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Effective detection of Dengue fever virus using an Aptazyme approach

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Traditional viral detection methods can take hours to days before results may be obtained for an infected patient. We are developing a novel, rapid Dengue virus (DENV) diagnostic approach using an aptazyme complex consisting of a previously described anti Dengue virus hammerhead ribozyme and the theophylline-sensing aptamer (theoD-hRAz). Hammerhead ribozymes (hRz) have been used to target a number of RNA viruses, including HCV and HIV-1, in a sequence specific manner without the need for native cellular cofactors. RNA aptamers, RNA-based ligand binding molecules, have been generated to bind a wide range of ligands with high affinity and selectivity. Targeting of the DENV-2 NGC genome resulted in a conformational change of the module that connects the hRz to the theophylline aptamer. This conformational change leads to an alteration in aptamer structure allowing for binding of theophylline resulting in a purple to clear color transition of the reacted sample indicating detection of DENV RNA. *In vitro* cleavage analysis of *in vitro* transcribed theoD-hRAz against Dengue virus type 2 New Guinea strain (DENV-2 NGC) showed the hRz portion of the aptazyme retains targeting and catalytic activities in the presence of an appended theophylline-specific aptazyme. The presence of theoD-hRAz RNA is confirmed by RT-PCR. The theophylline aptamer has a published Kd<1μM. Equilibrium-filtration experiments, used to determine aptamer-ligand affinity, showed the theophylline aptamer was able to effectively and specifically bind the ligand, theophylline, while appended to the hRz. Viral RNAs isolated from Vero cells infected with DENV-2 NGC at increasing M.O.I, were titrated into a reaction mixture containing theoD-hRAz conjugated to agarose beads to establish the utility and limits of specificity of our engineered theoD-hRAz. RT-PCR analysis confirmed the presence of DENV in the reaction mixture. These results validate a unique approach towards coupling effective DENV targeting with the ligand binding capabilities of an RNA aptamer for the rapid detection of viral RNAs.