low specificity for melioidosis, such as the indirect hemagglutination assays, has also led to misdiagnoses, overuse of antibiotics that are effective against *B. pseudomallei*, placing patients at risk of avoidable adverse drug reactions, an incorrect mortality of melioidosis reported to policy makers, and, thus, a lack of public health responses against melioidosis in Thailand [12].

Chembio Diagnostic Inc, in collaboration with FIND (Foundation for Innovative New Diagnostics), developed a multiplex lateral flow immunoassay (DPP® Fever Panel II Assay) that is able to detect serum immunoglobulin M (IgM) and specific microbial antigen of the most common agents of acute febrile illness (AFI) in Asia. Here, we evaluated the DPP® Fever Panel II Asia Antigen System and DPP® Fever Panel II Asia IgM System for diagnosis of malaria, dengue and melioidosis.

## 4. Methods

We previously conducted a prospective observational (non-interventional) study of community-acquired infection and sepsis in Sunpasitthiprasong Hospital, Ubon Ratchathani province, northeast Thailand [8]. From March 2013 to February 2017, we enrolled 5,001 adult patients (≥18 yr of age) who were admitted with a primary diagnosis of suspected or documented infections as determined by the attending physician, were within 24 h of hospital admission and had at least three sepsis diagnostic criteria documented in their medical record. We excluded patients who were suspected of having hospital-acquired infections determined by the attending physician, had a hospital stay within 30 days prior to this admission or were transferred from another hospital with a total duration of hospitalization >72 hours. Blood was drawn from all patients at the time of enrolment for culture and polymerase chain reaction (PCR). Patients who were positive for malaria, dengue or melioidosis were selected as cases in the current study. Dengue and malaria were diagnosed by a nested PCR assay as described previously [6, 7, 13]. Patients who were culture positive for *B. pseudomallei* from any clinical specimens were selected as cases of melioidosis. Patients with blood cultures positive for *Staphylococcus aureus*, *Escherichia coli* or *Klebsiella pneumoniae*, or cases of the other diseases were selected as controls.

Serum samples obtained in the first 24 hours of admission were frozen at -80°C and for the purposes of the current study thawed and tested using the DPP® Fever Panel II Asia antigen and IgM multiplex assays. The DPP® Fever Panel II Asia **antigen** assay (Chembio Inc; lot no FPIIAAG010821/A; Figure 1A) was developed as a multi-line lateral flow for the simultaneous qualitative detection and differentiation of specific febrile illness antigens; including (1) Chikungunya, (2) pan *Plasmodium* antigen lactate dehydrogenase (pLDH), (3) Dengue NS1, (4) Zika NS1, (5) *Plasmodium falciparum* (Pf) Histidine rich protein

II antigen (HRPII) and (6) *Burkholderia* Capsular Polysaccharide antigen (CPS). The DPP® Fever Panel II Asia **IgM** assay (Chembio Inc; lot no FPIIAAG010821/A; Figure 1B) was developed for the simultaneous qualitative detection and differentiation of specific IgM antibodies for (1) Chikungunya, (2) Zika, (3) *Leptospira*, (4) *Orientia tsutsugamushi*, (5) *Rickettsia typhi* and (6) Dengue. The tests were performed as described by the manufacturer. For antigen assay, 50 µL of serum sample was directly added in the sample well and following immediately added by 100 µL (4 drops) of sample buffer into the SAMPLE + BUFFER well (Well #1; Figure 1A). For IgM assay, 10 µL of serum sample was mixed with 150 µL of sample buffer in separately tube and used for 100 µL to add into the SAMPLE + BUFFER well (Well #1; Figure 2A). At 5 minutes of incubation at room temperature for both systems, 300 µL (12 drops) of running buffer was added into the BUFFER well (Well #2). The test results were interpreted using the DPP® Micro Reader 2 between 20 and 25 minutes after buffer addition to Well #1 (Figure 1C and 1D). Tests were labelled with the patient code and performing date. The laboratory technician who were unaware of the final diagnosis performed the tests.

The lowest OD cut-off values that gave a specificity of ≥95% for each individual test line were selected. The sensitivity of diagnostic tests was defined as the proportion of case patients who had positive test results. The specificity of diagnostic tests was defined as the proportion of control patients who had negative test results. A receiver operating characteristic (ROC) curve was created to monitor the shifting of the positive cut-off value of true-positive (sensitivity) and false positive (1-specificity) rates. When assessing diagnostic tests in combination, results were considered positive if either test was positive. All analyses were performed using Stata version 14 (Stata Corp LP, College Station, TX, USA). The final database with data dictionary are publicly available online (https://figshare.com/XXX).

## 5. Results

From March 2013 to February 2017, 5,001 adult patients presenting with community-acquired infections or sepsis were enrolled and followed for 28 days. A total of 153 and 126 were PCR positive for malaria or

dengue, and were included as malaria and dengue cases, respectively. A total of 193 patients were culture positive for *B. pseudomallei* and thus included as melioidosis cases, and 268 patients who were blood culture positive for *E. coli* (n=189), *K. pneumoniae* (n=26) and *S. aureus* (n=53) without malaria, dengue and melioidosis were included in this study as bacteraemia controls. Serum was not available for all patients. Overall, 614 patients were included in the analyses; including 143 malaria cases (70 Pf, 63 *Plasmodium vivax* (Pv) and 10 mixed Pf and Pv), 98 dengue cases, 177 melioidosis cases and 200 patients with bacteraemia were included in the analyses. Of 414 cases of malaria, dengue or melioidosis, 5 had mixed infections including melioidosis plus dengue (n=3), Pv malaria plus dengue (n=1) and melioidosis plus *K. pnuemoniae* bacteraemia (n=1).

Figure 2A and 2B show quantitative values of antigen-detection and IgM-detection read by the DPP® Fever Panel II Asia antigen and IgM assays, respectively. For example, the median value of pLDH among malaria cases was 50 (IQR 19-125; range 4-326). Areas under the ROC curves (AUROCC) of pLDH for malaria diagnosis were 0.90 (95%CI 0.86 to 0.93; Figure 3A) and AUROCC of HRPII Malaria Antigen test for Pf-malaria diagnosis was 0.97 (95% 0.94-1.00; Figure 3B). AUROCC of Dengue NS1 Ag test and Ab test for dengue diagnosis were 0.80 (95% 0.74-0.86; Figure 3C) and 0.65 (95%CI 0.60-0.71; Figure 3D), respectively. AUROCC of *Burkholderia* CPS Ag for melioidosis diagnosis was 0.62 (95%CI 0.57-0.67; Figure 3E).

Using a positive cut-off value of 19 for pLDH Malaria Antigen test, the test had a sensitivity of 76% (108/143 malaria case patients) and a specificity of 99.0% (464/471 non-malaria control patients; Table 1). Of 80 and 63 patients with Pf and Pv mono infections, pLDH Malaria Antigen test had a sensitivity of 73% (58/80 PF case patients) and 79% (50/63 PV-mono malaria case patients). Using a positive cut-off value of 9 for HRPII Malaria Antigen test, the test had a sensitivity of 94% (75/80 Pf malaria case patients) and a specificity of 98.0% (524/534 non-Pf malaria control patients). Among 10 non-Pf malaria control patients who had HRPII Malaria Antigen test ≥9, 2 had Pv malaria, 2 were dengue cases, 3 were melioidosis cases and 3 were *K. pneumoniae* bacteraemia cases. The combination of pLDH and HRPII Malaria Antigen test had a sensitivity of 91% (130/143 malaria case patients) and a specificity of 97% (458/471 non-malaria control patients).

Using a positive cut-off value of 22 for Dengue NS1 Antigen test, the test had a sensitivity of 55% (54/98 dengue case patients) and a specificity of 95.0% (491/516 non-dengue control patients). Using a positive cut-off value of 56 for Dengue Antibody test, the test had a sensitivity of 11% (11/98 dengue case patients) and a specificity of 95.0% (491/516 non-dengue control patients) for dengue. Of 98 dengue case patients, 5 (5%) were positive for both Dengue NS1 Antigen and Antibody tests, 49 (50%) were positive only Dengue NS1 Antigen and 6 (6%) were positive only Dengue Antibody test. The combination of Dengue NS1 antigen test and Dengue antibody test had a sensitivity of 60% (60/98 malaria case patients) and a specificity of 91% (472/516 non-dengue control patients).

Using a positive cut-off value of 8 for *Burkholderia* antigen test, the test had a sensitivity of 27% (47/177 melioidosis case patients) and a specificity of 97% (424/437 non-melioidosis control patients). Using a positive cut-off values ranging from 7 to 36, Chikungunya antigen test, Zika antigen test, Chikungunya antibody test, Zika antibody test, *Leptospira* antibody test, *Orientia tsutsugamushi* antibody test, *Rickettsia typhi* antibody test gave a specificity of 95%.

Of all 414 cases with malaria, dengue or melioidosis, 236 (57%) had an accurate diagnosis made by DPP® Fever Panel II Asia. All five cases with mixed infections had an inaccurate diagnosis made; including a positive result for pLDH but negative results for Dengue NS1 Ag and IgM Ab for the case with a mixed infection of malaria and dengue and all negative test results for malaria, dengue and melioidosis for the remaining four cases. Of all 200 bacteraemia controls without malaria, dengue and melioidosis, 178 (89%) had accurate negative results for all malaria, dengue and melioidosis.

## 6. Discussion

This study of patients hospitalized within 24 hours with community-acquired infection and sepsis at a referral hospital in northeast of Thailand demonstrates that the DPP® Fever Panel II Asia could be used to

diagnose malaria, dengue and melioidosis comparable to other established RDTs for these tropical diseases. The DPP® Fever Panel II Asia can perform for all of the diseases in the array altogether and with relatively lower amount of blood compared to using RDTs for each specific disease separately.

The sensitivity of pLDH and HRPII as measured in the DPP® Fever Panel II Asia Antigen System was comparable with findings from previous studies evaluating other leading malaria rapid tests [14-17]. Among Pf malaria cases, the sensitivity of HRPII is higher than that of pLDH (94% vs. 73%), which is consistent with previous findings [16]. The sensitivity of pLDH among non-Pf malaria in our setting (79%) was comparable to that of CareStart® Malaria pLDH (79%), but relatively lower than that of OptiMal-IT® pLDH (90%), previously evaluated in Southeast Asia [17].

The sensitivity and specificities of the DPP® Dengue NS1 Antigen and IgM Antibody were comparable to the performance of other RDTs in previous studies [18-22]. Nonetheless, the sensitivity and specificities of the DPP® Dengue IgM test was relatively low in our setting. If a cut-off value that gave a specificity of ≥95% were selected, the Dengue Antibody test offered only a modest improvement in overall sensitivity when combined with NS1 as compared with NS1 alone.

The sensitivity of *B. pseudomallei* CPS Ag of DPP (27%) was comparable to the sensitivity of *B. pseudomallei* CPS detection of Active Melioidosis Detect® lateral flow immunoassay developed by InBios (31%) for the diagnosis of melioidosis [23]. Melioidosis is often fatal and difficult to diagnose and treat [2]. This is because *B. pseudomallei* is intrinsically resistant to commonly used antibiotics and treatment with effective antibiotics; including ceftazidime or carbapenem, is required. Nonetheless, preserving high specificity (e.g. ≥95%) of RDTs for melioidosis is crucial [12]. Therefore, *B. pseudomallei* CPS Ag testing with limited sensitivity but high specificity could be used to guide antibiotic treatment against *B. pseudomallei* in melioidosis-endemic countries. In addition, a recent study also shows that positivity of *B. pseudomallei* CPS Ag in serum samples obtained within 24 hours of admission was associated with severe infections, bacteraemia and mortality [24].

One of the strengths of this study is the inclusion of cases and controls drawn from a large prospective observational study of patients presenting with community-acquired infection and sepsis in northeast Thailand, representing a real-world setting [8]. Additionally, all serum samples were drawn within 24 hours of admission to the study hospital which is ideal for evaluating point-of-care diagnostic tests.

A limitation of this study is that positive predictive and negative predictive values could not be estimated because of the case-control study design. Quantitative PCR assays for malaria and dengue were not performed. The cut-off values selected were arbitrary. However, with the open-access data set provided, different cut-off values could be selected for different settings. Our study population could not evaluate sensitivity and specificities of other pathogens the DPP® Fever Panel II Asia is designed to test for including Chikungunya infection, Zika infection, leptospirosis, scrub typhus and murine typhus as there were insufficient numbers of patients with these infections in the cohort. Other work is currently underway to evaluate the performance of the assay in detecting these infections.

In conclusion, the DPP® Fever Panel II Asia offers the potential for rapid, simultaneous point of care testing for both antigen and antibodies for some of the key causes of febrile illness in Southeast Asia. We found the performance of the panels for the diagnosis of malaria, dengue and melioidosis to be similar to other established rapid tests for these pathogens, although as with other tests the accuracy for dengue was modest and the sensitivity for melioidosis was low. Notwithstanding these limitations and the need for further evaluations for its performance in diagnosing other pathogens on the panel, the DPP® Fever Panel II Asia system could substantially improve the management of febrile illness in much of rural Southeast Asia where access to laboratory testing is often unavailable.

# 7. Author statements

## 7.1 Authors and contributors

PA and DL developed the first draft of the manuscript. YL, XX, XX TEW, YL and DL conceptualized the study. VH, GW and PT conducted the clinical study. PA performed the diagnostic test evaluation. PA and DL analyzed the data. All co-authors revised the manuscript.

## 7.2 Conflicts of interest

The authors declare no conflict of interest.

## 7.3 Funding information

The study was funded by the Wellcome Trust (090219/Z/09/Z and 220211/A/20/Z) to DL, National Heart, Lung and Blood Institute, National Institutes of Health (R01HL113382) to TEW, and National Science and Technology Development Agency (NSTDA) (P-16-51225) to SW. DL was supported by an intermediate fellowship from the Wellcome Trust (101103/Z/13/Z). Stuart funding; Joel funding. The DPP® Fever Panel II Asia was provided by Chembio Inc. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript. For the purpose of open access, the author has applied a CC BY public copyright licence to any Author Accepted Manuscript version arising from this submission.

## 7.4 Ethical approval

The study protocol and related documents were approved by Sunpasitthiprasong Hospital Ethics Committee (039/2556), the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (MUTM2012-024-01), the University of Washington Institutional Review Board (42988) and the Oxford Tropical Research Ethics Committee at the University of Oxford (OXTREC172-12). Written, informed consent was obtained from participants prior to enrollment.

### 7.5 Consent for publication

This study does not contain details, images, or videos related to any individuals, and do not need consent for publication.


### 7.6 Acknowledgements

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## 8. References


1. Southeast Asia Infectious Disease Clinical Research N. Causes and outcomes of sepsis in southeast Asia: a multinational multicentre cross-sectional study. Lancet Glob Health. 2017;5(2):e157-e67.
2. Wiersinga WJ, Virk HS, Torres AG, Currie BJ, Peacock SJ, Dance DAB, et al. Melioidosis. Nat Rev Dis Primers. 2018;4:17107.
3. WHO. World malaria report 20202020. Available from: https://www.who.int/publications/i/item/9789240015791.
4. Zeng Z, Zhan J, Chen L, Chen H, Cheng S. Global, regional, and national dengue burden from 1990 to 2017: A systematic analysis based on the global burden of disease study 2017. EClinicalMedicine. 2021;32:100712.
5. Limmathurotsakul D, Golding N, Dance DA, Messina JP, Pigott DM, Moyes CL, et al. Predicted global distribution of Burkholderia pseudomallei and burden of melioidosis. Nat Microbiol. 2016;1:15008.
6. Teparrukkul P, Hantrakun V, Imwong M, Teerawattanasook N, Wongsuvan G, Day NP, et al. Utility of qSOFA and modified SOFA in severe malaria presenting as sepsis. PLoS One. 2019;14(10):e0223457.
7. Teparrukkul P, Hantrakun V, Day NPJ, West TE, Limmathurotsakul D. Management and outcomes of severe dengue patients presenting with sepsis in a tropical country. PLoS One. 2017;12(4):e0176233.

8. Hantrakun V, Somayaji R, Teparrukkul P, Boonsri C, Rudd K, Day NPJ, et al. Clinical epidemiology and outcomes of community acquired infection and sepsis among hospitalized patients in a resource limited setting in Northeast Thailand: A prospective observational study (Ubon-sepsis). PLoS One. 2018;13(9):e0204509.

9. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-10.

10. Bisoffi Z, Pomari E, Deiana M, Piubelli C, Ronzoni N, Beltrame A, et al. Sensitivity, Specificity and Predictive Values of Molecular and Serological Tests for COVID-19: A Longitudinal Study in Emergency Room. Diagnostics (Basel). 2020;10(9).

11. Borges LP, Martins AF, Silva BM, Dias BP, Goncalves RL, Souza DRV, et al. Rapid diagnosis of COVID-19 in the first year of the pandemic: A systematic review. Int Immunopharmacol. 2021;101(Pt A):108144.

12. Hinjoy S, Hantrakun V, Kongyu S, Kaewrakmuk J, Wangrangsimakul T, Jitsuronk S, et al. Melioidosis in Thailand: Present and Future. Trop Med Infect Dis. 2018;3(2):38.

13. Paris DH, Imwong M, Faiz AM, Hasan M, Yunus EB, Silamut K, et al. Loop-mediated isothermal PCR (LAMP) for the diagnosis of falciparum malaria. Am J Trop Med Hyg. 2007;77(5):972-6.

14. White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM. Malaria. Lancet. 2014;383(9918):723-35.

15. Poti KE, Sullivan DJ, Dondorp AM, Woodrow CJ. HRP2: Transforming Malaria Diagnosis, but with Caveats. Trends Parasitol. 2020;36(2):112-26.

16. Li B, Sun Z, Li X, Li X, Wang H, Chen W, et al. Performance of pfHRP2 versus pLDH antigen rapid diagnostic tests for the detection of Plasmodium falciparum: a systematic review and meta-analysis. Arch Med Sci. 2017;13(3):541-9.

17. Ashley EA, Touabi M, Ahrer M, Hutagalung R, Htun K, Luchavez J, et al. Evaluation of three parasite lactate dehydrogenase-based rapid diagnostic tests for the diagnosis of falciparum and vivax malaria. Malar J. 2009;8:241.

18. Raafat N, Blacksell SD, Maude RJ. A review of dengue diagnostics and implications for surveillance and control. Trans R Soc Trop Med Hyg. 2019;113(11):653-60.

19. Pan-ngum W, Blacksell SD, Lubell Y, Pukrittayakamee S, Bailey MS, de Silva HJ, et al. Estimating the true accuracy of diagnostic tests for dengue infection using bayesian latent class models. PLoS One. 2013;8(1):e50765.

20. Blacksell SD, Jarman RG, Gibbons RV, Tanganuchitcharnchai A, Mammen MP, Jr., Nisalak A, et al. Comparison of seven commercial antigen and antibody enzyme-linked immunosorbent assays for detection of acute dengue infection. Clin Vaccine Immunol. 2012;19(5):804-10.

21. Blacksell SD, Jarman RG, Bailey MS, Tanganuchitcharnchai A, Jenjaroen K, Gibbons RV, et al. Evaluation of six commercial point-of-care tests for diagnosis of acute dengue infections: the need for combining NS1 antigen and IgM/IgG antibody detection to achieve acceptable levels of accuracy. Clin Vaccine Immunol. 2011;18(12):2095-101.

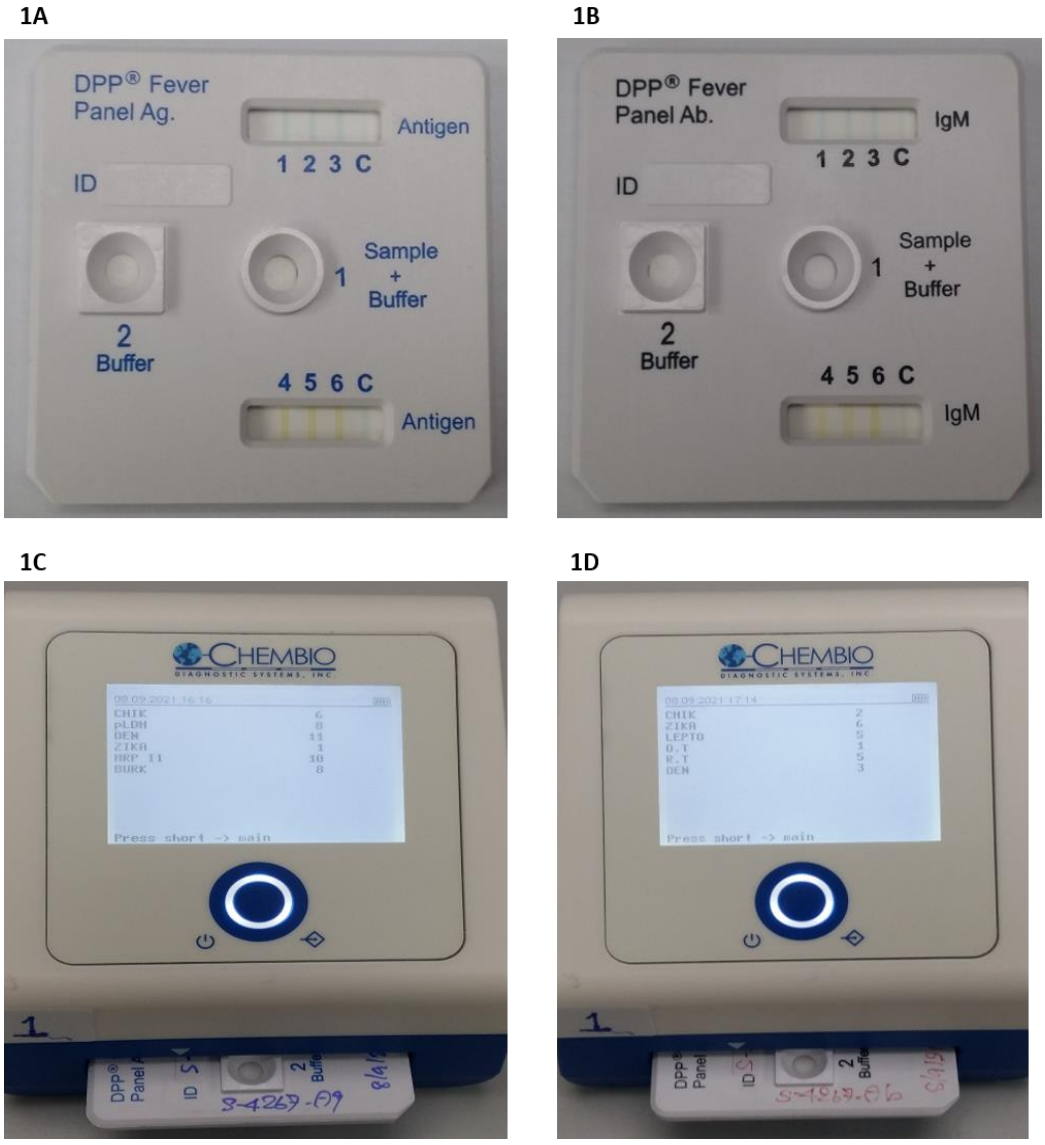
22. Blacksell SD, Bell D, Kelley J, Mammen MP, Jr., Gibbons RV, Jarman RG, et al. Prospective study to determine accuracy of rapid serological assays for diagnosis of acute dengue virus infection in Laos. Clin Vaccine Immunol. 2007;14(11):1458-64.

23. Wongsuvan G, Hantrakun V, Teparrukkul P, Imwong M, West TE, Wuthiekanun V, et al. Sensitivity and specificity of a lateral flow immunoassay (LFI) in serum samples for diagnosis of melioidosis. Trans R Soc Trop Med Hyg. 2018;112(12):568-70.

24. Amornchai P, Hantrakun V, Wongsuvan G, Wuthiekanun V, Wongratanacheewin S, Teparrakkul P, et al. Evaluation of antigen-detecting and antibody-detecting diagnostic test combinations for diagnosing melioidosis. PLoS Negl Trop Dis. 2021;15(11):e0009840.

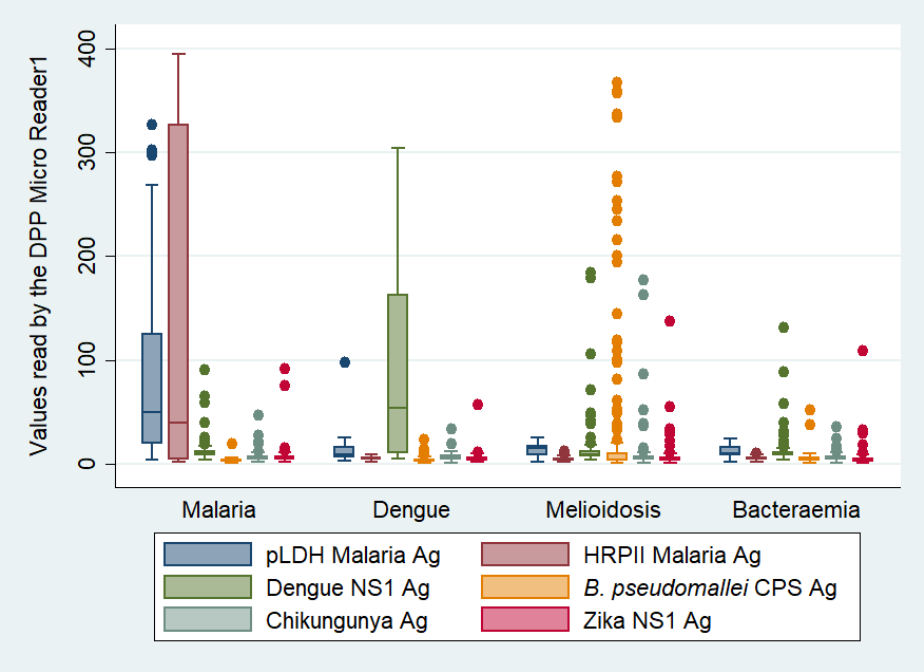
## 9. Figures and tables

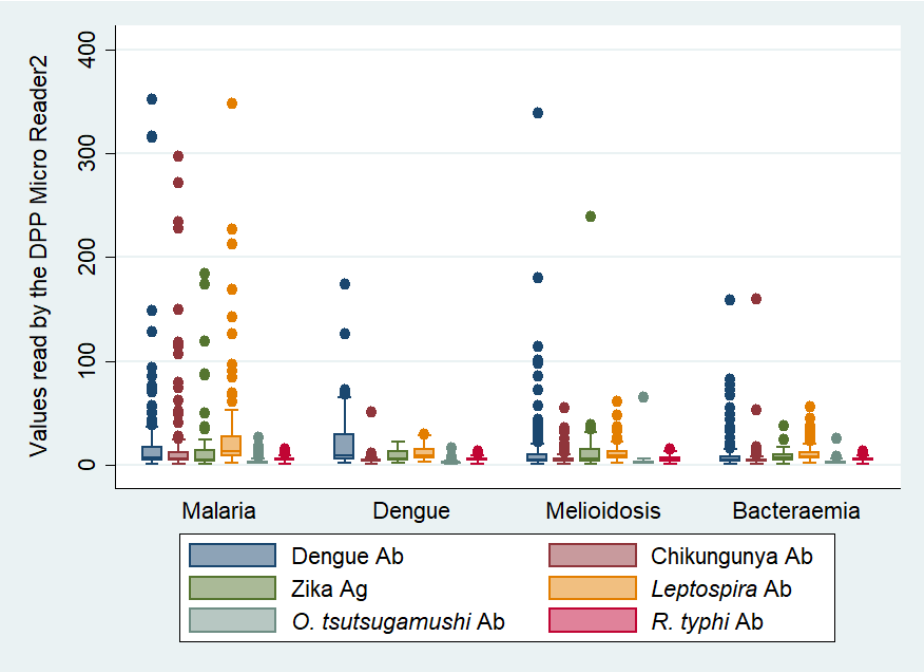
**Figure 1: DPP® Fever Panel II Asia Antigen System and IgM System**




**Footnote of Figure 1:** (A) DPP® Fever Panel II Asia Antigen System, (B) DPP® Fever Panel II Asia IgM System, (C) Example test result of the antigen system read by DPP® Micro Reader 2, and (D) Example test result of the IgM system read by DPP® Micro Reader 2

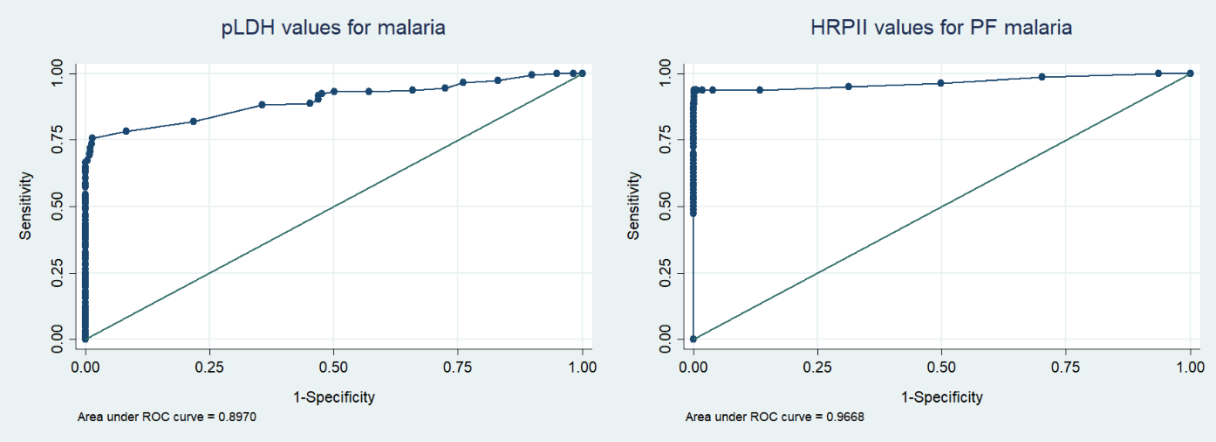
**Figure 2: Quantitative test results for antigen-detection (2A) and IgM-detection (2B) by DPP® Fever Panel II Asia Antigen System and IgM System among patients with malaria, dengue, melioidosis and bacteraemia.**

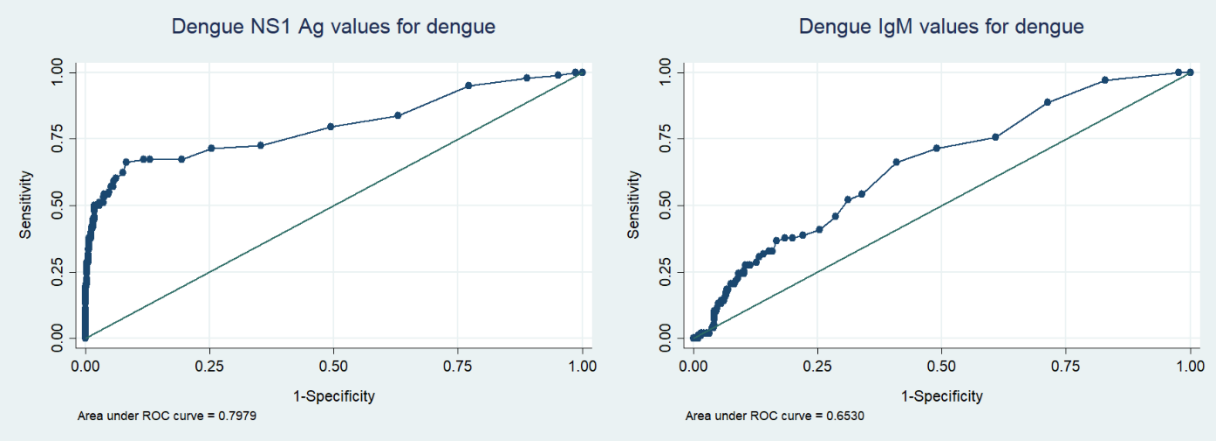


**Footnote of figure 2.** Boxes show 25th, 50th, and 75th percentiles. Bottom and top whiskers show 25th percentile minus 1.5 times the interquartile range (IQR) and 75th percentile plus 1.5 times the IQR, respectively. Patients with bacteraemia included patients with blood culture positive for *E. coli*, *K. pneumoniae* and *S. aureus*.

**Figure 3. Receiver operating characteristic curves of DPP® Fever Panel II Asia Antigen System and IgM System for diagnosis of malaria, dengue and melioidosis**



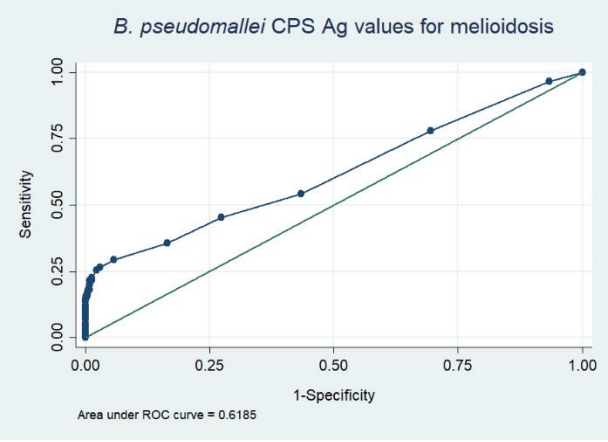



**Table 1. Sensitivity and specificity of DPP® Fever Panel II Asia Antigen System and DPP® Fever Panel II Asia IgM System among patients with malaria, dengue, melioidosis and bacteraemia**

| Panels | Cut-off values | Sensitivity | Specificity |
|---|---|---|---|
| **Antigen (Ag)** | | | |
| pLDH Malaria Ag test | ≥ 19 | 76% (108/143 malaria cases) | 99% (464/471 non-malaria controls) [a] |
| HRPII Malaria Ag test | ≥ 9 | 94% (75/80 PF malaria cases) | 98% (524/534 non-PF malaria controls) [b] |
| Dengue NS1 Ag test | ≥ 22 | 55% (54/98 dengue cases) | 95% (491/516 non-dengue controls) [c] |
| *B. pseudomallei* CPS Ag test | ≥ 8 | 27% (47/177 melioidosis cases) | 97% (424/437 non-melioidosis controls) [d] |
| Chikungunya Ag test | ≥ 12 | Not applicable | 95% (586/614 patients) [e] |
| Zika NS1 Ag test | ≥ 11 | Not applicable | 96% (580/614 patients) [e] |
| **Antibody (Ab)** | | | |
| Dengue Ab test | ≥ 56 | 11% (11/98 dengue cases) | 95% (491/516 non-dengue controls) [c] |
| Chikungunya Ab test | ≥ 21 | Not applicable | 95% (585/614 patients) [e] |
| Zika Ab test | ≥ 18 | Not applicable | 95% (585/614 patients) [e] |
| *Leptospira* Ab test | ≥ 36 | Not applicable | 95% (585/614 patients) [e] |
| *O. tsutsugamushi* Ab test | ≥ 7 | Not applicable | 97% (595/614 patients) [e] |

| | | | |
|---|---|---|---|
| *R. typhi* Ab test | ≥ 10 | Not applicable | 95% (584/614 patients) [e] |
| **Used as a combination** | | | |
| pLDH + HRPII Malaria Ag test | ≥ 19, ≥ 9 | 76% (108/143 malaria cases) | 99% (464/471 non-malaria controls) [a] |
| Dengue NS1 Ag + Ab test | ≥ 22, ≥ 56 | 61% (60/98 dengue cases) | 91% (472/516 non-dengue controls) [c] |

**Footnote of Table 1.** Patients with bacteraemia included patients with blood culture positive for *E. coli*, *K. pneumoniae* and *S. aureus* [a] included patients with bacteraemia, dengue and melioidosis [b] included patients with bacteraemia, *Plasmodium vivax* malaria, dengue and melioidosis [c] included patients with bacteraemia, malaria and melioidosis [d] included patients with bacteraemia, malaria and dengue [e] included patients with bacteraemia, malaria, dengue and melioidosis