Critical Reviews in Biotechnology

ISSN: 0738-8551 (Print) 1549-7801 (Online) Journal homepage: http://www.tandfonline.com/loi/ibty20

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To cite this article: Jane Ru Choi, Jie Hu, ShuQi Wang, Hui Yang, Wan Abu Bakar Wan Abas, Belinda Pingguan-Murphy & Feng Xu (2016): Paper-based point-of-care testing for diagnosis of dengue infections, Critical Reviews in Biotechnology, DOI: 10.3109/07388551.2016.1139541

To link to this article: http://dx.doi.org/10.3109/07388551.2016.1139541

Critical Reviews in Biotechnology

http://informahealthcare.com/bty
ISSN: 0738-8551 (print), 1549-7801 (electronic)

Crit Rev Biotechnol, Early Online: 1–12
 DOI: 10.3109/07388551.2016.1139541

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REVIEW ARTICLE

Paper-based point-of-care testing for diagnosis of dengue infections

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Abstract

Dengue endemic is a serious healthcare concern in tropical and subtropical countries. Although well-established laboratory tests can provide early diagnosis of acute dengue infections, access to these tests is limited in developing countries, presenting an urgent need to develop simple, rapid, and robust diagnostic tools. Point-of-care (POC) devices, particularly paper-based POC devices, are typically rapid, cost-effective and user-friendly, and they can be used as diagnostic tools for the prompt diagnosis of dengue at POC settings. Here, we review the importance of rapid dengue diagnosis, current dengue diagnostic methods, and the development of paper-based POC devices for diagnosis of dengue infections at the POC.

Keywords

Cost-effective, dengue infections, early diagnosis, paper-based device, point-of-care (POC), rapid, user-friendly

History

Received 6 April 2015
Revised 7 December 2015
Accepted 11 December 2015
Published online 12 February 2016

Introduction

Dengue infections are amongst the fastest spreading mosquito-borne diseases in the world.[1] Approximately 100 million dengue infections are reported annually, affecting approximately 2.5 billion of people in tropical or subtropical countries, especially in Southeast Asia, the Western Pacific, and South America.[2,3] Generally, there are four serotypes of dengue viruses (DENVs), which are transmitted via the bites of Aedes mosquitoes (genus Flavivirus, family Flaviviridae; amongst which *Aedes aegypti* is particularly problematic). These mosquitoes can carry any of the four serotypes of DENV.[2] During the primary dengue infection, fever, rashes and musculoskeletal pain may appear within 3–14 days after the mosquito bite. In the secondary dengue infection, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DHS) may occur, which significantly increase the risk of death.[4] Since there is no vaccine to prevent primary dengue infection, early diagnosis of secondary dengue infections is a key to the prevention of severe clinical outcomes.

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Various laboratory techniques, such as viral culture, enzyme-linked immunosorbent assay (ELISA), and real time-quantitative polymerase chain reaction (RT-qPCR), have been widely used in developed countries to detect DENV,[5] antigens (Ag)/antibodies (Ab),[6] and nucleic acids,[7] respectively. However, these techniques are labor-intensive, costly, and time-consuming,[8] which are not suitable for POC testing. With the advances in microfluidics technologies, lower cost chip-based microfluidic devices have been introduced to substitute the conventional assays for dengue detection, but these improvements often compromise the simplicity of the fabrication process.[9] In contrast, paper-based devices such as lateral flow assays (LFAs) are rapid (less than 30 min), affordable (US$0.10–3.00 per test), and easy-to-use (e.g. visual detection), which are broadly applicable to both developed and developing countries.[10] Clearly, development of paper-based microfluidic assays holds great promise for developing prompt diagnosis of dengue infection in resource-limited settings.

In the present review, we focus on the most recent advances in paper-based diagnostic devices for diagnosis of dengue infections, which is distinct from previous review papers where evaluations of commercial dengue rapid diagnostic tests (RDTs) are summarized.[9,11,12] We first discuss the importance of early diagnosis, and then summarize the currently available diagnostic techniques, and chip-based devices for dengue diagnosis. Subsequently, we emphasize the

beneficial use of paper based-POC devices for detecting dengue infections as compared to other RDTs, in terms of the detection of target Ag, Ab, and nucleic acids. We also discuss the existing challenges and future perspectives for developing paper-based POC for prompt diagnosis of dengue infections.

The importance of early and rapid diagnosis of dengue infections

Early and prompt diagnosis is crucial to reduce the risk of severe dengue complications. Recovery from dengue infection of any serotype can provide life-long immunity against that particular serotype. However, the protective immunity may cause severe clinical manifestations in the secondary infection of other three DENV serotypes.[13,14] For example, severe dengue infections are usually associated with the primary infection of DENV-1, followed by secondary infection of DENV-2 or DENV-3.[3,15,16] Furthermore, other risk factors such as age, immune status, and underlying disease can potentially lead to severe DENV infections and even death. For instance, children, elderly and patients who are immunocompromised or diabetic are more prone to developing severe dengue complications. Therefore, timely supportive treatment is of great importance to slow down the disease progression and to prevent death.[4]

Identification of effective diagnostic biomarkers enables timely and accurate diagnosis of dengue infection. Appropriate diagnostic biomarkers can be used to determine the two stages of primary dengue infection. The first stage is the early febrile stage where the patient is viremic following the infection. The second stage is the defervescence stage when the Ab begins to be produced (Figure 1). Following the mosquito bite, DENV primarily infects the keratinocytes and replicates inside Langerhans cells while migrating to adjacent lymph nodes.[17] The virus then spreads via the bloodstream and replicates inside several types of blood cells, particularly the platelet, which results in viremia.[18] After 3 days following the mosquito bite, the virus particle, viral RNA and dengue nonstructural protein 1 (NS1), an Ag produced by DENV can be readily detected, which cause the stimulation of inflammatory cytokines production, resulting in dehydration, severe fever, headache, joint and muscle pains, and skin rash.[4] This stage is termed febrile stage. Defervescence stage starts about 5 days after the mosquito bite. At this stage, the increase of Ag triggers the immune response, followed by the stimulation of T cells, which in turn stimulate B cells to produce immunoglobulin M (IgM) and immunoglobulin G (IgG). At the febrile stage, if rapid treatment is given to keep the patient well hydrated, the risk of further disease progression would be reduced. In contrast, late treatment would result in an increased risk of severe clinical manifestations.

In the secondary dengue infection, there is a dramatic increase in numbers of DENV load as compared to primary infection. Pre-existing IgG Abs in the acute phase are actively produced by memory B cells with a dramatic increase in the amount over the next 2 weeks. However, these pre-existing Abs are unable to neutralize the different serotypes of second infecting virus (heterologous DENV). Instead, these Abs mediate the Ab-virus complex to bind to the receptors of circulating monocytes, FcγR, which increases the severity of the disease.[19–21] This phenomenon is known as Ab-dependent enhancement (ADE), which commonly causes fatal DHF or DSS.[15,22] The preexisting Abs cross-react with platelets and endothelial cells, causing coagulopathy and an increase in vascular permeability. Gastrointestinal mucosal bleeding and bruising may happen which represents the features of DHF or DSS.[23] In some cases, high viral load over-stimulates the production of cross-reactive T cells. T cells produce high levels of inflammatory cytokines, which also ultimately lead to the plasma leakage and coagulopathy, and result in severe dehydration. This phenomenon is termed T-cell original antigenic sin.[18,24] Both phenomena, especially ADE, appear to be the major cause of fatal DENV infections. DHF or DSS is a more severe syndrome during secondary infection, which causes increased vascular permeability, resulting in plasma leakage, shock, severe bleeding, and organ failure.[25] DHF or DSS has a mortality rate of 2–5% when treated using fluid replacement

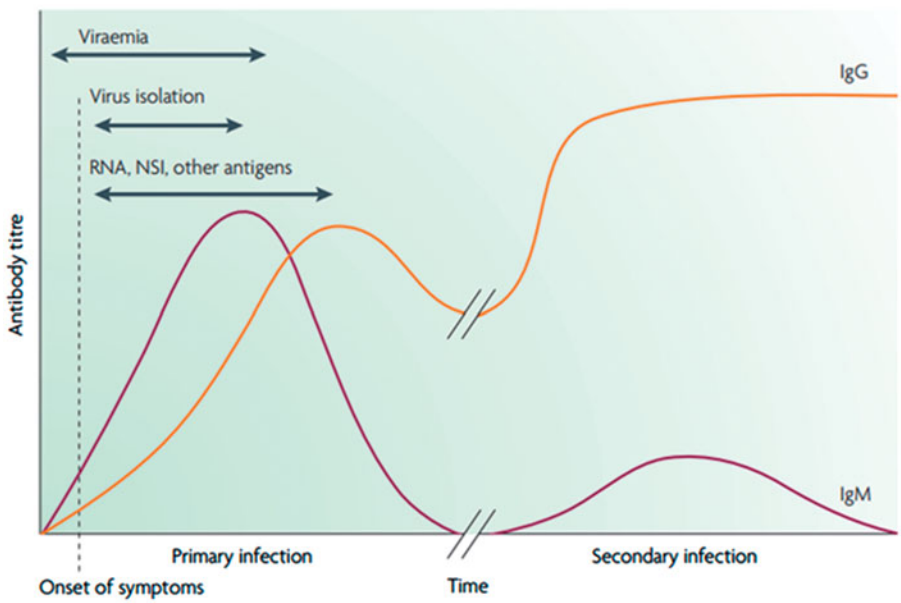

Figure 1. Major diagnostic markers for dengue infections. Major diagnostic markers for dengue infections. During the primary infection, the number of DENV, RNA, and NS1 increase dramatically at early stage of infections, followed by an increase in the IgM level. The IgG level rises dramatically following the secondary infection[2].

therapy or other supportive treatment regimens, but can reach as high as 50% when left untreated. Therefore, the high mortality rate of DHF or DSS highlights the need for rapid diagnosis and treatment for severe dengue infections.[26]

Formerly, laboratory-based assays, including viral culture, ELISA and RT-qPCR have been introduced to diagnose dengue infections[4] (Table 1). Viral isolation represents the ''gold standard'' for conventional dengue diagnosis. However, this technique is time-consuming, costly, and requires high-end instrumentations and skilled personnel.[9] Besides that, ELISA (particularly *IgM* Ab capture-enzyme-linked immunosorbent assay (MAC-EIA)) and molecular techniques (e.g. reverse transcription-PCR (RT-PCR), reverse transcription loop-mediated isothermal amplification (RT-LAMP) and RT-qPCR) have also been routinely used in the diagnosis of dengue infections in endemic areas. MAC-EIA enables differentiation between primary and secondary infections, but it is equipment-dependent without capability for serotype identification. On the other hand, molecular techniques including the RT-PCR and RT-qPCR allow rapid serotypes identification. However, highly trained operators and high-end equipment are required, which is not feasible in resource-poor settings where dengue is endemic.[30] Taken together, even though the conventional techniques could successfully diagnose dengue infections, they fail to accomplish the requirement for POCT in terms of rapidity, portability and affordability. Since then, low-cost chip-based microfluidic devices have been introduced to improve the assays. However, most microfluidic devices for dengue diagnosis are made of glass,[31,32] silicon,[33] or polymer (e.g. polydimethylsiloxane (PDMS); 34] which require complicated fabrication processes and additional pumping mechanisms to drive bioreactions.[35] Considering the cost and the simplicity of the dengue detection techniques, the development of a low-cost and uncomplicated device is vital.

Paper-based POC diagnostics for diagnosis of dengue infections

Fundamental of paper-based devices

Utilization of paper-based substrates has become an alternative to the conventional laboratory-based and chip-based assays. Paper, usually made of natural cellulose fibers, is abundant, inexpensive, easily used and biodegradable, representing a promising tool for on-site rapid diagnosis.[36–38] With simple capillary effect, this device allows the flowing of biological sample on paper, avoiding the need for external power sources, and can readily be manufactured on a large scale via standardized coating or inkjet printing techniques. Desired biomolecules (e.g. Ag, Ab, or nucleic acid) can be incorporated to paper substrates and their functions can be maintained.[39] Additionally, white colored paper is particularly suitable for implementing colorimetric tests with visually detectable colorimetric changes. The strong correlation between the color intensity and the concentration of analyte allows semi-quantitative or even quantitative measurements.[40]

To date, paper-based devices for diagnosis of various diseases have gained significant interest.[41–44] A variety of

Table 1. Comparison of dengue diagnostic techniques.

	Virus isolation	Antigen detection	Nucleic acid detection	Serological detection		RDT (e.g. NS1 test strip)
Diagnostic test	Cell culture	ELISA for NS1 detection	Reverse transcription and amplification (e.g. PCR, RT-qPCR)	EIA for IgM detection	EIA for IgG detection	LFA
Time	1–2 weeks	6 h	4 h	6 h	6 h	10–15 min
Costs ($)	3–6	5–19	20	5–19	5–19	1
Specificity (%)	100	100	100	52–100	63.5–100	92.5–100
Sensitivity (%)	40.5	67.1–98.3	58.9–100	20.22–99.0	7.8–88.9	45.9–88.06
Sample collection period (post-onset)	Days 1–5	Days 1–5	Days 1–5	After 5 days	After 15 days	Days 1–5
Serotypes identification	No	No	Yes	No	No	No
Ability to differentiate between primary and secondary infection	No	No	No	Yes	Yes	No
Expertise required	Yes	Yes	Yes	Yes	Yes	No
Special equipment/facilities required	Yes	Yes	Yes	Yes	Yes	No
References	(Chua et al., 2011)	[11, Chaterji et al., 2011, Puttikhunt et al., 2011]	[Waggoner et al., 2013, 27,28]	[11, Barde et al., 2012, Chua et al., 2011]	[11, Barde et al., 2012, Chua et al., 2011]	[Fry et al., 2011, 29, Pan-ngum et al., 2013]

RT-qPCR: real time-quantitative polymerase chain reaction; NS1: nonstructural protein 1; IgG: immunoglobulin G; IgM: immunoglobulin M; ELISA: enzyme-linked immunosorbent assay; EIA: enzyme immunoassay; RDT: rapid diagnostic test; LFA: lateral flow assay.

paper-based devices are commercially available for rapid testing of infectious diseases, such as human immunodeficiency virus (HIV), hepatitis C virus (HCV), dengue, malaria, and chikungunya.[45–47] The possibility to detect the target analyte (e.g. Ab or Ag or nucleic acid) in paper-based devices by the naked eye mainly arises from the use of labeling substances, typically colloidal gold nanoparticles.[48] Colloidal gold nanoparticles have been commonly used in paper strips due to their monodispersity properties, ease of preparation of the desired particle size, and ability to present a stable optical signal.[49] Stability of gold nanoparticles can be achieved by tagging them with biomolecules (e.g. Ab or Ag or nucleic acid), which can then be specifically bound to the target analyte. These biomolecules are attached to the gold nanoparticle by non-covalent interactions, namely hydrophobic interaction and van der Waals forces.[50] Nanoparticles eventually create a visually detectable color intensity based on the amount of analyte.[51] However, the major factors that affect the outcome of this assay include the type of the paper strip, different color perceptions, and the environmental factors such as humidity and lighting.[52] Such a qualitative measurement is less desirable when the quantitative level of analyte is critical for proper diagnosis or treatment.

In an effort to move toward the quantitative measurement based on the results of paper-based assay, imaging devices such as mobile phones, portable scanners, and electrochemical readers have been introduced.[52,53] The development of advanced mobile technologies and POC diagnostics provides great opportunity to produce well-integrated paper-based diagnostic devices. The production of a low-cost, handheld universal mobile electrochemical detector (μMED) that couples a variety of electrochemical analysis directly to the mobile phone, is particularly useful in resource-limited settings. For example, a smartphone with the price as low as US$100 with an electrochemical reader with the price tag of <US$80 can be readily available. The ability of μMED to perform a wide range of electrochemical techniques (e.g. amperometry, voltammetry, and potentiometry), high compatibility with a paper-based device and universal compatibility with any generation of mobile technology, makes it broadly applicable in the developing world.[54] This handheld device can be potentially used to detect DENV infections in resource-limited settings. The data can be transferred from remote areas to the off-site laboratory, analyzed by trained personnel, and then returned to the onsite personnel or end-users for decision-making.

Paper-based assays offer a relatively simple and quick procedure for dengue diagnosis, including the dipstick, the immune fluorescent Ab assay, the dot plot assay, and so forth.[55] To further simplify the diagnosis, LFAs have been recently developed. Today, protein-based and molecular-based diagnostics have been integrated into paper-based devices to detect both viral protein (Ag or Ab) and nucleic acids, respectively. These technologies offer close-to-patient testing, thus, reduces the number of clinical visits and the cost of healthcare, allowing more efficient diagnosis and treatment of patients.[29]

Paper-based POC testing via antigen–antibody interactions

Protein-based diagnostics (i.e. immunochromatographic techniques based on Ag–Ab interactions) have been widely used to detect the various target analytes for diagnosis of several diseases (e.g. acute myocardial infarction [30], and nephropathy).[56] There are two formats of LFAs based on the principle of Ag–Ab interactions, termed the sandwich format and competitive format. In the most commonly used sandwich format, the presence of an analyte (e.g. Ab or Ag) leads to the formation of analyte–gold conjugate, which migrates along the paper strip via capillary force and forms a visible red–purple band at the test line after binding to the corresponding capturing molecule. The analyte-free gold conjugates will be captured by different capturing molecules at the control line, forming another visible red band. The control band must be appear to confirm the validity of the assay. Thus, the appearance of red band in both control and test regions indicates a positive result whereas the appearance of red band only in the control region shows a negative result. For example, a rapid immunochromatographic test strip based on the sandwich format has been developed for detecting dengue virus Ab.[55] The target Ab reacts with the colloidal gold-NS1 to form a complex, which diffuses across the nitrocellulose membrane to bind to the highly specific anti-IgG and/or anti-IgM at their respective test line. The unreacted gold-NS1 complex further reacts with the IgG or IgM at the control line to form a visible line. A positive IgM and a negative IgG indicate a primary infection, whereas a positive IgG with negative IgM or positive at both lines shows a secondary infection. A negative result is indicated by no visible line formation in both test regions.[55]

In the competitive format, the conjugated particles (e.g. gold-labeled Ab) are able to bind to the capturing molecules deposited at both test and control lines. The analyte competes for the binding sites of the capturing molecules deposited on both test and control lines. In contrast to the outcome of the sandwich format, a positive result is indicated by a red line formation in the control region whereas a negative result is indicated by red line formation in both control and test regions. In general, sandwich format assays are utilized for an analyte with multiple Ag epitopes, while competitive format assay are designed to detect an analyte with a single Ag epitope.[57]

Paper-based immunoassay kits have become widely available for diagnosis of dengue infections.[28] The commercial dengue diagnostic kits are listed in Table 2. Dengue NS1 Ag Strip (e.g. Panbio Dengue Early Rapid NS1 and SD Bioline Dengue NS1 Ag (Figure 2A)) have been used for detection of dengue Ag NS1 at the early stage of dengue infection whereas One Step Dengue IgG & IgM Rapid Test kits, namely, Panbio Dengue Duo Cassette and SD Dengue IgG/IgM (Figure 2B), have been made commercially available for the detection of anti-dengue IgM and IgG. Recently, the Panbio Early Rapid NS1 and Duo assay kit, SD Bioline Dengue Duo NS1 and IgM/IgG Combo Device (Figure 2C) have become available to detect both NS1 and Ab simultaneously. In the most commonly used Panbio Dengue Duo Rapid Strip Test, the proteins from four serotypes of DENV, which represent

DOI: 10.3109/07388551.2016.1139541

Table 2. Commercially available chromatographic assays for diagnosis of dengue infections.

Commercial product	Analyte	Sample type	Sample volume (μL)	Assay time (min)	Sensitivity (%)	Specificity (%)	Storing temperature (°C)	References
Panbio Dengue Early Rapid	NS1	Serum, plasma, whole blood	50	15	45.9–88.06	92.5–100	22–30	(29, Fry et al., 2011, Pan-ngum et al., 2013, Ferraz et al., 2013)
Panbio Dengue Duo Cassette	IgG/IgM	Serum, plasma, whole blood	10	15	60.0–70.0	94.0–100	2–30	(29, Pan-ngum et al., 2013)
Panbio Early Rapid NS1 and Duo assay kit	NS1 & IgG/IgM	Serum, plasma, whole blood	50	15	87.0–93.0	92.5–100	2–30	(29, Fry et al., 2011, Pan-ngum et al., 2013, Ferraz et al., 2013)
SD Bioline Dengue NS1 Antigen	NS1	Serum, plasma, whole blood	100	15	44.4–70.6	73.4–100	2–30	(29,47, Tontulawat et al., 2011, Ferraz et al., 2013, Andries et al., 2012, Osorio et al., 2010)
SD Dengue IgG/IgM	IgG/IgM	Serum, plasma, whole blood	10	15	53.5–79.2	89.4–100	2–30	(29,47, Tontulawat et al., 2011)
SD Bioline Dengue Duo NS1 and IgM/IgG Combo Device	NS1 & IgG/IgM	Serum, plasma, whole blood	NS1 – 100 IgG/IgM – 10	15	80.7–85.7	83.9–97.9	2–30	(29,47, Andries et al., 2012, Osorio et al., 2010, Tricou et al., 2010)

NS1: nonstructural protein 1; IgG: immunoglobulin G; IgM: immunoglobulin M.

the N-terminal of the respective envelope glycoprotein, have been incorporated into the paper matrix. Following a single application of serum sample, IgM and IgG can be both detected to distinguish primary and secondary infections. Cuzzubbo et al.[55] demonstrated a good specificity and sensitivity of the Panbio Duo Rapid Test comparable to ELISA. These point-of-care testing (POCT) devices produce qualitative readouts which enable rapid self-diagnosis by end-users. The application of such paper-based diagnostic tests would be attractive in resource-limited settings owing to economic cost and high assay speed.

In fact, the use of protein-based LFAs has been limited by their poor specificity, which might be due to the cross-reactivity toward Ab against other flaviviruses (yellow fever or Japanese encephalitis). This normally occurs in flaviviruses infected patients or in flaviviruses vaccinated patients due to the production of cross-reactive Ab.[2] Even though there are a number of commercially available test strips for dengue detection, several studies have been performed not only to improve the specificity, but also the sensitivity and multiplexing capabilities of paper-based devices based on the principle of Ag–Ab interaction. For instance, in specificity improvement, a 2D paper network has been suggested to produce specific multiplexed immuoassays for dengue detection.[58] A specific protein removal module has been developed to enhance the specificity of the IgM detection assay. Given that both IgM and IgG are able to bind to the same Ag, to avoid the potential interference of specific Ab detection, protein G-coated membrane was used to remove IgG and to specifically detect IgM (Figure 2D).

Nowadays, most dengue diagnostic assays have used the whole virus Ag for Ab detection, which manifest cross-reactivity toward Ab against other flaviviruses as mentioned, resulting in false positive result. Customized recombinant Ags consisting of selected epitopes, which specifically recognize dengue IgG Ab, have been used to replace the whole virus Ags to address these concerns.[62–64] This technique can be implemented into LFA to specifically detect IgG for rapid and accurate dengue diagnosis.

In addition to specificity improvement, the multiplex capability of the device has been improved by developing a rapid mixing and dilution module on the device to achieve a wide range of dilution which is fundamental to sample processing (Figure 2E).[59] Osborn et al.[59] further suggested the technique of sensitivity improvement by timing of reagent flow via chemical and physical flow control technique. Chemical technique can be applied by drying the dissolvable sugar on paper to allow fluidic time delays for dengue detection (Figure 2F). For instance, following the Ag–Ab reaction, the test region was rinsed by phosphate-buffered saline (PBS), followed by the addition of gold enhancement solution to enhance the optical signal. The automated timing-controlled device is created by the application of dissolvable sugar barrier which enhanced the sensitivity of the assay. The physical technique which involves the development of origami card (i.e. NS1 Ag card) with multiple inlet legs has also been suggested for fluidic control. The card consists of three reagent pads, NS1–Ab complex, PBS, and gold enhancement solution, connected to the wicking pad. This technique controls the flow sequence of each reagent toward the test

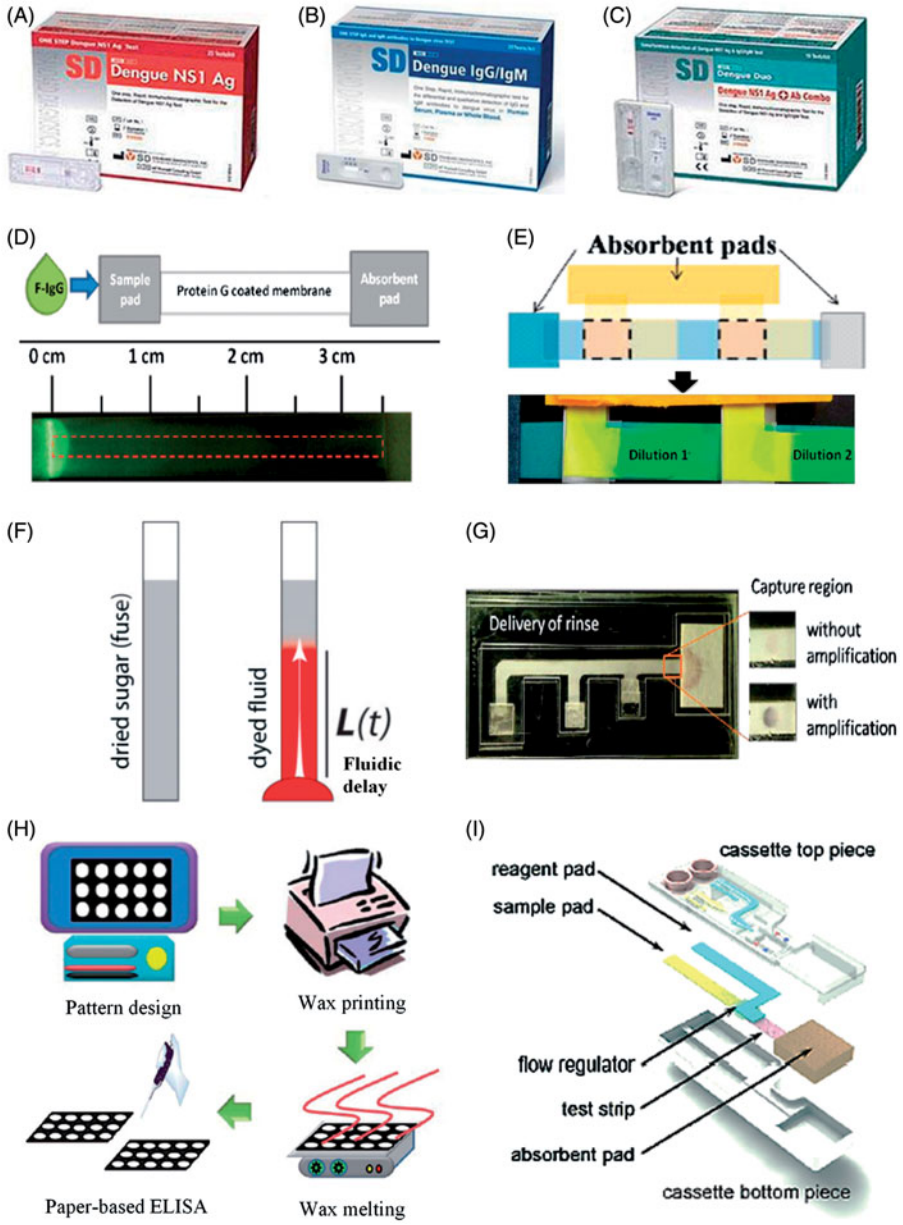

Figure 2. The paper-based detection of Ag–Ab interactions for diagnosis of dengue virus infections. The commercially available (A) SD Bioline Dengue NS1 Antigen, (B) SD Dengue IgG/IgM, and (C) SD Bioline Dengue Duo NS1 and IgM/IgG Combo Device.[11] A 2D paper network for (D) IgG removal,[58] (E) rapid mixing and dilution,[59] (F) fluidic delayed created by dried sugar,[58] and (G) multiple inlet legs-origami card,[58] for assay enhancement. The idea of (H) creating paper-based ELISA to detect viral envelope protein and NS1 detection[60] and (I) stacking flow immunoassay to detect IgG from salivary fluid[61].

region coated with polyclonal Abs, based on their distance from the test region. Similar to the chemical technique, the signal enhancement solution is allowed to flow across the capture zone following the Ab–Ag reaction, thus enhancing the sensitivity of the assay (Figure 2G). In addition to the LFA assay, Wang et al.[60] have successfully developed a paper-based ELISA with filter paper via the technique of wax printing and wax melting to form complete hydrophobic barriers (Figure 2H). The study has successfully detected the envelope protein and NS1 at as low as 100 pg/mL.

To date, the existing dengue Ag or Ab detection techniques require the collection of patient's blood sample. A current study has reported the development of a stacking flow immunoassay to detect dengue-specific IgG directly from salivary fluid, instead of blood sample [61] (Figure 2I). This technique involves the guiding of the samples and reagents to the test strip through two different paths, enabling the removal of the salivary substances (i.e. mucin and other proteinaceous substances) from the sample before reacting with the reagents. These substances would cause particle aggregation and nonspecific adsorption when directly applied to the commercially available lateral flow device. Unlike blood samples, saliva could be collected more easily without causing pain. Therefore, the special design of stacking flow device enables the regulation of fluid flow, which addressed the challenges encountered while using the conventional test strips.

In addition to the need for improvement in sensitivity, specificity and multiplex capability, the accuracy of quantification represents another challenge. Multiple-line or ''barcode'' LFA have been developed to achieve a quantitative readout, where appropriate clinical treatment can be given based on the severity of the diseases. For example, the level of C-reactive protein (CRP) biomarker indicates the severity of inflammation, predicted by the number of visible lines at the test zone, which is critical for diagnosis of bacterial infection and heart disease.[65] This technique can be potentially applied to semi-quantify the DENV to determine the severity of dengue infection. Apart from the conventional

colorimetric-labeled Ag–Ab detection, enzyme-based barcode LFAs have also been demonstrated, which quantify the hydrogen peroxide (H_2O_2) produced from enzymatic reactions that control the conversion rate of 3,3′,5,5′-tetramethylbenzidine (TMB) to blue color complex, allowing accurate POC diagnosis.[66] This technique can also be used to quantitatively measure the amount of DENV Ag corresponding to the severity of the infections for appropriate treatment.

In short, rapid onsite diagnosis of dengue infections currently relies on commercially available paper-based immunoassay kits which detect DENV Ag or Ab in human plasma and serum.[67] To date, there are no relevant commercially available POC devices, which have been specifically designed for the detection of dengue viral nucleic acid. Although the currently available paper-based immunoassay kits have been introduced, they are less sensitive as compared to nucleic acid amplification technique (NAAT). Therefore, researchers have made efforts to integrate highly sensitive and specific molecular diagnostic approaches in paper-based devices for better diagnosis of dengue infections.

Paper-based POC testing via nucleic acid hybridization

In fact, the aforementioned protein-based diagnostic techniques are normally used at the late stage of infection. Instead, molecular diagnostic techniques directly detect the presence of nucleic acids at the early stage of infection, displaying a higher sensitivity and specificity for the diagnosis of infectious diseases compared to Ag–Ab interactions.[68,69] NAAT, as a widely used molecular diagnostic technique consists of three main steps: target nucleic acid extraction, amplification, and detection. The processes are labor-intensive and time-consuming and require complicated equipment for the detection of fluorescence and chemiluminescence signals. The paper-based nucleic acid lateral flow (NALF) assay involves the use of detector probes and capture probes which are complementary to the target nucleic acid sequence. The production of a red band on the test line enables the visual detection of target DNA without instrumentation. The results can be quantitatively analyzed based on the intensity of the red bands.[43] High sensitivity of NALF assay (i.e. lower detection limit) is important to allow detection of low concentration of DENV in early cycles of nucleic acid amplification.

To date, there is no any NALF available on the market, including those for dengue diagnosis. This may be due to the lack of sample pretreatment and amplification technologies in the device, which represent the critical steps of NAAT prior to the detection stage. In fact, there are several commercially available cards such as the fast technology analysis (FTA) card [70] and the IsoCode card,[71] which play an important role in all sample pretreatment processes, such as sample collection and storage, target separation, and extraction. These cards provide an acceptable matrix for the whole blood sample collection, stable storage of the original genetic sequences or Ag for future target amplification (for nucleic acid) and detection. However, the paper-based devices for target amplification have not yet become commercially available. With the advances in fabrication technologies of paper, several studies have also demonstrated the fabrication of paper-based microfluidic devices for sample pretreatment and DNA amplification by wax printing,[72,73] craft-cutting, or paper folding [74] with simple and readily available paper materials such as standard Whatman No. 1 chromatography papers,[75] filter papers,[76,77] and glass fibers.[78] These technologies have to be further simplified and improved before being brought into the market in order to detect serious dengue infections.

In fact, there have been several attempts to improve NALF for dengue infection, mainly in terms of assay sensitivity and multiplex capability.[79] To improve the multiplex capability of NALF, Baeumner et al.[80] have developed a paper-based DNA–RNA hybridization system with amplification of dye-encapsulating liposome for rapid detection of DENV (Figure 3A). By applying an isothermal nucleic acid sequence-based amplification (NASBA) technique, the amplification of low level of viral RNA was performed using a simple water bath. A generic DNA probe is coupled to the liposome, and the amount of liposome-target sequence complexes present in the test line is directly proportional to the amount of target analyte. With the conserved capture probe, a single generic biosensor is capable to detect all four serotype of DENV. However, serotype-specific biosensor would yield false positive result due to the cross-reactivity among the serotypes. To date, the accurate identification of DENV serotype with a device still remains challenging. A multiple analyte detection device can be potentially developed for simultaneous detection of DENV 1–4, which offers an attractive alternative to expensive, complex, and instrument-dependent conventional RT-qPCR. For example, with the design of fluorogenic oligonucleotide probes, a paper-based device has been developed to detect multiple target nucleic acids, hepatitis B virus, HCV, and HIV 1.[42]

Erwin [81] have demonstrated the application of competitive assay to detect the nucleic acid of DENV (Figure 3B). In contrast to the noncompetitive assay, the absence of red line indicates the positive result whereas the presence of a red line indicates the negative result. However, the device possesses some limitations such as low sensitivity and lack of assay validity. The lack of control line in the design failed to show the validity of the assay. To reach the clinically relevant values (10^{-15}–10^{-11} M), the sensitivity of the assay could be improved by the methods such as metal ion-based (i.e. gold or silver)[78] or enzyme-based signal enhancement techniques.[84,85]

One of the metal ion-based signal enhancement techniques is the use of oligonucleotide-linked gold nanoparticles (AuNP) aggregates.[27] Amplification probe (AP) and complementary probe (CP) could hybridize with each other, assembling both AuNP-AP and complementary probe-gold nanoparticle-detector probe (CP-AuNP-DP) to form AuNP aggregates, resulting in a 2.5-fold signal enhancement. Another group has also enhanced the sensitivity of the nucleic acid biosensor with the introduction of molecular beacons (MBs), oligonucleotide probes that report the presence of specific nucleic acid.[86] The introduction of MBs is particularly attractive due to their high selectivity in oligonucleotide recognition, which produced a higher sensitivity of the dry-reagent strip-type nucleic acid biosensor (DSNAB) as

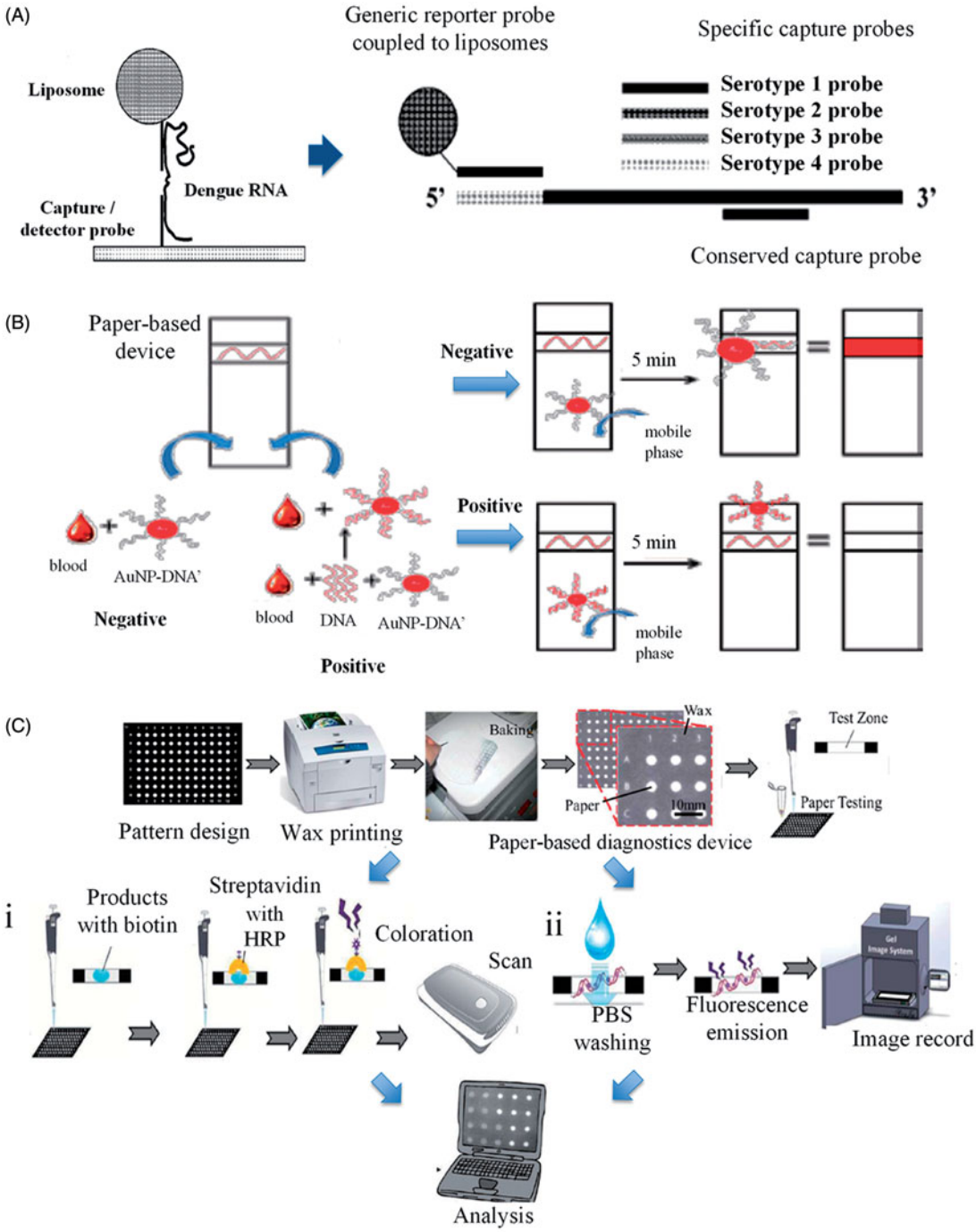

Figure 3. The paper-based detection of nucleic acid hybridization for diagnosis of dengue infections. Nucleic-acid detection in LFA using (A) dye-encapsulating liposome noncompetitive format[80] and (B) competitive format.[81] Paper-based well plates was used to detect the nucleic acids via (Ci) colorimetric[82] and (Cii) fluorescence[83] detection techniques.

compared to conventional dye-labeled MBs and DNA-AuNP-based DSNABs. Besides, the specificity and sensitivity of visual detection of single nucleotide polymorphism (SNP) have been enhanced by using a hairpin oligonucleotide (HO)-gold nanoparticle. The unique recognition characteristic of HO to specific DNA and single-base mismatched DNA, offers a higher assay sensitivity with 500 times lower detection time and shorter preparation time of HO-AuNP conjugates (8 h) as compared to MB-AuNP conjugates (50 h) in their previous work.[87] In another study, this group has further developed an ultrasensitive nucleic acid detection device using an enzyme-based signal enhancement technique, HRP-AuNP dual labels, and is able to detect 0.01 pM of target DNA without using instrumentation, showing a great promise for POC diagnosis.[84] The techniques can be used to fabricate highly sensitive paper-based devices for diagnosis of dengue infection.

Besides that, Lo et al.[83] have also demonstrated the development of a paper-based diagnostic device for early diagnosis of dengue infections at the molecular level. The device is composed of Whatman No. 1 chromatography paper, which was fabricated by using the wax printing technique. The amplification of nucleic acid (i.e. DENV 2 RNA) was performed via RT-LAMP, followed by the

detection of amplified products (DNA) with the device using a colorimetric method[82] (Figure 3Ci). This technique enables the amplification process to be performed at a constant temperature (i.e. isothermal condition) with a shorter assay time (1 h) without the need for thermal cycling as required in PCR. However, it was relatively unstable due to the low binding affinity of biotin–DNA complex to the streptavidin–HRP complex in the paper-based system, and low sensitivity of the device. To this end, this group has further developed a stable approach using DNA ladders and fluorescent probes in the same paper-based platform[83] (Figure 3Cii). This device requires a relatively small amount of sample for accurate DNA detection based on the fluorescence signal. The study provides great insight into the functional development of a combination of a small-scale heating device and a simple paper-based device, which is suitable for POC diagnostics.

Conclusion and future perspectives

Since there are no specific vaccines or treatments available for dengue infections, rapid and accurate diagnosis is critical in providing appropriate health measures to improve healthcare. Early diagnosis is important to identify the patients with secondary infection and to initiate immediate treatment for improved clinical outcomes. In general, the time-consuming serological tests (i.e. ELISA) and complex laboratory assay (i.e. RT-qPCR) are unsuitable for rapid diagnosis in resource-limited dengue endemic areas. The most recent commercially available rapid diagnosis assays, Dengue NS1 Ag strip and One Step Dengue IgG & IgM Rapid Test kits have helped shortening the turnaround time for rapid diagnosis, but their performance is yet to be improved to fully rule out other diseases with similar conditions. Therefore, as an alternative to the existing technologies, the trend of using paper-based diagnostic devices has become a new approach for early diagnosis of dengue infections. The device is simple, portable, and has high potential to achieve the objective for rapid and accurate diagnosis with high availability in the areas with poor infrastructure.

In this review, the advantages of using rapid paper-based diagnostic devices for dengue diagnosis have been discussed as compared to the existing techniques, but further improvements are required to bring the technology to the next level. For example, the multiplexing capability of the device is useful for rapid dengue diagnosis. Given that dengue infection involves a broad range of clinical symptoms, and the levels of virus, Ag, IgG, and IgM are greatly dependent on the timing of sample collection, simultaneous detection of two analytes representing two different stages of the infections are significant for accurate diagnosis. For instance, the concurrent detection of nucleic acid or NS1 at the febrile stage and IgG or IgM at the defervescence stage, enables specific detection of dengue infections. In conjunction with the mobile phone technology, the rapid and accurate target quantification allows better clinical follow-up and timely treatment.

Furthermore, current NAAT, which involves three main steps: nucleic acid extraction, amplification, and detection, plays an important role in disease diagnosis for prompt medical decision. The nucleic acid-based LFA is inapplicable in resource-limited settings without the integration of simple nucleic acid extraction and amplification technologies. Several studies have demonstrated the extraction technique using the FTA card,[70,88] nucleic acid amplification by plastic- and tape-covered glass fiber, and nucleic acid detection by the most commonly used LFA or paper-based microfluidic devices.[87,27] However, the integration of the three main steps into one single paper-based device for dengue diagnosis has not yet been reported. This sample-to-answer molecular diagnostic device is highly required in resource-limited settings. Additionally, another challenge in the development of paper-based device would be the stability of the device, which is particularly related to the reagent storage. The capability of reagent storage makes the device user-friendly, circumventing the need for laboratory supplies such as pipettes or tubes in low resource settings.

Given the fact that suitable therapeutic management can be provided following a definite diagnosis of infections, the development of such a portable and simple device is crucial for diagnosis at the very early stage of infection to save lives. Indeed, for the real-life use in dengue endemic areas, the performance of such a cost-effective device should be validated against the conventional and well-established methods. Specific international control and validation of the device should be considered to avoid the possibility of false positive results arousing from sample preparation, reverse transcription, DNA amplification, and detection.[89,90] The newly established assay should be standardized internationally to achieve a high quality assay performance.

Declaration of interest

The authors report no declarations of interest.

This work was financially supported by the Major International Joint Research Program of China (11120101002), the Key (Key grant) Project of Chinese Ministry of Education (313045), and the International Science & Technology Cooperation Program of China (2013DFG02930). J.R.C., B.P.-M., and W.A.B.W.A. received funding from the Ministry of Higher Education (MOHE), Government of Malaysia under the high impact research (UM.C/HIR/MOHE/ENG/44). F.X. was also partially supported by the China Young 1000-Talent Program and Program for New Century Excellent Talents in University (NCET-12–0437).

References

[1] Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature 2013;496:504–507.
[2] Guzman MG, Halstead SB, Artsob H, et al. Dengue: a continuing global threat. Nat Rev Microbiol. 2010;8:S7–S16.
[3] Halstead SB. Dengue. Lancet 2007;370:1644–1652.
[4] Peeling RW, Artsob H, Pelegrino JL, et al. Evaluation of diagnostic tests: dengue. Nat Rev Microbiol. 2010;8:S30–S38.
[5] Shah I, Katira B. Clinical and laboratory profile of dengue, leptospirosis and malaria in children: a study from Mumbai. Arch Dis Child. 2007;92:561–1510.
[6] Shu PY, Chen LK, Chang SF, et al. Comparison of capture immunoglobulin M (IgM) and IgG enzyme-linked immunosorbent assay (ELISA) and nonstructural protein NS1 serotype-specific IgG ELISA for differentiation of primary and secondary dengue virus infections. Clin Diagn Lab Immunol. 2003;10:622–630.

[7] Lai YL, Chung YK, Tan HC, et al. Cost-effective real-time reverse transcriptase PCR (RT-PCR) to screen for Dengue virus followed by rapid single-tube multiplex RT-PCR for serotyping of the virus. J Clin Microbiol. 2007;45:935–941.
[8] Martinez AW, Phillips ST, Whitesides GM, et al. Diagnostics for the developing world: microfluidic paper-based analytical devices. Anal Chem. 2010;82:3–10.
[9] Teles FS. Biosensors and rapid diagnostic tests on the frontier between analytical and clinical chemistry for biomolecular diagnosis of dengue disease: a review. Anal Chim Acta 2011;687: 28–42.
[10] Hawkins KR, Weigl BH. 2010. Microfluidic diagnostics for low-resource settings. Available from http://dx.doi.org/10.1117/12.845500.
[11] Blacksell SD. Commercial dengue rapid diagnostic tests for point-of-care application: recent evaluations and future needs? J Biomed Biotechnol. 2012;2012:151967.
[12] Fatima A, Wang J. Review: progress in the diagnosis of dengue virus infections and importance of point of care test: a review. Pak J Pharm Sci. 2015;28:271–280.
[13] Dejnirattisai W, Jumnainsong A, Onsirisakul N, et al. Cross-reacting antibodies enhance dengue virus infection in humans. Science 2010;328:745–748.
[14] Simmons CP, Farrar JJ, Nguyen V, et al. Dengue. N Engl J Med. 2012;366:1423–1432.
[15] Guzman A, Isturiz RE. Update on the global spread of dengue. Int J Antimicrob Agents 2010;36:S40–S42.
[16] Ong A, Sandar M, Chen MI, et al. Fatal dengue hemorrhagic fever in adults during a dengue epidemic in Singapore. Int J Infect Dis. 2007;11:263–267.
[17] Wu SJ, Grouard-Vogel G, Sun W, et al. Human skin Langerhans cells are targets of dengue virus infection. Nat Med. 2000;6: 816–820.
[18] Martina BE, Koraka P, Osterhaus AD. Dengue virus pathogenesis: an integrated view. Clin Microbiol Rev. 2009;22:564–581.
[19] Huang KJ, Yang YC, Lin YS, et al. The dual-specific binding of dengue virus and target cells for the antibody-dependent enhancement of dengue virus infection. J Immunol. 2006;176:2825–2832.
[20] Kou Z, Quinn M, Chen H, et al. Monocytes, but not T or B cells, are the principal target cells for dengue virus (DV) infection among human peripheral blood mononuclear cells. J Med Virol. 2008;80:134–146.
[21] Ubol S, Halstead SB. How innate immune mechanisms contribute to antibody-enhanced viral infections. Clin Vaccine Immunol. 2010;17:1829–1835.
[22] Halstead SB. Neutralization and antibody-dependent enhancement of dengue viruses. Adv Virus Res. 2003;60:421–467.
[23] Nachman RL, Rafii S. Platelets, petechiae, and preservation of the vascular wall. N Engl J Med. 2008;359:1261–1270.
[24] Mangada MM, Rothman AL. Altered cytokine responses of dengue-specific CD4+ T cells to heterologous serotypes. J Immunol. 2005;175:2676–2683.
[25] Morens DM, Fauci AS. Dengue and hemorrhagic fever: a potential threat to public health in the United States. JAMA 2008;299: 214–216.
[26] Alvarez M, Rodriguez-Roche R, Bernardo L, et al. Dengue hemorrhagic fever caused by sequential dengue 1-3 virus infections over a long time interval: Havana epidemic, 2001–2002. Am J Trop Med Hyg. 2006;75:1113–1117.
[27] Hu J, Wang L, Li F, et al. Oligonucleotide-linked gold nanoparticle aggregates for enhanced sensitivity in lateral flow assays. Lab Chip 2013;13:4352–4357.
[28] Pok KY, Lai YL, Sng J, et al. Evaluation of nonstructural 1 antigen assays for the diagnosis and surveillance of dengue in Singapore. Vector Borne Zoonotic Dis. 2010;10:1009–1016.
[29] Blacksell SD, Bell D, Kelley J, et al. Prospective study to determine accuracy of rapid serological assays for diagnosis of acute dengue virus infection in Laos. Clin Vaccine Immunol. 2007;14:1458–1464.
[30] Vickers A. Parametric versus non-parametric statistics in the analysis of randomized trials with non-normally distributed data. BMC Med Res Methodol. 2005;5:35.
[31] Kumbhat S, Sharma K, Gehlot R, et al. Surface plasmon resonance based immunosensor for serological diagnosis of dengue virus infection. J Pharm Biomed Anal. 2010;52:255–259.
[32] Ngo HT, Wang HN, Fales AM, et al. DNA bioassay-on-chip using SERS detection for dengue diagnosis. Analyst 2014;139: 5655–5659.
[33] Huang MJ, Xie H, Wan Q, et al. Serotype-specific identification of Dengue virus by silicon nanowire array biosensor. J Nanosci Nanotechnol. 2013;13:3810–3817.
[34] Lien KY, Lee WC, Lei HY, et al. Micro reverse transcription polymerase chain reaction systems using super-paramagnetic beads for virus detection. Paper presented at the 1st IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS'06); 2006 Jan 18–21; Zhuhai. p. 668–673.
[35] Nilghaz A, Wicaksono DH, Gustiono D, et al. Flexible microfluidic cloth-based analytical devices using a low-cost wax patterning technique. Lab Chip 2012;12:209–218.
[36] Bruzewicz DA, Reches M, Whitesides GM. Low-cost printing of poly(dimethylsiloxane) barriers to define microchannels in paper. Anal Chem. 2008;80:3387–3392.
[37] Martinez AW, Phillips ST, Wiley BJ, et al. FLASH: a rapid method for prototyping paper-based microfluidic devices. Lab Chip 2008;8:2146–2150.
[38] Zhao W, Ali MM, Aguirre SD, et al. Paper-based bioassays using gold nanoparticle colorimetric probes. Anal Chem. 2008;80: 8431–8437.
[39] Zhao W, van der Berg A. Lab on paper. Lab Chip 2008;8: 1988–1991.
[40] Zimmermann M, Schmid H, Hunziker P, et al. Capillary pumps for autonomous capillary systems. Lab Chip 2007;7:119–125.
[41] Choi, JR, Hu, J, Feng, S, et al. Sensitive biomolecule detection in lateral flow assay with a portable temperature-humidity control device. Biosens Bioelectron. 2015;79:98–107.
[42] Dineva MA, Candotti D, Fletcher-Brown F, et al. Simultaneous visual detection of multiple viral amplicons by dipstick assay. J Clin Microbiol. 2005;43:4015–4021.
[43] Mao X, Ma Y, Zhang A, et al. Disposable nucleic acid biosensors based on gold nanoparticle probes and lateral flow strip. Anal Chem. 2009;81:1660–1668.
[44] Zhu J, Zou N, Zhu D, et al. Simultaneous detection of high-sensitivity cardiac troponin I and myoglobin by modified sandwich lateral flow immunoassay: proof of principle. Clin Chem. 2011;57:1732–1738.
[45] Lee S, Yearwood G, Guillon G, et al. Evaluation of a rapid, point-of-care test device for the diagnosis of hepatitis C infection. J Clin Virol. 2010;48:15–17.
[46] Prat CM, Flusin O, Panella A, et al. Evaluation of commercially available serologic diagnostic tests for Chikungunya virus. Emerg Infect Dis. 2014;20:2129.
[47] Wang S, Xu F, Demirci U. Advances in developing HIV-1 viral load assays for resource-limited settings. Biotechnol Adv. 2010; 28:770–781.
[48] Feng S, Choi JR, Lu TJ, et al. State-of-art advances in liquid penetration theory and flow control in paper for paper-based diagnosis. Adv Porous Flow 2015;5:16–29.
[49] Li Y, Liu S, Yao T, et al. Controllable synthesis of gold nanoparticles with ultrasmall size and high monodispersity via continuous supplement of precursor. Dalton Trans. 2012;41: 11725–11730.
[50] Eichmann SL, Bevan MA. Direct measurements of protein-stabilized gold nanoparticle interactions. Langmuir 2010;26: 14409–14413.
[51] Dungchai W, Chailapakul O, Henry CS. Use of multiple colorimetric indicators for paper-based microfluidic devices. Anal Chim Acta 2010;674:227–233.
[52] Martinez AW, Phillips ST, Carrilho E, et al. Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. Anal Chem. 2008; 80:3699–3707.
[53] Nie Z, Deiss F, Liu X, et al. Integration of paper-based microfluidic devices with commercial electrochemical readers. Lab Chip 2010;10:3163–3169.
[54] Nemiroski A, Christodouleas DC, Hennek JW, et al. Universal mobile electrochemical detector designed for use in resource-limited applications. Proc Natl Acad Sci USA 2014;111: 11984–11989.

[55] Cuzzubbo AJ, Endy TP, Nisalak A, et al. Use of recombinant envelope proteins for serological diagnosis of Dengue virus infection in an immunochromatographic assay. Clin Diagn Lab Immunol. 2001;8:1150–1155. 10.1128/cdli.8.6.1150-1155.2001.

[56] Omidfar K, Kia S, Larijani B. Development of a colloidal gold-based immunochromatographic test strip for screening of micro-albuminuria. Hybridoma (Larchmt) 2011;30:117–124.

[57] Posthuma-Trumpie G, Korf J, van Amerongen A. Lateral flow (immuno)assay: its strengths, weaknesses, opportunities and threats. A literature survey. Anal Bioanal Chem. 2009;393: 569–582.

[58] Yager P, Fu E, Liang T, et al. A two dimensional paper network for comprehensive dengue detection at the point of care. 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences; 2011; 2092–2095.

[59] Osborn JL, Lutz B, Fu E, et al. Novel sample processing modules for enhanced paper-based diagnostics. 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences; 2011 Oct 2–6; Seattle, WA, USA. p. 500–502.

[60] Wang HK, Tsai CH, Tang CT, et al. Monitoring the disease activity via the antibody-antigen recognition in paper. International Conference on Nano/Micro Engineered and Molecular Systems; 2013 Apr 7–10; Suzhou. p. 229–232.

[61] Zhang Y, Bai J, Ying JY. A stacking flow immunoassay for the detection of dengue-specific immunoglobulins in salivary fluid. Lab Chip 2015;15:1465–1471.

[62] Heeren T, D'Agostino R. Robustness of the two independent samples t-test when applied to ordinal scaled data. Stat Med. 1987; 6:79–90.

[63] Jekel J, Katz D, Elmore J. Epidemiology, biostatistics, and preventive medicine. Philadelphia (PA): W.B. Saunders Company; 2001.

[64] Sawilowsky S. Comments on using alternative to normal theory statistics in social and behavioural science. Canadian Psychol. 1993;34:432–439.

[65] Leung W, Chan CP, Rainer TH, et al. InfectCheck CRP barcode-style lateral flow assay for semi-quantitative detection of C-reactive protein in distinguishing between bacterial and viral infections. J Immunol Methods 2008;336:30–36.

[66] Fung KK, Chan CP, Renneberg R. Development of a creatinine enzyme-based bar-code-style lateral-flow assay. Anal Bioanal Chem. 2009;393:1281–1287.

[67] Bessoff K, Phoutrides E, Delorey M, et al. Utility of a commercial nonstructural protein 1 antigen capture kit as a dengue virus diagnostic tool. Clin Vaccine Immunol. 2010;17:949–953.

[68] Craw P, Balachandran W. Isothermal nucleic acid amplification technologies for point-of-care diagnostics: a critical review. Lab Chip 2012;12:2469–2486.

[69] Yu AC, Vatcher G, Yue X, et al. Nucleic acid-based diagnostics for infectious diseases in public health affairs. Front Med. 2012;6: 173–186.

[70] Lange V, Arndt K, Schwarzelt C, et al. High density FTA plates serve as efficient long-term sample storage for HLA genotyping. Tissue Antigens 2014;83:101–105.

[71] Kudo T, Oshima T, Kure S, et al. Mutation detection of GJB2 using IsoCode and real-time quantitative polymerase chain reaction with SYBR green I dye for newborn hearing screening. Laryngoscope 2004;114:1299–1304.

[72] Rosenfeld T, Bercovici M. 1000-fold sample focusing on paper-based microfluidic devices. Lab Chip 2014;14:4465–4474.

[73] Yang X, Forouzan O, Brown TP, et al. Integrated separation of blood plasma from whole blood for microfluidic paper-based analytical devices. Lab Chip 2012;12:274–280.

[74] Luo L, Li X, Crooks RM. Low-voltage origami-paper-based electrophoretic device for rapid protein separation. Anal Chem. 2014;86:12390–12397.

[75] Blasco H, Garrigue MA, De Vos A, et al. Filter paper saturated by urine sample in metabolic disorders detection by proton magnetic resonance spectroscopy. Anal Bioanal Chem. 2010;396: 1205–1211.

[76] Quraishi R, Lakshmy R, Mukhopadhyay AK, et al. Analysis of the stability of urea in dried blood spots collected and stored on filter paper. Ann Lab Med. 2013;33:190–192.

[77] Randriamparany T, Kouakou K, Michaud V, et al. African Swine fever diagnosis adapted to tropical conditions by the use of dried-blood filter papers. Transbound Emerg Dis. 2014. doi:10.1111/tbed.12295. [Epub ahead of print].

[78] Rohrman BA, Richards-Kortum RR. A paper and plastic device for performing recombinase polymerase amplification of HIV DNA. Lab Chip 2012;12:3082–3088.

[79] Choi JR, Tang R, Wang S, et al. Paper-based sample-to-answer molecular diagnostic platform for point-of-care diagnostics. Biosens Bioelectron. 2015;74:427–439.

[80] Baeumner AJ, Schlesinger NA, Slutzki NS, et al. Biosensor for dengue virus detection: sensitive, rapid, and serotype specific. Anal Chem. 2002;74:1442–1448.

[81] Erwin, P. Nucleic-acid based lateral flow strip biosensor via competitive binding for possible Dengue detection. J Biosens Bioelectron. 2012;3:128.

[82] Lo SJ, Yang SC, Yao DJ, et al. Molecular-level dengue fever diagnostics via a combination of RT-LAMP and paper-based devices. Paper presented at the IEEE International Conference on Nanotechnology Materials and Devices Conference (NMDC); 2012 Oct 16–19; Waikiki Beach, HI, USA. p. 84–87.

[83] Lo SJ, Yang SC, Yao DJ, et al. Molecular-level dengue fever diagnostic devices made out of paper. Lab Chip 2013;13: 2686–2692.

[84] He Y, Zhang S, Zhang X, et al. Ultrasensitive nucleic acid biosensor based on enzyme-gold nanoparticle dual label and lateral flow strip biosensor. Biosens Bioelectron. 2011;26: 2018–2024.

[85] Parolo C, de la Escosura-Muñiz A, Merkoçi A. Enhanced lateral flow immunoassay using gold nanoparticles loaded with enzymes. Biosens Bioelectron. 2013;40:412–416.

[86] Mao X, Xu H, Zeng Q, et al. Molecular beacon-functionalized gold nanoparticles as probes in dry-reagent strip biosensor for DNA analysis. Chem Commun. 2009;7:3065–3067.

[87] He Y, Zeng K, Gurung AS, et al. Visual detection of single-nucleotide polymorphism with hairpin oligonucleotide-functionalized gold nanoparticles. Anal Chem. 2010;82:7169–7177.

[88] Liang X, Chigerwe M, Hietala SK, et al. Evaluation of fast technology analysis (FTA) cards as an improved method for specimen collection and shipment targeting viruses associated with bovine respiratory disease complex. J Virol Methods 2014; 202:69–72.

[89] Guzman MG, Kouri G. Dengue diagnosis, advances and challenges. Int J Infect Dis. 2004;8:69–80.

[90] Teles FR, Prazeres DM, Lima-Filho JL. Trends in dengue diagnosis. Rev Med Virol. 2005;15:287–302.

[91] Andries AC, Duong V, Ngan C, et al. Field evaluation and impact on clinical management of a rapid diagnostic kit that detects dengue NS1, IgM and IgG. PLoS Negl. Trop. Dis. 2013;7:e2116.

[92] Barde P, Godbole S, Bharti P, et al. Detection of dengue virus 4 from central India. The Indian journal of medical research. 2012;136:491.

[93] Chaterji S, Allen JC, Chow A, et al. Evaluation of the NS1 rapid test and the WHO dengue classification schemes for use as bedside diagnosis of acute dengue fever in adults. The American journal of tropical medicine and hygiene. 2011;84:224–228.

[94] Chua K, Mustafa B, Abdul Wahab A, et al. A comparative evaluation of dengue diagnostic tests based on single-acute serum samples for laboratory confirmation of acute dengue. Malaysian Journal of Pathology. 2011;33:13–20.

[95] Ferraz FO, Bomfim MR, Totola AH, et al. Evaluation of laboratory tests for dengue diagnosis in clinical specimens from consecutive patients with suspected dengue in Belo Horizonte, Brazil. Journal of Clinical Virology. 2013;58:41–46.

[96] Fry SR, Meyer M, Semple MG, et al. The diagnostic sensitivity of dengue rapid test assays is significantly enhanced by using a combined antigen and antibody testing approach. PLoS Neglected Tropical Diseases. 2011;5:e1199.

[97] Hue KDT, Tuan TV, Thi HTN, et al. Validation of an internally controlled one-step real-time multiplex RT-PCR assay for the detection and quantitation of dengue virus RNA in plasma. Journal of Virological Methods. 177:168–173.

[98] Osorio L, Ramirez M, Bonelo A, et al. Comparison of the diagnostic accuracy of commercial NS1-based diagnostic tests for early dengue infection. Virology Journal. 2011;7:361.

[99] Pan-ngum W, Blacksell SD, Lubell Y, et al. Estimating the true accuracy of diagnostic tests for dengue infection using bayesian latent class models. PloS One. 2013;8:e50765.
[100] Puttikhunt C, Prommool T, Nathaporn U, et al. The development of a novel serotyping-NS1-ELISA to identify serotypes of dengue virus. Journal of Clinical Virology. 2011;50:314–319.
[101] Tontulawat P, Pongsiri P, Thongmee C, et al. Evaluation of rapid immunochromatographic NS1 test, anti-dengue IgM test, semi-nested PCR and IgM ELISA for detection of dengue virus. Southeast Asian Journal of Tropical Medicine and Public Health. 2011;42:570.
[102] Tricou V, Vu H, Quynh N, et al. Comparison of two dengue NS1 rapid tests for sensitivity, specificity and relationship to viraemia and antibody responses. BMC Infectious Diseases. 2010;10:142.
[103] Wang SM, Sekaran SD. Evaluation of a commercial SD dengue virus NS1 antigen capture enzyme-linked immunosorbent assay kit for early diagnosis of dengue virus infection. Journal of Clinical Microbiology, 2010;48:2793–2797.
[104] Waggoner JJ, Abeynayake J, Sahoo MK, et al. Single-reaction, multiplex, real-time RT-PCR for the detection, quantitation, and serotyping of dengue viruses. PLoS Neglected Tropical Diseases, 2013;7:e2116.