DEVELOPMENT OF ONE TIME MULTI-COLOR CHART DETECTION OF DENGUE VIRUS USING MOBILE CAMERA ANALYSIS VIA DNA LABEL FREE PROBE

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Keywords:

DENV, G4 Quadruplex, probe IDT (integrated DNA technology), ABTS , Diagnostic kit ,UV visible spectrophotometer, mobile camera analysis, color chart.

Introduction:

Deoxyribonucleic acid (DNA), which serves as a genetic information storage mechanism for all living species as well as many viruses, has allowed scientists to identify essential organisms based on sequences that are unique to each organism. As the continuous threat of diverse organisms and viruses against humans has become a critical public issue, medical diagnostics can take advantage of these valuable properties. DNA-based detection methods, in which the unique DNA sequences of target organisms or viruses are identified, are appealing alternatives for the diagnosis of infection. In general, the detection of any biological information in an in vitro system necessitates several critical components. This contains target organism segments that are unique, target binders that capture the segments, and, last but not least, a reporter system that can detect the presence of target organisms. The fact that DNA-based sensors are very reliant on the target DNA and DNA anchor/probe meeting and interacting strongly is an appealing feature. Chemical modification and labelling, whether in combination with fluorescent dyes, dye pigments, nanoparticles, or enzymes, can produce a variety of readouts in many DNA-based detection systems. The use of the oligonucleotide itself as a reporter for the creation of visual signals is a good but less expensive alternative to the use of modified oligonucleotides. This is possible thanks to the use of a higher-order DNA structure known as the G-quadruplex. Several dengue diagnosis platforms have been studied and developed to date in the quest for the perfect identification approach. NS1 antigen detection, IgM and IgG antibody detection and other DNA amplification techniques are the major platforms. While there are numerous DENV detection methods available, no single methodology has been proven to meet the requirements of being sensitive, specific, quick, and economical all at the same time. This scenario necessitates the development of alternate detection techniques to bridge the gap in DENV diagnosis. The final outcome is to development of a new detection or diagnostic kit for dengue disease.

Objectives:

- Design and production of the probe to the specific Dengue serotype using Bioinformatics tool named integrated DNA technology .(IDT).
- Maintainance of probes in a stable environment.
- Optimization of color in UV visible Spectrophotometer and mobile camera for color analysis .
- Testing of spiked and non-spiked DNA in simulated blood samples.
- Using a split G4 configuration, a simple label-free colorimetric method for Dengue and detection as effectively constructed.
- preparation of ABTS buffer (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid which acts as colouring agent. DMSO acts as oxidizing agent .
- Absorbance was calculated at 350-800 nm using Biospetrophotometer.

Methodology:

Flow chart

1µM concentration of target sequences with an equal concentration of Probe A & B were mixed in a total mixture of 15µL containing a final concentration of 3M Betaine and 50% DMSO

Mixtures were then heated at 95°C for 10 minutes to dissociate the secondary structure

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The mixed solution was gradually cooled to room temperature prior to adding 1M KCl and2µM hemin to a final volume of 25µL for G4-Hemin DNAzyme formation

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Samples were then incubated for another one hour in the dark

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85µL of ABTS buffer containing H2O2 was added to make up the total reaction volume to 110µL andthe reaction of the sample was left in the dark

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After one hour the color change of the ABTS-H2O2 reaction was observed and the absorbance was measured at the wavelength scan of 350-800 nm

Results: DENV2

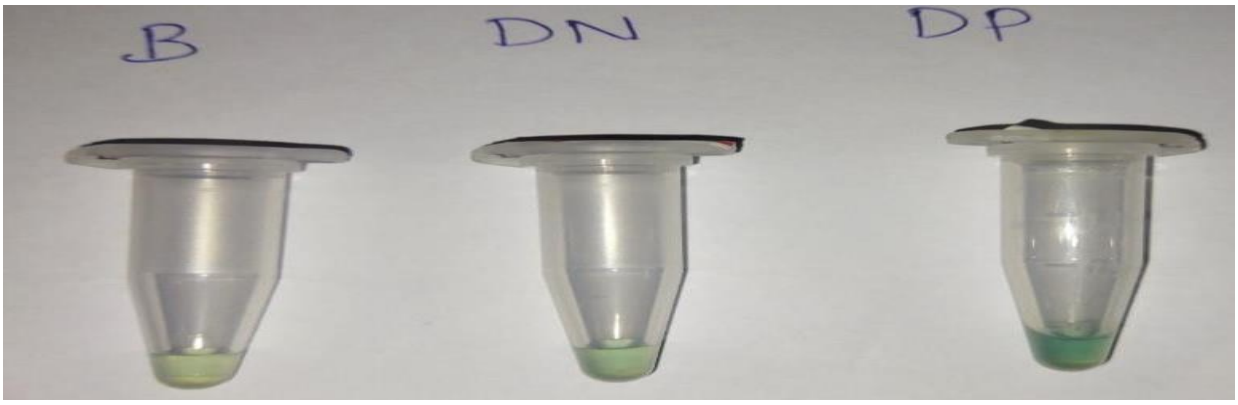
Visualization of Colorimetric assay following addition ABTS buffer. The color changes from blue to green after adding the ABTS buffer in the DENV-2 serotype. B-Blank, DN-Dengue Negative, DP-Dengue Positive.

Positive control:

Sensitivity assessment tests for Probe A & B were used to determine the target strand for the DENV-2 serotype. The sensitivity of the colorimetric assay was assessed using the different concentrations of the target strand. The specificity test was performed in duplicate and repeated in 5 times.

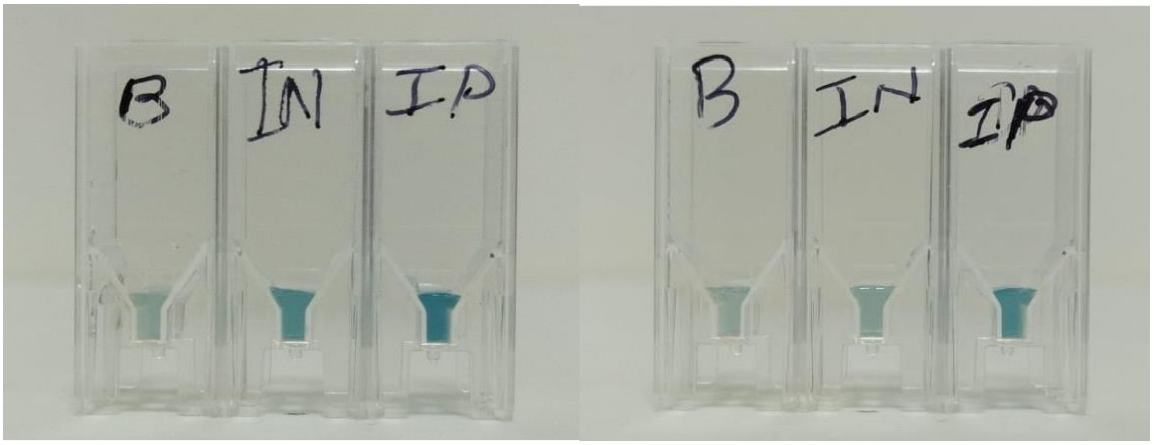
(a) 1µM conc of target strand (Dengue)

(b)1.4µM conc of the target strand



Photograph of colorimetric detection of target strand by probes A & B at different concentrations of target strand. Conditions: Fig. a shows the 1µM concentration of the target strand and Fig. b shows the 1.4 µM concentration of the target strand.

Results: IFI6 Host Biomarker



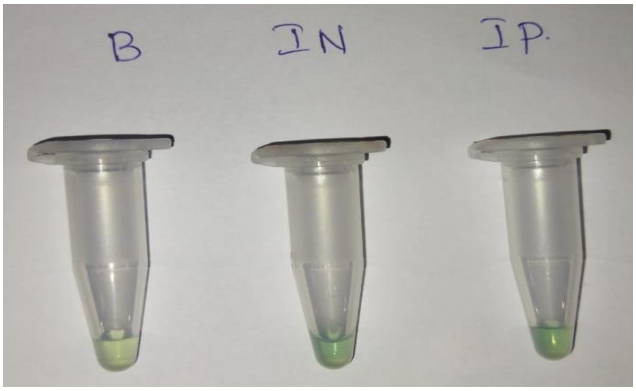
Visualization of Colorimetric assay following addition ABTS buffer. The color changes from blue to green after adding the ABTS buffer in the IFI6

B-Blank, IN – IFI6 Negative, IP – IFI6 Positive.

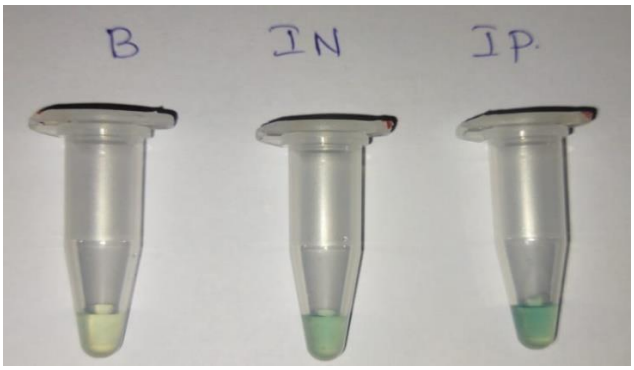
Positive control:

Sensitivity assessment tests for Probe A & B were used to determine the target strand for the biomarker IFI6 . The sensitivity of the colorimetric assay was assessed using the different concentrations of the target strand. The specificity test was performed in duplicate and repeated in 5 times.

A) 1µM conc



(B)1.4µM conc



Photograph of colorimetric detection of target strand by probes A & B at different concentrations of target strand. Conditions: Fig. a shows the 1µM concentration of the target strand and Fig. b shows the 1.4 µM concentration of target strand.

Transmittance graphs: DENV2 and HOST BIOMARKER IFI6

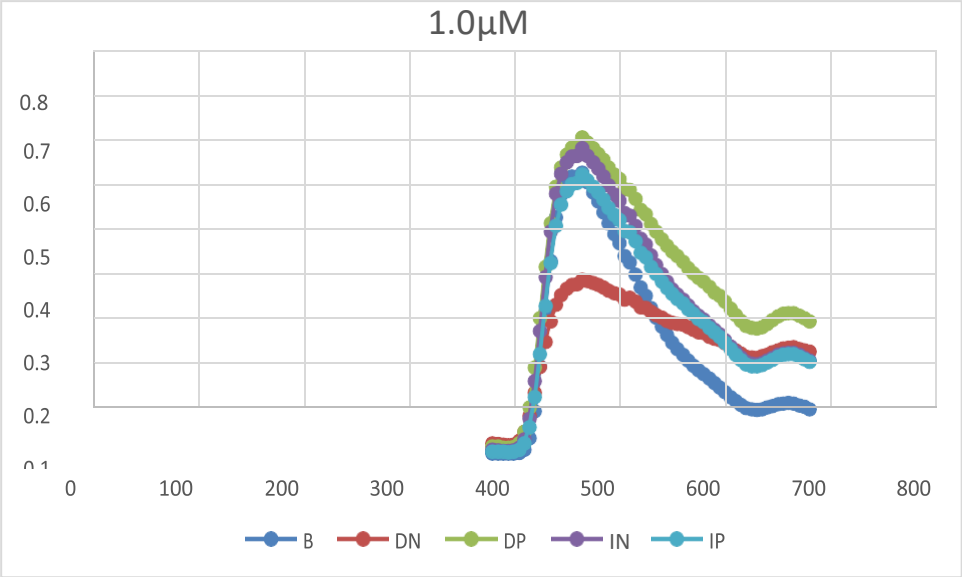


Chart 1: Transmittance (%) data for DENV-2,IFI6 Standard Concentrations of 1.0 µM

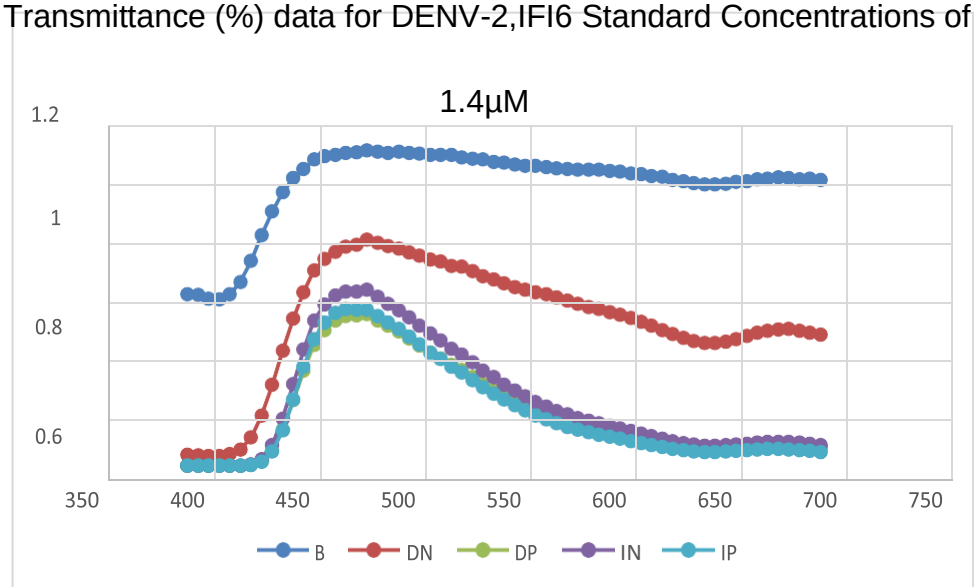


Chart 2: Transmittance (%) data for DENV-2 ,IFI6 Standard Concentrations of 1.4 µM

Abstract

Nucleic acids have a unique ability to organise themselves into non-canonical forms,such as the Gquadruplex (G4), a four-stranded DNA molecule that has been used for diagnostic and therapeutic purposes. Using a split G4-hemin DNAzyme design, we show that G4 can differentiate dengue virus (DENV) depending on its serotype (DENV-2). Upon target DNA strand hybridization, two distinct Grich oligonucleotides are brought together to form a three-way junction architecture, permitting the development of a G4 structure. By developing a DNAzyme that can catalyse H2O2-mediated oxidation of 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, G4 generation in complexation with hemin can offer a signal readout (ABTS). This causes a colour change, which serves as a sensor platform for DENV colorimetric detection. Betaine and dimethyl sulfoxide were used in our method to improve G4 generation by increasing target-probe hybridization. The technology demonstrates how to split G-quadruplex topologies can be used to construct future DNA-based detection and serotyping systems.