

EVALUATION OF THE DENGUE NS1 AG STRIP FOR DETECTING DENGUE VIRUS ANTIGEN IN WILD-CAPTURED AND LABORATORY-REARED *Aedes Aegypti*

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Dengue is a serious public health concern worldwide. *Aedes aegypti* is the main vector of DENV, and *Aedes albopictus* acts as a secondary vector of Dengue Virus (da Costa *et al.*, 2017; Guzman *et al.*, 2016). Dengue incidence has been high in recent years and is present in more than 128 countries (Brady *et al.*, 2012). The majority of its range is in the tropics and subtropics (Mutsuddy *et al.*, 2019). Dengue Hemorrhagic Fever (DHF) is particularly concerning in Southeast Asia, where it poses a major threat to children's health (Rohani *et al.*, 2008). Up to 390 million dengue cases are thought to occur each year, and approximately 2.5 billion people are at risk for infection worldwide (Pal *et al.*, 2014). It poses serious threat to many countries of Asia including Bangladesh. The dengue virus is widespread throughout Bangladesh and significantly contributes to morbidity and mortality (Bhowmik *et al.*, 2023). The epidemiology of dengue in Bangladesh is experiencing a subtle shift (Bashar *et al.*, 2020 and 2013). In 2023, Bangladesh experienced the highest number of dengue cases and deaths in history. According to the Health Emergency Operation Center and Control Room of the Directorate of General Health Services, the reported dengue cases have surpassed 300,000, with deaths exceeding 1,500(DGHS).

In the absence of vaccines or therapeutic options, the prevention of dengue fever largely depends on the surveillance and control of mosquito vectors (Tan *et al.*, 2011; Voge *et al.*, 2013). Current surveillance systems are insufficient to implement timely vector control measures to prevent outbreaks. Early dengue virus detection is crucial for dengue outbreaks prediction. Many techniques are available for DENV detection. Like through cell culture and immunofluorescence techniques (da Cruz Santos *et al.*, 2020), enzyme-linked immunosorbent assay

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(ELISA) (Pollak *et al.*, 2023), hemagglutination inhibition (HI), complement (fixation tests (CF), neutralization tests, and ELISA (Zhang *et al.*, 2015), real-time reverse transcription-polymerase chain reaction (RT-PCR), Dengue NS1 Ag Kit or other nucleic acid detection methodologies (Kao *et al.*, 2005).

In Bangladesh, a Dengue NS1 Ag Kit like qDetect™ Dengue NS1 Ag Test Device is used for DENV detection from patient samples only which works based on the principle of ICT and targets the Non-structural protein 1 (NS1) that is present in blood within the first seven days following the onset of fever (Raihan *et al.*, 2025). In many countries like India, Mexico, Colorado Dengue NS1 kits are using as an entomovirological tool to identify DENV not only from blood samples but also from Field-caught *Aedes spp.* .

Currently, there is a significant gap in research conducted in Bangladesh regarding the utilization of the NS1 antigen kit for detecting the Dengue virus in *Aedes aegypti* mosquitoes. The present study aimed to evaluate the efficacy of the Dengue NS1 Ag Kit (qDetect™ Dengue NS1 Test Device) to detect the dengue virus from both wild-captured adult and laboratory-reared *Aedes aegypti* and to find out the sensitivity of qDetect™ Dengue NS1 Test Device in detecting DENV in *Aedes aegypti* mosquitoes.

This study was conducted in the Insect Rearing & Experimental Station (IRES) for six months from June 2024 to November 2024 , a dedicated facility of the Department of Zoology, Jahangirnagar University, Savar, Dhaka-1342. The geographical location of this site is 23.874903 N and 90.266568 E, recorded using GPS essentials apps in device. The study was conducted in a region characterized by a tropical monsoon climate, which is marked by significant seasonal variations. Mosquito specimens were collected from five designated locations in the North Dhaka area (Plate 1.). The study protocol was approved by the Biosafety, Biosecurity, and Ethical Clearance Committee of Jahangirnagar University. Ref No: BBECJU/M 2024/ 03 (89).

Aedes aegypti mosquitoes were systematically collected on a weekly basis from five major areas of Dhaka North City: Uttara, Mirpur 1, Mirpur 2, Gulshan, and Mohammadpur, which have been notably affected by dengue fever. Adult mosquitoes were captured utilizing light traps and Gravitraps, thereby ensuring a robust and efficient sampling procedure. Following collection, the mosquitoes were transported to the IRES for subsequent experimentation. The *Aedes aegypti* mosquitoes were identified using a stereo microscope and taxonomical keys, facilitating the accurate categorization of these species (Rueda, 2004; Le Goff *et al.*, 2012). To preserve the integrity of the specimens for DENV NS1 antigen detection tests, the collected mosquitoes were meticulously preserved at -70°C, which effectively dehydrates them and facilitates their preparation for analysis.

Aedes aegypti mosquitoes were reared at $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $75\% \pm 5\%$ humidity (Imam and Seikh, 2014). Adult females, aged 3 to 5 days, were provided blood meals for egg laying. A conical filter paper was placed in distilled water overnight for egg deposition. After hatching, larvae were fed yeast powder, and adults were maintained on a 10% sugar solution. (Gerberg et al., 1994).

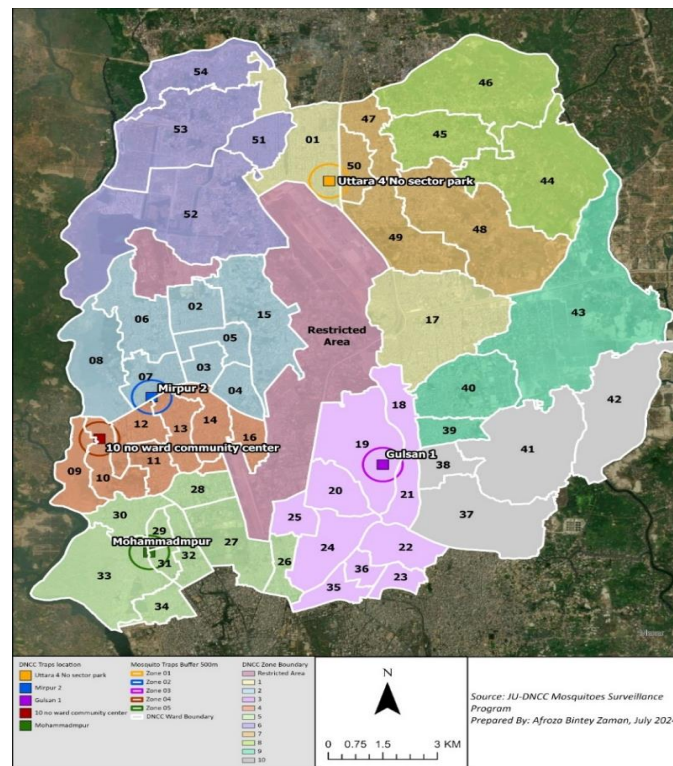


Plate 1. The map of the sampling area

Fifty male and one hundred fifty female *Aedes aegypti* mosquitoes were placed in a cage to facilitate mating after emerging from the pupa stage. Five days later, the females were fed with dengue-infected blood using an artificial membrane feeding method with Parafilm to simulate animal skin, allowing them to pierce it for blood. Six milliliters of blood were introduced, and warm water at approximately 37°C circulated through glass cones to keep the blood at the right temperature. After feeding, the engorged mosquitoes were removed and placed in a new cage with a glucose solution. Three days post-feeding, they were separated using an aspirator and anesthetized by freezing.

Following the identification of field-caught mosquitoes each pool comprised ten mosquitoes and subsequently, each mosquito pool was transferred into a 1.5 ml Eppendorf tube. To determine the sensitivity of the qDetect™ Dengue NS1

Test Device, we created ten pools comprised of dengue virus (DENV)-infected and uninfected *Aedes aegypti* mosquitoes in varying ratios. Forty-five mosquitoes were artificially infected and grouped into nine pools, consisting of infected *Aedes aegypti* mosquitoes ranging from one to nine (Voge *et al.*, 2013).

Phosphate-buffered saline (PBS) solution was prepared to achieve a physiological pH range of 7.2 to 7.4 using hydrochloric acid and was monitored with a pH meter. Each pool received 50 µl of PBS, which was then homogenized to ensure uniformity, as noted by Tan *et al.* (2011). The samples were centrifuged at 4000 rpm for 8 minutes. Using the dropper provided with the kit, the supernatant was carefully collected without disturbing the pellet. For the dengue NS1 antigen test, three drops of the supernatant were transferred to the designated well of a qDetect™ Dengue NS1 test device.

Tabulation, analysis, graphs and percentages of raw data were carried out using Microsoft Excel 2019. The percentages of sensitivity of **qDetect™** for DENV positive mosquito pool were calculated using the following formula (Wang and Sekaran, 2010):

$$\text{Sensitivity Percentage} = a / (a+c) \times 100\%$$

a = No. of pools containing DENV-positive mosquito

c = No. of false negative pools

A total of 1729 *Aedes aegypti* mosquitoes were captured during the study period from the selected five locations. From these mosquitoes, 160 pools were created for subsequent analysis. Of these pools, 44 (approximately 27.5%) were identified as positive for DENV (Figure 1).

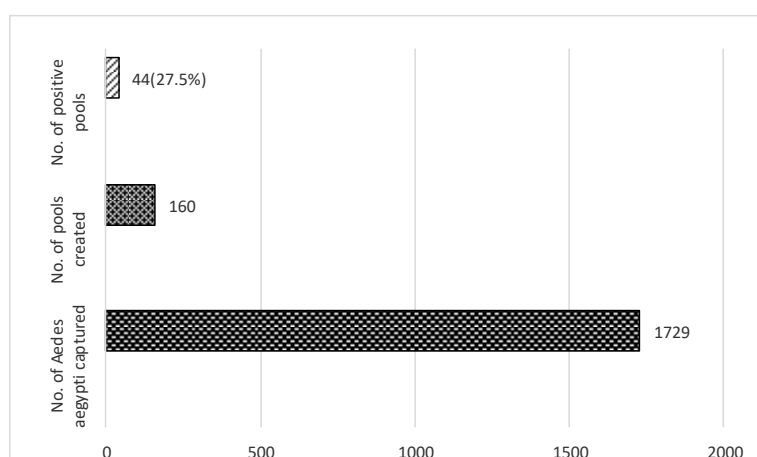


Fig. 1. No. of DENV-positive pools found from the field-caught *Aedes aegypti* during the study period

The result clearly indicated that this NS1 Test Device showed 100% sensitivity in detecting Dengue NS1 Ag in laboratory-controlled infected pools of *Aedes aegypti* mosquitoes even when the pool of 10 *Aedes aegypti* mosquito having decreasing ratio of DENV infected mosquitoes compared to uninfected mosquitoes. In all tested scenarios, ranging from 9 infected mosquitoes and 1 uninfected mosquito down to just 1 infected mosquito and 9 uninfected mosquitoes within a pool the NS1 Kit consistently yielded a positive result (Table1).

Table 1. Evaluation of the sensitivity of qDetect™ Dengue NS1 Test Device in detecting NS1 Ag from Infected *Aedes aegypti*

Mosquito type	Pool size(n)	Infected: Uninfected (N)	Result	Sensitivity (%)
Laboratory Controlled	10	9:1	+	100%
	10	8:2	+	
	10	7:3	+	
	10	6:4	+	
	10	5:5	+	
	10	4:6	+	
	10	3:7	+	
	10	2:8	+	
	10	1:9	+	

N = Number of mosquitoes; n = Number of mosquitoes used in pool construction

The present study evaluated the effectiveness of Dengue NS1 antigen detection kits in identifying the dengue virus in *Aedes aegypti* mosquitoes. Here it is clearly indicated that this qDetect™ Dengue NS1 Test Device can detect DENV from both field-caught and laboratory-reared *Aedes aegypti* and can be used as an entomovirological tool and these findings coincide with the findings of Chao *et al.*, (2015); Lau *et al.*, (2017) and Tan *et al.*, (2011), who similarly highlighted the potential of the NS1 kit for the detection of DENV from *Aedes aegypti* mosquito and surveillance of dengue virus (DENV) in wild mosquito populations.

Our result also showed that this device exhibits high sensitivity in terms of detecting dengue virus from *Aedes aegypti*. This device can detect DENV from mosquito pool even when the ratio of infected and uninfected mosquitoes within a pool of 10 was as low as 1:9. This observation suggests the potential utility of the kit for surveillance purposes, as it is capable of detecting a small number of

infected mosquitoes within a pool with small amount of DENV. This finding is supported by the research conducted by Abraham and Kumar (2021), who noted that the NS1 Antigen Kit can identify the dengue virus from the infected blood-fed and unfed mixed mosquito pools of *Aedes aegypti*. They observed its high sensitivity and specificity for virus detection, being able to detect as little as 750 femtograms of NS1 protein.

During the study, the results indicated that the average time for positive detection from field collected mosquitoes was approximately two to three hours for the appearance of lines in the test region. This finding contrasts with the results reported by Tan *et al.*, (2011), which noted that a positive result for wild-caught mosquitoes could be obtained in under one hour. The variation in the timing for results may be attributed to the quantity of virus present within *Aedes aegypti*. Additionally, the color of the test lines differed under various conditions: a red line appeared for clinical samples, light pink for laboratory-controlled infected mosquitoes, and faint purple for field collected mosquitoes.

In summary, the qDetect™ Dengue NS1 Test Device presents a valuable and highly sensitive tool for entomovirological surveillance of DENV in mosquito populations. It demonstrated efficacy and the ability to detect low levels of infection make it a significant asset in ongoing efforts for dengue prevention. Integrating this kit in a surveillance system can help in early dengue virus detection, which is crucial for implementing timely vector control measures to prevent dengue outbreaks.

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