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# Development of a Saliva-Based Lateral Flow Assay for SARS-Cov-2 with the Potential to Quantify Viral Load

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the deadliest virus in the last 50 years, with the USA having the highest reported deaths and cases at over 1.1 million and over 100 million, respectively, as of June 1, 2023. Identifying infected people remains the primary method of stopping the spread of the virus, either using real-time, quantitative polymerase chain reaction instruments or at-home lateral fow assay (LFA) antigen tests. Herein we describe a simple four step at-home -2 LFA test that provides three advantages over current LFAs. The test employs 1) saliva sampling, 2) three antibodies to bind the virus to the LFA Test Line, and 3) a smartphone to quantify the refectance of the Test Line in terms of Ct values. The use of saliva samples eliminates the pain and fear of nasopharyngeal sampling, especially for children. The use of three antibodies yielded 100% correct sensitivity, specifcity, predicted positive and predicted negative for samples with Ct values of 29 and below. The use of a smartphone to measure refectance allowed calculating the Ct values for 16 samples with an average error and standard deviation of 0.58±0.43 for samples with Ct values below 26. The smartphone also adds the capability of sharing the results to trab' Industrial Park Road, Unit 8, Middletown, CT 06457, USA, 860-635-9800; E-mail: stu@rta.biz

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(LOD), - , , . LOD, , SARS-R C 2, I USA-WA1/2020, 1.6 105 TCID50 L, , - 10, 100, 200, 400 5 20 LFA (T 2). I FDA, 100% , T L 20 , LOD 1.6 103 TCID50 L. - , , 12 , 3 - , 7 LFA . N T L , - FDA (T 3). S , , , , SARS-C V-2 1.6 103 TCID50 L 3 . N FDA T L SARS-C V-2 (T 4). A LFA , , , , 50 38 . - , SASR-C V-2 C 11.6 32.5 (L B B B ). A LFA 3 RTA . 100% T L , 29 38 (76.32%) T L . A 9 C 30-32.5 (T 5). S 100% C 30, . COVID-19 S A A -H T C 29 (F 3 T 6).

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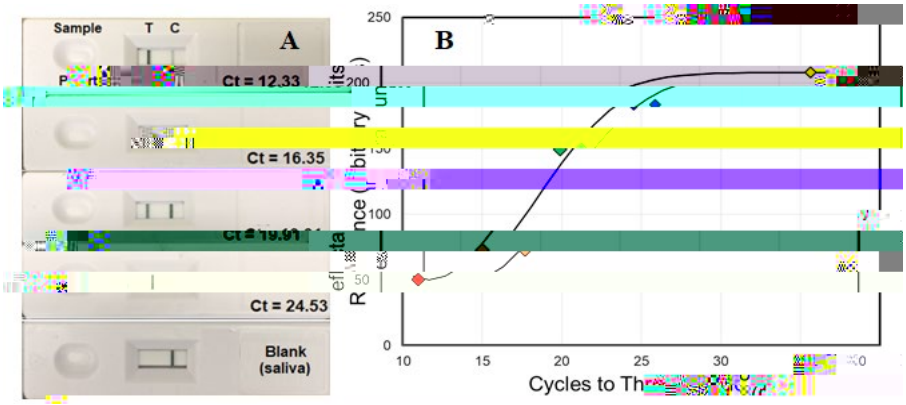
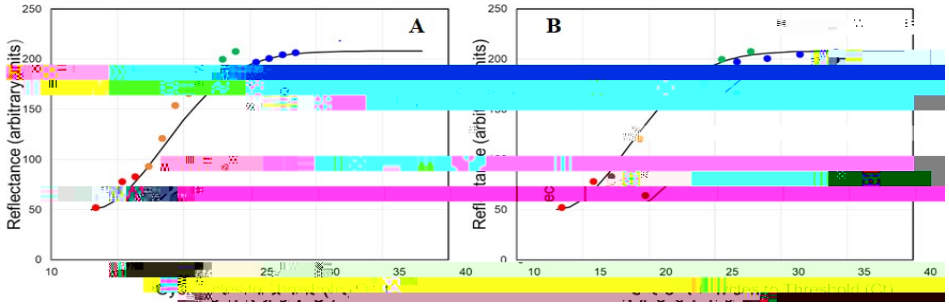



: A) Photograph of 5 cassettes. First 4 cassettes prepared using 4 purchased Sars-CoV-2 samples with Ct values as indicated, and 1 cassette prepared using purchased de-identified saliva without the virus. B) Plot of reflectance values of the LFA Test Line for 4 samples and a saliva blank (diamonds) fit with Equation 1 shown as a black line. See text for measurement conditions.


: Photograph of 21 cassettes. Four cassettes across the top prepared using 4 purchased COVID-19 samples with Ct values as indicated, 4 successive cassettes of diluted samples below each purchased sample, and 1 cassette of a sample prepared using purchased de-identified saliva without the virus. Ct values calculated using the measured reflectance at the Test Line (T, left side of the viewing port) and the Equation 1 are shown on each cassette. See text for conditions.




: Plot of reflectance values of the LFA Test Line for the 16 diluted samples calculated using Equation 1. Diluted samples are represented as red, orange, green, and blue circles, prepared from Ct values of 12.33, 16.35, 19.91, and 24.53, respectively. B) Plot of the same samples, but the Ct values corrected by multiplying by 1.086. Equation 1 is shown as a black line in both figures. See text for measurement conditions.


: Photograph of 21 cassettes reordered based on calculated Ct values from measured reflectance and blank saliva sample. Continuous loss of intensity (increasing reflectance and corresponding Ct values) of Test Line (T, left side of the viewing port) is apparent. Original cassettes for samples with Ct values of 12.33, 16.35, 19.91, and 24.53, are red, orange, green and blue framed, respectively. See text for measurement conditions.

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