Supplementary Materials: Label-Free Electrochemical Detection of *S. Mutans* Exploiting Commercially Fabricated Printed Circuit Board Sensing Electrodes

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Selection and validation of the anti-S. mutans antibody by ELISA

There are some reports [1–3] of the detection of *Streptococcus mutans* using custom-made monoclonal antibodies specific to the bacteria. We used the only commercially available anti-*S. mutans* polyclonal antibody (ab31181) from Abcam (USA). We validated our anti-*S. mutans* antibody by a series of ELISA experiments. We found 0.11 μg/mL and 0.20 μg/mL to be the optimal concentrations of the primary and secondary antibodies respectively.

To detect the bacteria, we performed the tests 1, 3, 5, and 7 in triplicate, as shown in Figure S1, using a 96-well plate. Tests 3, 5, and 7 are various negative controls as explained by the schematic diagram. We added 100 μL of the *S. mutans* culture to the wells to perform tests 1, 5 and 7, and incubated at room temperature for 90 min. The plate was washed twice with warm 1X PBS. We then added 200 μL of the blocking buffer and incubated overnight at 4 °C. The plate was washed twice with 200 μL of the washing buffer (0.01 mol/L PBS, pH 7.4, mixed with 0.05% Tween-20). We next added 100 μL of the primary antibody to the wells 1, 3 and 5, and incubated the plate at room temperature for 2 h. The plate was then washed four times. We then added 100 μL of the HRP-coupled secondary antibody to the wells in row 1 (containing both bacteria and antibody), row 3 (negative control without bacteria, but with antibody) and row 7 (negative control with bacteria, but without antibody) and incubated the plate at room temperature for 2 h. After this step, the plate was washed four times with 200 μL of the washing buffer. The plate was then left at room temperature for 3–5 min to allow it to dry. To each well, we then added 100 μL of the 3,3′,5,5′-tetramethylbenzidine (TMB) substrate, covered the plate with aluminium foil and incubated it away from light for 45 min. We then stopped the reaction by adding equivalent volumes of the stop solution to each well and immediately measured the absorbance at 450 nm using a SpectraMax® M2^{e} Multi-Mode Micro Plate Reader.

Figure S2 shows the absorbance readings. We obtained the absorbance maxima of the test sample (test 1) at 450 nm as 0.077. The absorbance values were comparatively lower in the negative controls. The difference in absorbance values between the actual test (i.e. test 1) and the negative controls (tests 3 to 7) shows that the primary antibody binds to our strain of *S. mutans*.

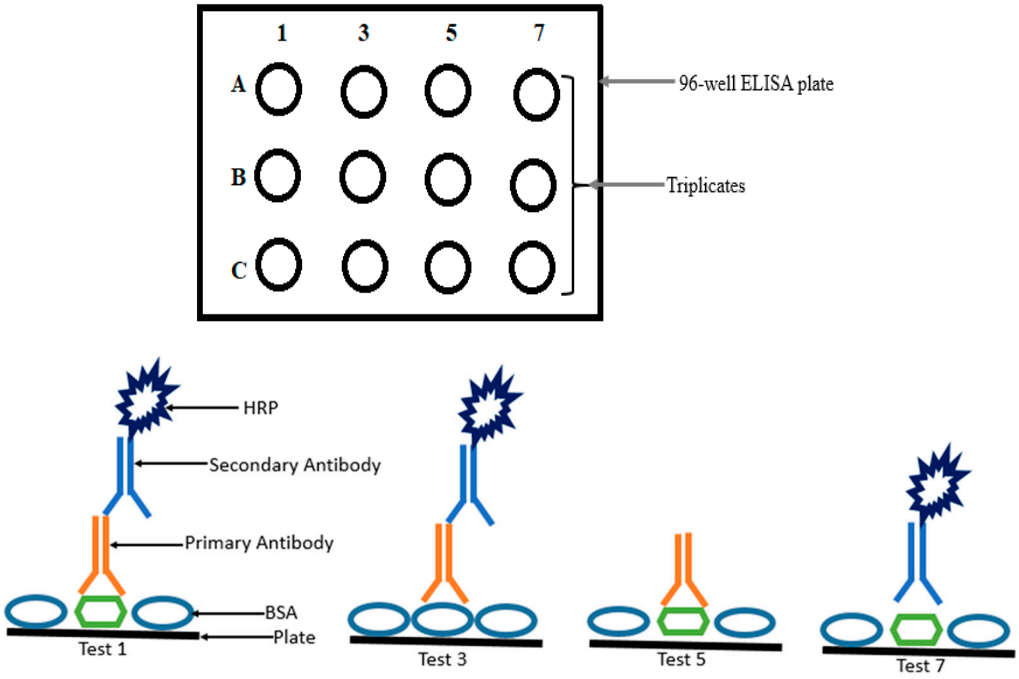

Figure S1. Schematic diagram of the ELISA set-up showing the configuration of the wells. (Top) The well configurations used for the tests numbered 1, 3, 5 and 7. A, B, and C indicate replicates. (Bottom) The test and the various negative controls. Test 1 contains all reagents and the target bacteria (green hexagon). Test 3 does not contain any bacteria, test 5 has no secondary antibody and test 7 contains no primary antibody. Hence tests 3, 5 and 7 are used as negative controls.

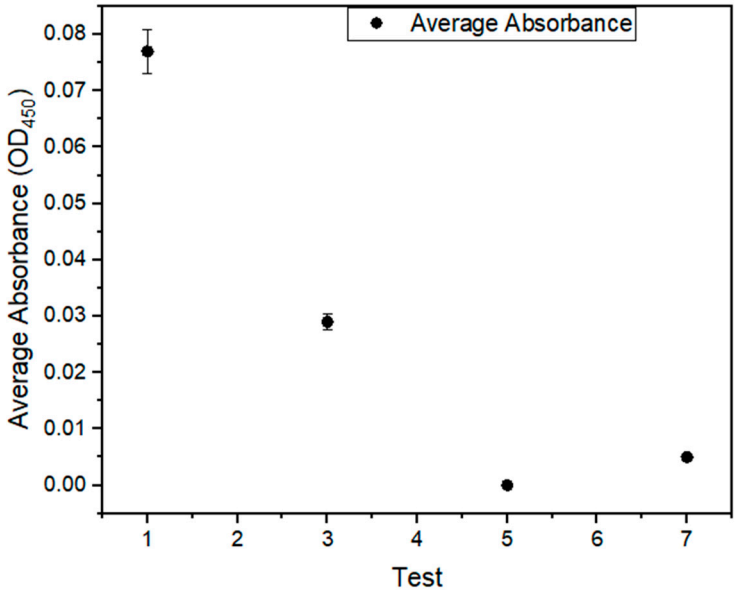

Figure S2. Average absorbance values (n = 3) measured at 450 nm for different tests. The error bars represent the standard deviation of the data.

References

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