Point-of-care (POC) assay to detect dengue exposure

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Abstract

Dengue virus (DENV) infection is a life-threatening disease. About 40% of the world population lives in high-risk dengue-endemic areas, and the global incidence has significantly increased in the last three decades. Early DENV detection is critical but also challenging since the primary infection is asymptomatic. The dengue vaccine exists but is less effective and potentially dangerous if the person has no previous exposure to DENV. However, the current immunoassay-based technologies used to distinguish primary and secondary infections are not qualified for point-of-care (POC) because they are neither rapid nor cost-effective. This work aims to develop a dengue-specific POC assay that can detect circulating biomarkers to confirm dengue exposure as early as possible to facilitate a high-throughput vaccination program.

Keywords: Dengue, fever, viral infection, DENV, detection, vaccine, NS1, IgM, Sensor, electrochemical, aptamer, point-of-care (POC).

Significance | Prediction of COVID-19 patient's fate

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Introduction

Dengue is an acute systemic viral infection transmitted between humans by *Aedes mosquitoes* (mainly *Aedes aegypti* in **Figure 1** and a lesser extent, *Aedes albopictus*).(Bhatt et al., 2013) This viral disease can cause life-threatening illness in tropical and subtropical regions.(Gibbons, Streitz, Babina, & Fried, 2012) For example, the frequency of dengue infection has been a persistent challenge and concern for US military personnel since World War II.(Gibbons et al., 2012) The dengue vaccine (CYD-TDV) is highly effective at protecting against infections, but it is recommended for individuals that test positive for a newly acquired infection or for those that have had a prior infection. Therefore, the clinical efficacy of a dengue vaccine is significantly increased when individuals are properly diagnosed with the infection prior to its administration. Clinical trials have confirmed that the dengue vaccine is less effective when administered to individuals who have no previous dengue exposure.(Department of Defense (DOD); Gibbons et al., 2012; Shepard, Undurraga, Halasa, & Stanaway, 2016; Sridhar et al., 2018) Without a rapid test to confirm infection, the vaccine can cause serious health issues.(Shepard et al., 2016) Time-sensitive dengue vaccination programs in dengue-endemic regions are crucial and depend on the availability of a cost-effective, reliable, and rapid dengue test. There is a clear, urgent need for a rapid, point-of-care (POC) assay that will measure dengue exposure almost instantly after the first and/or secondary infection in remote resource-limited areas.(Department of Defense (DOD)) Using rapid screening results, the healthcare personnel can provide immediate vaccination support or further

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Figure 1. Dengue exposure by Aedes aegypti mosquito bite. A rapid POC test is an urgent unmet need to detect dengue

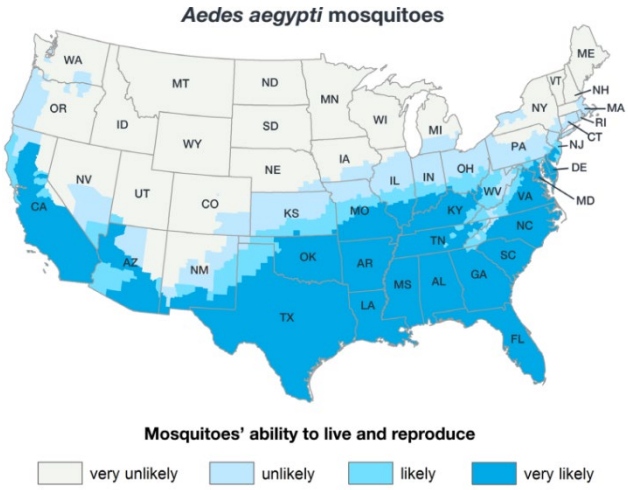

Figure 2. CDC's best estimate of the potential range of Aedes aegyptiand in the United States based on previous data.

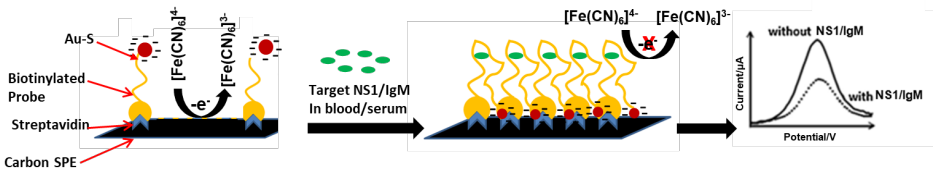

Figure 3. An Experimental set up using Streptavidin-modified SPEs, and a redox probe (ferricyanide) showed electrochemical detection of DENV-NS1/DENV-IgM via aptamer-functionalized gold nanoparticles

diagnostic assessment, or precision health counseling/treatment.(Department of Defense (DOD)) The World Health Organization (WHO) also endorses the development of rapid tests for dengue exposure as a critical step towards reducing mortality and facilitating early clinical intervention with dengue protection prior to severe infection.(WHO, 2019) Current test protocols for US military personnel involve the collection and testing of serum samples before and after deployment to screen for dengue infection. More frequent, on-site testing using a rapid POC test would enable better screening of deployed personnel since dengue infections can occur anytime during deployment. (Hesse et al., 2017) There is an increased risk for severe dengue disease associated with secondary infections during prolonged or repeat deployments to dengue-endemic areas.(Hesse et al., 2017) For example, there are around 11% dengue seroprevalence rate observed in 500 U.S. Special Forces soldiers during their deployment in South America in 2006-2008.(Caci et al., 2014) A rapid screening assay can help to counsel the risk of being undiagnosed during deployment, also minimizing any infection risk during the time gap between pre-and post-deployment monitoring process and further confirm any necessity of vaccination.(Hesse et al., 2017) That's why there is an urgent unmet need for early and rapid diagnostics of this lethal infection prior to receiving the vaccination.(Department of Defense (DOD); WHO, 2009)

Early diagnosis of DENV infection is crucial not only to the warfighter but also to the public. This infectious disease is a growing threat to people that live in or travel to dengue-classified endemic regions.(Castellanos & Coronel-Ruiz, 2014) According to the WHO and Center for Disease Control and Prevention (CDC), about 400 million individuals are infected with dengue virus (DENV) around the world annually.(Bhatt et al., 2013; Center for Disease Control and Prevention (CDC); Robinson et al., 2019; WHO, 2019) Out of these infections, 0.5 million cases develop dengue hemorrhagic fevers which lead to ~25,000 deaths every year.(Bhatt et al., 2013; Castellanos & Coronel-Ruiz, 2014; Center for Disease Control and Prevention (CDC); Rey, 2014; Robinson et al., 2019; WHO, 2019) This mortality rate would be less if the infection could be identified early. The dengue-related global annual cost is approximately 8.9 billion USD.(Shepard et al., 2016) In May 2019, the CDC, the National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), and the Division of Vector-Borne Diseases (DVBD) reported that about 3 billion people (~40% of the world's total population) live in high risk of dengue transmission areas.(Center for Disease Control and Prevention (CDC)) Dengue virus disease has now spread to more than 120 countries, including the United States (most recently to Hawaii, Florida, and Texas) and its territories of Puerto Rico, the US Virgin Islands, and American Samoa.(Center for Disease Control and Prevention (CDC); Hesse et al., 2017; Rey, 2014) **Figure 2** shows the high-risk dengue prevalent areas in the United States. Extensive research has been performed to discover several target biomarkers for confirming dengue exposure. According to the CDC, current literature, as well as commercially available kits approved/non-approved by U.S. FDA, dengue specific- non-structural protein 1 (NS1) for primary infection and immunoglobin (M) IgM/ immunoglobin (g) IgG for secondary infection is recommended to confirm dengue exposure.(Anand et al., 2016; CDC, 2019) Most of the commercial kits target these antigen/antibody biomarkers for early DENV detection.(CDC, 2019) NS1 in combination with IgM assay, offers the most sensitive and cost-effective diagnostic modality for dengue.(Anand et al., 2016) Currently there is no sensitive instrument that is capable of point-of-care (POC) measurement of dengue-specific NS1 and IgM in the blood/blood serum.(Balmaseda et al., 2017)

Diseases caused by DENV: DENV is a 50 nm virus that consists of 11 Kb genome bases that encodes a large polyprotein that is subsequently cleaved into three structural proteins (capsid, prM, and E) and a non-structural protein (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5). This virus particle consists of an RNA-capsid protein complex surrounded by a bilayer lipid membrane.(Hasan, Sevvana, Kuhn, & Rossmann, 2018; Modis, Ogata, Clements, & Harrison, 2004) There are four serotypes of dengue virus (DENV) reported: DENV-1, DENV-2, DENV-3, and DENV-4. One more serotype recently found in Malaysia shows similarities to DENV-4.(Darwish, Sekaran, & Khor, 2018) Approximately ~83% polyprotein homology is identical between each serotype, with only subtle differences observed in their surface protein.(Ngono & Shresta, 2018; X. Xu et al., 2016) Each serotype can cause the full spectrum of clinical manifestations following DENV infection, ranging from asymptomatic infection to severe dengue infection. Severe infections, including dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which primarily affect children but can affect adults as well. About 5%-20% of symptomatic (fever, severe headache, muscle, and joint pain, nausea and vomiting, eye pain, and rash) individuals with acute DHF progress to severe dengue manifested by bleeding, plasma leakage, shock, organ failure, and sometimes death.(Castellanos & Coronel-Ruiz, 2014; Robinson et al., 2019) While the first dengue infection is often asymptomatic, the severity of secondary infection can cause antibody-dependent enhancement (ADE) with variable contribution from aberrant activation of cross-reactive T-cells.(Robinson et al., 2019) This can lead a wide range of clinical manifestations, including potentially fatal DHF and DSS to children and adults in some cases compromising organs like the brain, liver, or heart.(Barniol et al., 2011; Castellanos & Coronel-Ruiz, 2014)

Target Biomarkers and Their Biological Levels: Asymptomatic patients are not recommended for any dengue virus detection test. DENV-NS1 is a highly conserved glycoprotein secreted in the blood immediately after the infection (at 0 days), so this is the only detectable reliable antigen during the acute phase (initial 1-7 days after symptom onset) of the infection.(Lai et al., 2019) After 7 days, DENV-NS1 will not be available for detection. So, DENV-NS1 is considered the ideal diagnostic biomarker for primary infection.(Alcon et al., 2002) The circulating DENV-NS1 concentration ranges from 7 to 284 ng/mL and in some cases up to 50 µg/mL.(Alcon et al., 2002; Allonso, Meneses, Fernandes, Ferreira, & Mohana-Borges, 2014) DENV-IgM or Immunoglobulin (G) (DENV-IgG) is a preferable biomarker for secondary infection during the convalescent phase (>7 Days Post Symptom Onset).(Lai et al., 2019) DENV-IgM is the first antibody that appears after infection (3-5 days) and persist for 30-60 days.(antibodies-online.com, 2022) During secondary infection, DENV-IgM level rises more slowly and reaches lower levels than during primary infection. DENV-IgG could be an alternative biomarker for DENV-IgM since it appears soon after infection (10-14 days) and stays in the blood for life. During secondary infection, DENV-IgG levels rise rapidly for 1 to 2 days.(Navarrete-Espinosa, Cuervo-Hernandez, & Vazquez-Martinez, 2008)

Current Technologies for Dengue exposure detection: Standardized immunological assays such as-Enzyme-linked Immunosorbent Assay (ELISA) for detection of viral antigen-antibodies, Plaque reduction neutralizing assay (PRNT) for specific neutralizing antibodies against dengue virus and CDC DENV-1-4 rRT-PCR Multiplex and Trioplex rRT-PCR assays are available for detecting dengue virus RNA.(Santiago et al., 2018) PRNTs are only available at the CDC or in highly specialized laboratories designated by CDC. PRNTs are cumbersome, requiring analysis of specimens in a central reference laboratory like CDC, thus making it not feasible for routine monitoring of dengue exposure and the vaccination recruitment program.(CDC, 2019; Department of Defense (DOD)) Despite the seemingly low detection limits, antibody-based ELISA assays are often non-specific to similarly structured targets and thus show lack of specificity. The currently used ELISA for DENV cross-reacts with other flaviviruses, particularly Zika Virus (ZIKV), along with Yellow-Fever and Japanese Encephalititis virus (JEV) vaccine-derived antibodies.(Brown et al., 2019; Priyamvada et al., 2016) Immunoassay methods also require skilled personnel and laborious sample preparation protocols in well-established laboratories that result in long turn-around-times (hours to days). A cost-effective, rapid POC assay to detect dengue exposure is an urgent and unmet need.(Department of Defense (DOD))

Why Electrochemical Detection? Electrochemical methods are faster and more viable alternative techniques as they do not require complex instrumentation or complicated sample preparation steps. Redox reactions occur on the sensing electrode at an analyte specific potential applied to the sensing electrode, and every analyte has a characteristic behavior even in a mixture of substances, like a fingerprint. Biotin-terminated DENV-NS1/DENV-IgM aptamer dually labeled with 3'-thiol and 5'-biotin is covalently attached to the streptavidin electrode. Also, the thiol terminated DENV-NS1/DENV-IgM aptamer is covalently attached to the gold nanoparticles (AuNPs). In the absence of DENV-NS1/DENV-IgM, the specific aptamer remains partially unfolded, so it allows ferricyanide (as the redox probe) to approach to electrode surface. In the presence of DENV-NS1/DENV-IgM, since the target-specific aptamer folds to bind with DENV-NS1/DENV-IgM and hence, AuNPs with negative charge at the end of the aptamer would come near the electrode surface, inhibiting the electron transfer and decreasing the redox probe $[Fe(CN)_6]^{4-/3-}$ peak current. With increasing DENV-NS1/DENV-IgM concentration, the peak current of ferricyanide decreases linearly. To detect the dengue specific analytes, various analytical methods such as differential pulse voltammetry (DPV), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques can be used **(Figure 3)**.

The high sensitivity and low detection limits ultimately depend on aptamer immobilization on the electrode surface and their covalent attachment to AuNPs.(Roushani & Shahdost-Fard, 2015) Multiple AuNPs can conjugate with one probe but the functionality and outcome of signal is not be influenced.(Roushani & Shahdost-Fard, 2015) DPV shows higher sensitivity than CV, so the optimal experimental conditions favor adjustment of DPV parameters.(Roushani & Shahdost-Fard, 2015) The changes of electrochemical current at different potentials depend on the type of electrode modification.(J. Xu, Wang, Xian, Jin, & Tanaka, 2003) We have previously used the SWV method for detection of short peptides in clinical samples at concentration values down to 1 pg/mL. To the best of our knowledge, there is no precedent to aptamer based direct electrochemical detection of dengue specific DENV-NS1 antigen and/or DENV-IgM antibody at low pg/mL levels.

Potential Application

Dengue infection can be lethal if misdiagnosed or untreated. The severity of the disease depends on rapid diagnosis and early therapeutic intervention. Currently, there is no rapid POC assay, so prohibiting early and accurate diagnosis and reducing the impact of the vaccination program. Many have developed complex and laborious ELISA/PRNT-based dengue detection kits/assays, but they require advanced and sophisticated laboratory facilities.

A real-time electrochemical sensor device to accurately detect DENV (primary vs. secondary infection) rapidly and cost-effectively would be a great alternative to existing solutions. In

addition to its specific military use for monitoring early dengue exposure, this device would apply to the general public, including people who live or travel to dengue-endemic areas. The potential market for such device is also not limited to military use; hospitals and clinics can benefit from performing routine check-ups in a minimally invasive fashion.

Author Contributions

M.R. conceived the study and designed the proposed strategy. M.R. wrote the manuscript. A.A. contributed intellectually to the scientific discussion and proofread the manuscript.

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Competing financial interests

The authors have no conflict of interest

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