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Quantifying SARS-Cov-2 At-Home Using a Lateral Flow Assay and a Smartphone

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Abstract

As of June 1, 2023, the SARS-CoV-2 virus has infected ~700 million people and caused ~7 million deaths worldwide. During the pandemic two types of diagnostic testing were developed in an efort to identify infected people with the goal of limiting the spread of the COVID-19 disease: real-time, quantitative polymerase chain reactions (PCR) that detect the SARS-CoV-2 genome, and lateral fow assays (LFAs) that detect the SARS-CoV-2 nucleocapsid and/or the spike antigens. PCR is used to quantify infection in terms of cycles-to-threshold of detection (Ct) values using saliva or nasopharyngeal samples. Unfortunately, the test employs expensive reagents and instruments and the sample collection-to-result remains 1 to 2 days. In contrast, iή Ōdꝑ ng ȝ ȝ ꝑ ꝑ ᴬng ˁ ή " ˁ gd1 -3- ! r infection information in 15 to 30 minutes. However, the home tests have two limitations: 1) they lack numerical data that indicates the person's infectious level, and 2) the results are not automatically supplied to agencies that track the number and location of cases. The latter information is crucial to stopping outbreaks. In an efort to provide a product that has the advantages of both methods, we present the development of a smartphone App that correlates the intensities of the test line of at-home LFAs measured by a smartphone camera to their corresponding PCR measured Ct values, with the added capability of sharing results with health agencies. The correlatdddd ˁ Rs values of 0.62±0.21. The smartpho infected, infected, contagious, and most importantly, highly contagious. As such, the combination lateral fow assay and smartphone is well suited for identifying, quantifying, and slowing the spread of current and future corona and other viruses.

Ke o d : COVID-19; SARS-CoV-2; Quantitative polymerase chain reactions (PCR); Lateral ow assays (LFAs)

In od c ion

Since the rst report of SARS-CoV-2 in December 2019 in Wuhan China [1], over 692 million cases and approximately 6.9 million deaths have been reported worldwide as of October 1, 2023 [2]. e rapid worldwide spread of the disease by inhalation of air and by contact with people and surfaces contaminated with virus-containing droplets, has been facilitated by the ~1 week incubation period, as well as by contagious, asymprapid antigen tests. PCR, the gold standard genetic analysis method, was developed to detect and quantify the SARS-CoV-2 genome. In use a saliva or nasopharyngeal sample is added to a PCR. e PCR employs nucleic acid primers to double the amount of SARS-CoV-2 ribonucleic acid (RNA) in the sample through each temperature cycle. e cycles are repeated until a uorescent dye attached to the primers increases su ciently to be detected, otherwise known as the cycles-to-threshold of detection (Ct). e Ct value is an indication of the concentration of the virus in the sample, and a person's viral load. e fewer cycles needed for detection (lower Ct value), the higher the viral load, and vice a versa. In general, PCR can detect a viral load of 10^3 to $10^{9.5}$ RNA copies/mL in a saliva or nasopharyngeal sample expressed as Ct values of 37 to 15, respectively, during the course of a person's infection from the 3rd to 25th day (Figure 1) [2]. Unfortunately, the test employs expensive primers, requires highly trained technicians in a well-equipped laboratory, takes 2-6 hours to perform, and as long as 2 days to provide results. During the delay contagious people may spread the virus to others. Furthermore, a single person's test may miss the ~6 to 11 day contagious window or report a positive detection for the post-contagious ~12 to 25 days [3].

An alternative method to PCR tests are rapid antigen tests. ese tests employ a lateral ow assay (LFA) test strip, in which antibodies are used to bind the SARS-CoV-2 nucleocapsid and/or the spike antigen proteins. In use a nasopharyngeal or saliva (oral uid) sample is added to a reagent that breaks down the body uid mucins freeing the SARS-CoV-2 virus antigens, which ows across the LFA when added, and bind to the antibodies at the test line. e antibodies also employ dyes or metal colloids to produce a visible color at the test line con rming the presence of the virus. Once the LFA tests became available, they displaced most of the PCR tests, because people could perform self-tests at home, and obtain results in 15 to 30 minutes. Furthermore, the LFAs are inexpensive and o en covered by health insurance. Unfortunately, the home LFA tests have two critical limitations: 1) they provide only positive and negative results, lacking numerical data that indicates a positive person's contagious level, and 2) unlike PCR data,

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the results are not automatically provided to health agencies, such as the US Centers for Disease Control (CDC), so that the number and location of cases can be tracked. In fact, in the US the number of PCR tests plummeted from a high of ~18 million the rst week of January to 6.4 million the last week of February 2022, a er free or insurance covered antigen tests became available. Furthermore, only 5% of the antigen tests were self-reported to the CDC. [4]

While the worldwide death rate of the pandemic has dropped considerably as of October 1, 2023, there remains a need for a test that can provide quantitative data like PCR as well as rapid, at-home results like LFAs, along with the ability to provide tracing data for government health agencies. Such data could be used to determine if an outbreak is expanding or waning, based on reported Ct values, i.e. low or high, respectively. e best approach would be to use a smartphone as the measurement device, as they are in widespread use.

e idea of using a smartphone to collect and transmit COVID-19 data is not new. Early during the COVID-19 pandemic, China researchers developed a smartphone App to identify subjects as infected or not (red or green displays, respectively) to control access to trains, subways, and buildings [5]. Downloading the App automatically retrieved the smartphone owner's medical data to produce the display. Similarly, several researchers developed Apps that used smartphone photographs of LFAs, coupled with arti cial intelligent, to improve the positive and negative sensitivity and precision statistics. [6] In addition, a broad range of LFA designs, ranging from quantum dots, orescent probes, and imbedded biosensors, were also developed to improve statistics. [7, 8] However, none of these smartphone methods provided quantitative information.

To overcome this limitation we have been developing a highly sensitive LFA in which the test line can be quanti ed using a smartphone displaying infectious and contagious levels with the ability to report Ct values to government health agencies. Here we present measurements of 19 LFA cassettes covering a range of 16.57 to 31.67 Ct values. To our knowledge, this is the rst report of a self-use, at-home test that quanti es the viral load using a smartphone with the ability to categorize infection levels and transmit Ct values.

Ma e ial and Me hod

Ma e ial

All reagents, such as non-ionic surfactant, polyvinylpyrrolidone, and tris (hydroxymethyl) aminomethane bu er, were obtained from Sigma-Aldrich (Allentown, PA), while COVID-19 antibodies were obtained from Meridian Bioscience (Cincinnati, OH) and de-identi ed, pooled saliva samples, with and without the SARS-CoV-2 virus were obtained from Lee Biosolutions (Maryland Heights, MO). Six of these samples with PCR Ct values of 21.07 to 31.67 were measured as unknowns. e virus samples were received in 2 mL plastic vials, and all sample preparations were performed in a Biosafety Level 2 cabinet following standard safety procedures. Non-cotton swabs, 1 mL plastic centrifuge tubes and a manually-set, auto pipetter were used for sample manipulation (all from VWR, Bridgeport, NJ). e LFA cassettes were of standard construction (nanoComposix, San Diego, CA), consisting of 1) a sample pad, 2) a conjugate pad containing probes consisting of synthesized gold nanoparticles coated with a dye and functionalized with mouse monoclonal SARS-CoV-2 nucleocapsid and spike antibodies as capture antibodies, 3) a test line functionalized with mouse monoclonal SARS-CoV-2 nucleocapsid/spike proteins, 4) a control line functionalized with goat anti-mouse IgG antibodies, and 5) a wicking pad, all on 6) a nitrocellulose support, and 7) enclosed in a plastic cassette containing a sample addition port and a viewing section.

Me hod

Re_ l

Previously, we developed an LFA to quantify the immunosuppressant drug tacrolimus. [9] e LFA required surface enhanced Raman active probes and a Raman spectrometer to perform the measurements. Based on this success we developed an LFA to quantify the SARS-CoV-2 virus antigens at the test line in terms of Ct values, again using Raman spectroscopy. [10] Unexpectedly, we noticed visible di erences at the test line for samples with di erent Ct values. Based on these di erences, an initial series of measurements were performed that suggested that a smartphone could be used to quantify the test line of an LFA in terms of Ct values. [11] is publication is a continuation of that work. Speci cally, the following series of re ectance measurements were performed to establish the ability of a smartphone to quantify the test line of an LFA in terms of Ct values: LFA test line repeatability, upper and lower re ectance range with time-dependent stability, measurement time optimization, calibration curve development and unknown sample analysis.

e repeatability of an LFA was determined by measuring the re ectance at the test line 20 times without the addition of a sample.

A calibration curve was prepared by diluting Sample 5434 eleven times in saliva by 50% (Figure 6). is dilution percent was chosen to mimic the factor of 2 nucleic acid replication achieved from one PCR cycle to the next. Each sample was prepared as previously described, added to a cassette, and the re ectance measured using the smartphone App from ~2 to 30 minutes. As before the scatter in the initial re ectance measurements stabilized from 10 to 30 minutes (Figure 7).

e e ciency of the dilution series was calculated by comparing the re ectance of 205.3 for the nal diluted sample to the purchased sample with the lowest concentration, i.e. Sample 5763 with a re ectance of 205. e ratio of the respective Ct values, 29.78/31.67 indicates an average dilution e ciency of 94%. is is attributed to sample loses during the successive sample transfers to produce the dilution series. e Ct values for the dilution series were corrected by multiplying their Ct values by the reciprocal of 0.94. A plot of the 10 to 30 minute average re ectance for the diluted samples as a function of original Ct values and corrected Ct values produced sigmoidal curves, red triangles and blue dots, respectively (Figure 8 and Table 1). e corrected data was t using the Avrami equation (Equation 1) [12], which has been adapted to many chemical and biological processes [13], including PCR plots of sample uorescence as a function of Ct values [14]. e equation describes the initial exponential increase in antibody binding at the test line and exponential decrease, when all of the bonding sites at the test line become occupied, viz:

Ct = 206-90•(exp[-0.037•(re ectance-21.8)2.15]) Equation (1)

e smartphone App, equipped with Equation 1, was then used to predict the Ct values from the LFA smartphone test line re ectance measurements of the 6 “unknown” samples (Figure 9, Table 2). Only a single re ectance measurement was made of each test line at 20 min a er addition of the sample to a cassette, as would be the case for an at-home measurement. e average error and standard deviation for the calculated 6 “unknown” Ct values were 0.62±0.21.

Figure 6: Photograph of LFA cassettes for Sample 5434 (Ct 18.78) and 11 successive diluted samples, each 50% of the former concentration. The decreasing visible intensity of the test line as a function of increasing Ct values is easily observed. Photograph of sample set was within 1 hours after the rst measurement of the rst cassette.

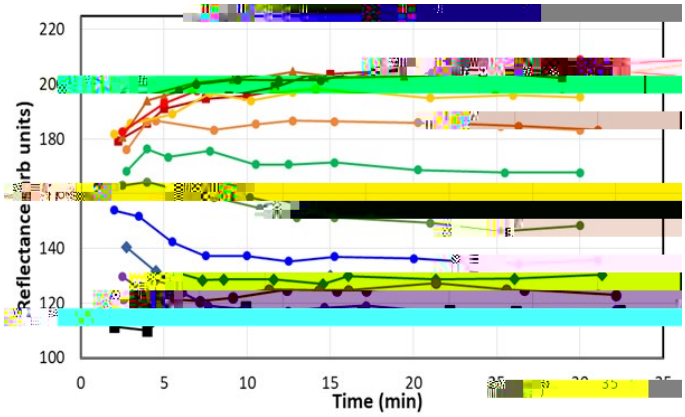

Figure 7: Plot of the measured re ectance at the LFA test lines as a function of time for the original Sample 5434 (Ct value of 18.78, vine 5nd 1

Di ç ion

e relationship presented here between the smartphone measured antigen test line re ectance and Ct values is supported by the >90% correlation between SARS-CoV-2 nucleocapsid antigen and RNA

concentrations in nasopharyngeal samples of 140 subjects by quantitative electrochemiluminescence immuno-assay and PCR measurements, respectively [15]. Furthermore, the Ct values can be categorized in terms of the level of infection [3].

For the current study, a Ct value less than 21 (re ectance less than 118), indicates in a general sense that a person is likely highly contagious, Ct values from 21 to 27 (re ectance 120 to 170) indicates likely contagious, Ct values greater than 27 (171 to 205 re ectance) indicates likely infected, and no Ct values are provided if the re ectance is greater than 210 and indicates likely not contagious. However, the latter value may represent pre- or post-contagious. A person who has not had any symptoms is likely in the pre-contagious stage, while a person who has had symptoms is likely in the post-contagious stage (Figure 1). Of course some people may not experience symptoms at any time during infection. Currently, the smartphone App provides the user with the above infectious/contagious categories, so they can make a decision regarding self-isolation. Upon downloading the App, the user's location is requested, such as a zip code in the US, while adding other information is optional, such as age, gender, race, and ethnicity. e user can also share the data with others as desired. Every test, positive or negative, can be automon d18(y n)4(o)11(t exp)-88s3ed. Everyaoyaoyaotes l3nl/30.1 Tw 0 2(ny n)4(o)4s 9(nr (r)19(n b(e)-5s c)-33(ac)-3(a)97.9

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