A SARS-CoV-2 Coronavirus Nucleocapsid Protein Antigen-Detecting Lateral Flow Assay

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Abstract

Inexpensive, simple, rapid diagnostics are necessary for efficient detection, treatment and mitigation of COVID-19. Currently, the primary diagnostic tool being utilized is reverse transcription polymerase chain reaction (RT-PCR). RT-PCR delivers results with good sensitivity and excellent specificity, but is expensive, prone to access challenges and is often slowed by transport to centralized testing laboratories. Antigen-based assays are inexpensive and can be rapidly mass-produced and deployed, with lateral flow assays (LFAs) being the most common inexpensive antigen test. To date, few antigen-detecting LFAs for COVID-19 have been commercialized. Herein, we present an open source LFA using commercially available antibodies and materials for the detection of SARS-CoV-2. Using an optical reader with comparable sensitivity to a visual read, the LFA yielded a Limit of Detection (LOD) of 23 $TCID_{50}$/mL (95% CI of 9.1 to 37 $TCID_{50}$/mL), equivalent to $1.4x10^5$ copies/mL (95% CI of $5.5x10^4$ to $2.3x10^5$ copies/mL) irradiated virus in pooled nasal matrix. This LOD meets the criteria suggested by WHO for diagnosis of acute SARS-CoV-2 infection in a point of care format. A clinical evaluation and further testing is ongoing.

Introduction

The novel coronavirus, SARS-CoV-2, was identified in China in late 2019 and is characterized by significant mortality, morbidity, and infectiousness.[1] In less than 8 months, COVID-19, the disease caused by SARS-CoV-2, has infected fifteen million people worldwide and caused over 600,000 deaths.[1,2]

Rapid testing measures are critical for identifying symptomatic and asymptomatic carriers, guiding treatment and quarantine recommendations and tracking viral spread. Testing is necessary to guide community protocols and planning, in order to reduce ongoing transmission. Rapid testing for SARS-CoV-2 is urgently needed early in the onset of infections in a community to effectively mitigate the spread of the virus.[3,4] The pandemic has created an unprecedented demand for RT-PCR testing in all countries. Direct antigen-based testing for SARS-CoV-2 is an alternative to RT-PCR and is likely the only viable and cost-effective solution for the majority of low and middle income countries (LMICs). Antigen tests to detect the presence of viral proteins can be performed directly on biological samples, such as tissue swabbed from the anterior nasal cavity, oropharynx, or even directly from saliva. Antigen tests are already used to detect influenza, strep throat, and other infectious diseases. LFA antigen tests can be rapidly produced in large volumes, i.e. billions of units/year. These tests are relatively inexpensive, require limited training and are easy to administer; results are delivered in minutes. Importantly, like RT-PCR and unlike serological tests, LFA antigen tests can identify active infections.

A recent report found that the average wait time for the results for a COVID-19 test in July 2020 in the US is 4 days.[5] This same report determined the mean and median waiting time 4.1 days and 3 days, respectively. Only 37% of people received test results within 2 days, and 21% waited more than 5 days. Such long response windows delay treatment determinations, obscure community spread and render contact tracing ineffective. The use case for an inexpensive, readily available SARS-CoV-2 assay is strong even if the assay's sensitivity is lower than current RT-PCR testing. Modeling suggests that providing decentralized, point-of-care testing with rapid feedback would have significantly greater impact on transmission vs. improving the absolute limit of detection of the assay.[6] Such models incorporate the observation that infectious viral particles have not been recovered below approximately 100 copies/mL.[7,8]

Rapid antigen tests are starting to be deployed commercially. However, few antigen tests for SARS-CoV-2 have been authorized by regulatory authorities worldwide. As of July 19th, 2020, only two such products have received emergency use authorization (EUA) from the US Food and Drug Administration.[9,10]

These two FDA EUA antigen assays are expensive, require instrumentation, and are currently available only to health care professionals. Efforts are underway to develop readily manufacturable antigen-based rapid diagnostic tests which do not rely on, or at most minimally rely on, access to instrumentation to meet an increasing global demand.[11]

In this paper we describe an LFA for the detection of SARS-CoV-2 nucleocapsid protein. We combined the results of our previously reported high-throughput robotic antibody screening efforts[12] with our experience building a half-strip LFA for SARS-CoV-2[13] in order to develop a complete LFA that utilizes anterior nares swabs. If supply or cost constraints limit the availably of our selected antibody pair, our antibody screening efforts may be utilized to guide the selection of alternates. We report our LOD against irradiated virus in pooled nasal matrix. Clinical evaluation of this device is on-going. All materials and reagents utilized in this LFA were chosen from commercially available sources to support development of an "open source" COVID-19 antigen LFA.

Materials and Methods

Latex block 6% casein preparation

Casein for blocking latex beads was prepared by first mixing 6.183 g of boric acid (Sigma, St Louis, MO, B0394) and 800 mL of Ultrapure water (10977-015, Invitrogen, Carlsbad, CA) with stirring. The pH was adjusted to 8.5 using NaOH. Next, 100 g of casein sodium salt powder (Sigma, St Louis, MO, C8654) was added to the solution with continuous stirring. Using a heat plate, the temperature of the solution was brought to 40°C and held overnight. The following day, the solution was diluted to 1 L using ultrapure water and filtered through a Nalgene 0.2 µm filter device (VWR 89176-982). If the filter clogged, the solution was transferred to a fresh unit until the entire solution was filtered. The concentration of casein in the filtered solution was determined by measuring the residual mass of a 1mL aliquot after drying. The solution was diluted in 100 mM borate to bring the final stock concentration to 6% w/v and aliquoted and frozen at -20°C.

Latex bead conjugation

For the test line conjugate, 400 nm carboxylic blue latex beads (CAB400NM, Magsphere, Pasadena CA, USA) were conjugated to Sino Biological 40143-R004 rabbit monoclonal antibody (Sino Biological, Beijing, China) at a weight:weight ratio of 30:1 (beads:antibody) using EDC/NHS coupling. For the control line conjugate, 400 nm carboxylic blue latex beads were conjugated to Chicken IgY (ChromPure 003-000-003, Jackson ImmunoResearch, West Grove PA) at a weight:weight ratio of 10:1 (beads:antibody) using EDC/NHS coupling.

Stock latex particles were vortexed and sonicated prior to use. The particles were then washed and resuspended in 0.1 M MES, pH 6.1 (Teknova, M225). The beads were covalently coupled to the antibodies in a two-step process. First, the beads are activated using freshly prepared EDC and NHS in MES. After 30 minutes, the beads were washed and resuspended in PBS pH 7.2. Antibodies were added at the ratios described above, and the activated beads were mixed with the antibodies for 3 hours with end-over-end mixing.

The conjugates were then quenched for thirty minutes using ethanolamine, again on the orbital shaker. Next, 6% casein was added to the mixture to a final concentration of 1% casein. The conjugates were blocked overnight at room temperature on the orbital shaker. The following day, conjugates were washed and stored in 50 mM borate with 1% casein (from the 6% latex block stock) as described under the latex bead casein block preparation. Concentrations for the completed conjugates were determined by measuring the absorbance at 560 nm for red and 660 nm for blue. Final stocks were stored at 4°C until use.

Antibody biotinylation

Sino Biologicals 40143-MM08 was first buffer exchanged into PBS to remove any interfering substances using Amicon filters (Sigma, St Louis, MO, 50 kDa MWCO, UFC5050). Specifically, the antibody was concentrated 20-fold and brought back to the original volume with PBS. This was done three times to remove sodium azide. The antibody was biotinylated at 1.5 mg/mL with 50 molar excess NHS-dPEG$_{12}$-biotin (10198, Quanta Biodesign, Plain City, OH, USA). Excess biotin was removed using Amicon filters again, this time with five total concentration and resuspension cycles. The concentration of the biotinylated antibody was determined using the A280. The final biotinylation molar ratio was determined to be 20 moles of biotin per mole of antibody using the QuantTag Biotin Quantification Kit (BDK-2000, Vector Laboratories, UK). If supply or cost constraints limit the utility of the above antibodies, our previously reported antibody screening effort may be used to select alternates.[12]

Nitrocellulose striping

The nitrocellulose, 25 mm CN95 (Sartorius), was striped with a test line at 8 mm from the edge of (upstream from the flow direction) and 13 mm from the upstream edge of nitrocellulose**.** The test line was striped at 1 µL/cm with 1 mg/mL polystreptavidin (Cat #10 120 050, Biotez, Berlin, DE) and the control line was striped at 1 µL/cm 0.5 mg/mL goat anti-Chicken IgY (Cat #703-005-155, Jackson ImmunoResearch). Both lines were striping using a Biodot Frontline dispenser (XYZ3060, Biodot, Irvine, CA).

Nitrocellulose blocking

Nitrocellulose strips were blocked in a solution containing 0.05%, 1-day aged casein, 5 mM borate, and 2% w/v sucrose.

One-day aged casein was prepared by adding 5 g of casein (Sigma, St Louis, MO, C7078) to 400mL of 0.31% (w/v) NaOH solution (Sigma, St Louis, MO, 415413) and stirring for 30 minutes. After the casein was fully dissolved, 1.2 g of boric acid (B0394) and 2.2 g of sodium tetraborate decahydrate (Sigma, St Louis, MO, S9640) were added to the stirred solution. The pH was adjusted to 8.5 with 1.0M HCl. Ultrapure water was added to bring the volume to 500 mL. The solution was filtered using a Nalgene 0.2 µm filter unit and placed in a 40°C oven for 24 hours. The solution was aliquoted and frozen at -20°C until use.

To block, striped nitrocellulose was placed with the long edge submerged in the blocking solution of 0.05%, 1-day aged casein, 5 mM borate, and 2% w/v sucrose. The solution was allowed to wick until the nitrocellulose was fully wet, at which point it was submerged fully in the blocking solution. The nitrocellulose remained in the blocking solution for 15 minutes, with continuous rocking. Finally, the nitrocellulose was removed from the blocking

solution and placed in a 25°C forced-air oven for 30 minutes or until dry. It was then immediately moved to an argon-purged desiccator for storage.

Conjugate pad blocking

A chopped glass fiber conjugate pad cut to 29 mm width (Ahlstrom-Munksjö, Helsinki, Finland, product 8951) and blocked with a solution of 0.05%, 1-day aged casein, 5 mM borate, and 0.2% w/v sucrose. The entire conjugate pad was submerged in the blocking solution for 15 minutes with continuous rocking. The nitrocellulose was removed and dried in a 25°C forced-air oven.

Sample pad blocking

The sample pad is a chopped glass fiber pad cut to 14 mm width (Ahlstrom-Munksjö, Helsinki, Finland, product 8964). The pad is blocked in 0.05%, 1-day aged casein, 5 mM borate, 0.2% w/v sucrose, 0.05% w/v PVP-40 (Sigma, St Louis, MO, PVP-40) and 1 mg/mL HBR-1 (Scantibodies, Santee, CA).   The   sample   pad   was   submerged completely in blocking solution with continuous rocking for 15 minutes and then dried at 25°C in a forced-air oven.

## Conjugate spraying and biotinylated antibody striping.

The test-line and conjugate-line latex beads were sonicated and diluted in 1-day aged casein with 10% sucrose and 2% trehalose to a final concentration of 0.06% test-line beads and 0.015% control-line beads. The bead solution was sprayed in three discreet locations on the conjugate pad, 10, 15 and 20 mm from the upstream edge of the nitrocellulose. The conjugate was sprayed at 4 μL/cm using the Biodot Airjet (ZX1010, Biodot, Irvine, CA). Biotinylated MM08 was similarly diluted in 1-day aged casein with 10% sucrose and 2% trehalose to a final concentration of 75 μg/mL. It was striped using the Biodot Frontline 5mm from the upstream edge at 4 μL/cm.

Conjugate pad lyophilization

The conjugate pad was then immediately transferred to a lyophilizer (Advantage Pro, SP Scientific, Stone Ridge, NY) with shelves pre-cooled to -40°C. As soon as all the conjugate pads were transferred to the lyophilizer, the lyophilization cycle was started. The cycle began with 15 minutes of freezing at -35°C with no vacuum. Next, with the shelf temperature held at -35°C, vacuum was applied until the pressure reached 100 mTorr. After one hour, the temperature was increased to 20°C over the course of 55 minutes, while maintaining 100 mTorr pressure. Finally, the vacuum was reduced to 1000 mTorr until the pads were removed and placed directly in a desiccant cabinet. LFAs were assembled without a dry room, which may negate the need for lyophilization.

LFA assembly

Blocked nitrocellulose was placed on an 80 mm backing card (Lohmann). A 22 mm Ahlstrom 320 wicking pad (Ahlstrom-Munksjo Oyj, Finland) was placed on top of the downstream edge of the nitrocellulose with a 5 mm overlap between the two materials. The sprayed and blocked conjugate pad was placed on top of the upstream edge of the nitrocellulose with a 2 mm overlap. Finally, the sample pad is placed on top of the upstream edge of the conjugate pad with a 2 mM overlap. Strips were cut to a 5 mm width using a Kinematic Matrix guillotine cutter (Kinematic Automation, Inc., Twain Harte, CA, USA). Strips were then placed in individual injection molded cassettes and stored in desiccator until use.
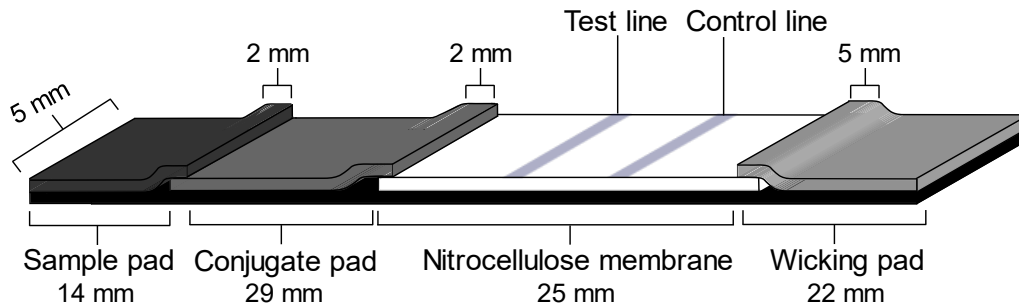
Figure 1 – An LFA was constructed using 14 mm of a sample pad (Ahlstrom 8964), 29 mm of a conjugate pad (Ahlstrom 8951), 25 mm of a nitrocellulose analytical membrane (CN95), and 22 mm of wicking pad (Ahlstrom 320). The test line and control line are striped at 8 and 14 mm from the downstream end of the nitrocellulose membrane.

LOD Running Protocol

Pooled nasal matrix was collected via nasal swabs collected by in-lab volunteers. All volunteers were confirmed negative by RT-PCR. Negative nasal matrix was collected using either Puritan Sterile Foam Tipped Applicator (25-1506 1PF TT MC) or Puritan PurFlock Ultra Elongated Flock Swab (25-3806-U BT) depending on availability. Volunteers self-swabbed by inserting the swab until resistance was met, rotating four times, and placing the swab into 500 µL of PBS with 2% IGEPAL CA-630 (Sigma, St Louis, MO, I8896). Nasal matrix samples were kept at 4°C until use. 450 µL of each sample was removed from the sample collection tube with a pipette and combined with all samples collected, to a total of 14 swabs from 9 volunteers. Gamma-irradiated SARS-CoV-2 (52287, lot number 70033322 BEI Resources), was diluted into the pooled nasal swab immediately before running. 200 µL of sample was added to each cassette using an exact-volume transfer pipette (Globe Scientific 139116). After 30 minutes, strips were read on a commercial lateral low reader (AX-2X-S, Axxin, Australia). Strips were also read visually by volunteers blinded to the irradiated virus concentration.

Limit of detection calculation

The data was fitted using a four-parameter logistic fit using the drc (Analysis of Dose-Response Curves) package in R.[14] The fit was weighted inversely to the square root of the signal. The test-line LFA reader score corresponding to the limit-of-detection was defined as

$$LOD_{LFA\ Reader\ Score} = \mu_0 + 1.645\ \sigma_0 + 1.645\ \sigma low\ positive$$

where μ_0 is the mean LFA reader score for the negative samples, σ_0 is the corresponding standard deviations, and $\sigma_{low\ positive}$ is the pooled standard deviation for the four lowest non-zero concentrations (0.25, 0.5, 1, 2 ng/mL).[15] The corresponding concentration and associated 95% confidence intervals were then calculated using the fitted curve.

Results and Discussion

A dose response curve was generated for the half-strip LFA using irradiated virus in pooled nasal matrix. The limit of detection was determined to be 23 TCID$_{50}$/mL (95% CI of 9.1 to 37 TCID$_{50}$/mL), equivalent to 1.4x10^5 copies/mL (95% CI of 5.5x10^4 to 2.3x10^5 copies/mL) for the lot of irradiated virus used, and was calculated in R using the drc package. For point of care test use cases, the WHO has proposed a minimal LOD of 10^6 genomic copies/mL and an

optimal of 10^4 genomic copies/mL.[16] The LOD for this assay, as tested, falls between these guidelines, and is superior to the analytical sensitivity of the commercially available antigen tests available as of early August, 2020.
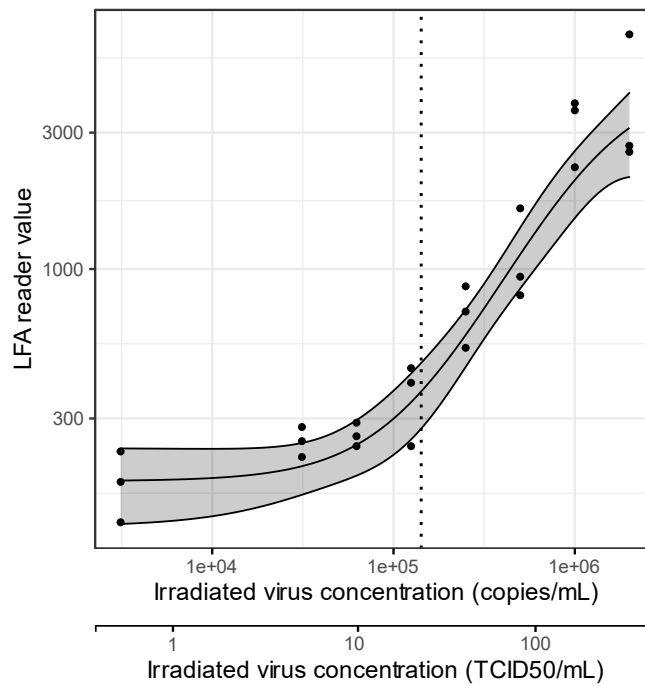
Figure 2 – The dose response curve for an LFA using irradiated virus, as measured using a commercially available optical LFA reader. The LOD is indicated by the dotted lines and was calculated as 23 $TCID_{50}$/mL (95% CI of 9.1 to 37 $TCID_{50}$/mL), equivalent to $1.4x10^5$ copies/mL (95% CI of $5.5x10^4$ to $2.3x10^5$ copies/mL). Using a conversion factor of 1:6,071,4 for $TCID_{50}$/mL to copies/mL for this lot of irradiated virus, the LOD in infectious units was 7.04 $TCID_{50}$/mL (95% CI of 3.33 to 10.75).

Table 1 – The LOD and test characteristics of this assay, two EUA authorized antigen tests in the US as of August 11, 2020, and a widely available antigen test in non-US markets. *Differences in LOD determination protocols as reported in the BD Veritor FDA submission make direct comparison with other tests problematic.

Test Name	Cat. No.	Read Mode	LOD $TCID_{50}$/ml	LOD copies/mL	Virus Type	Matrix Type
Global Health Labs	This paper	Chrom. by eye	23	$1.4x10^5$	Irradiated	Pooled Human Nasal Matrix
BD Veritor[10]	256082	Chrom. with reader	$1.4x 10^2$ *	$8.5x10^5$ *	Irradiated	Pooled Human Nasal Matrix
Sofia SARS Antigen FIA[9]	20374	Fluorescent with reader	$2.26x10^2$	$2.5x10^5 -$ $1.6x10^6$	Heat Inactivated	Nasopharyngeal matrix
Standard Diagnostics[3]	09COV30D	Chrom. by eye	$1.98x10^3$	N/A	"inactivated"	Nasopharyngeal matrix

As of August 2020, manufacturers have reported their LOD in terms of $TCID_{50}$. $TCID_{50}$ is the concentration at which 50% of cells are infected when a test tube or well plate upon which cells have been cultured is inoculated with a

diluted solution of viral fluid. The WHO target product profile lists its desired performance characteristics in terms of viral copies per mL. Unfortunately, lot to lot variability complicates a direct comparison of $TCID_{50}$ / mL and copies per mL.

The Sofia SARS Antigen FIA product insert indicates a LOD of 2.26×10^2 $TCID_{50}$/mL[9] of BEI product number NR-52286. The product insert claims that the starting concentration of the NR-52286 lot used had a $TCID_{50}$ of 3.4×10^5 per mL. To determine the copies per mL of this lot, we consulted the BEI reference data sheets, however no lot is shown with this starting concentration, and personal communication with BEI confirmed no such lot was made. For the three lots of NR-52286 with certificates of analysis as of August 8, 2020, the copies per mL per $TCID_{50}$ per mL ranges from 1106 to 7250. Therefore, we assume the LOD for the Sofia test in terms of copies per mL is between 2.5×10^5 and 1.6×10^6 copies per mL.

The BD Veritor product insert claims an LOD of 1.4×10^2 $TCID_{50}$/mL. Though a source is not referenced for the irradiated virus, we assume it is BEI NR-52287, lot 70033322 as the stock titer for this lot of BEI irradiated virus matches the stock titer in the certificate of analysis for this lot. The ratio of copies per mL per $TCID_{50}$ per mL for this lot is 6071 which corresponds to an LOD of 8.5×10^5 copies per mL. However, the LOD method utilized in the Veritor product insert utilizes serial dilutions which are added onto a swab, and then further diluted into the Veritor sample transfer buffer.[10] Without this additional dilution step the reported BD Veritor LOD would likely improve.

The Standard Diagnostics LFA reported an LOD of $1.25 \times 10^{\wedge}3.2 = 1.98 \times 10$ $TCID_{50}$ per mL. The strain utilized was SARS-CoV-2 (2019-nCOV) NCCP 43326/2020 / Korea, via the Korean National Culture Center for Pathogens, though insufficient information was available on this strain to determine the copies per mL contained wherein.

The SARS-CoV-2 LFA reported herein has an LOD of 23 $TCID_{50}$/mL (95% CI of 9.1 to 37 $TCID_{50}$/mL), equivalent to 1.4×10^5 copies/mL (95% CI of 5.5×10^4 to 2.3×10^5 copies/mL). Using a conversion factor of 1:6,071.4 for $TCID_{50}$/mL to copies/mL for this lot of irradiated virus, the LOD in infectious units was found to be 7.04 $TCID_{50}$/mL (95% CI of 3.33 to 10.75)

This LFA can be considered an open source design for an antigen-detecting point of care test for SARS-CoV-2, as it is designed entirely from commercially available materials. If antibody supplies for the antibodies chosen herein are limited, our previously reported antibody screening effort can be utilized to select alternate choices.[12] The information we present could be used by any organization to develop an LFA for both validation or towards large-scale production. This first-generation product can likely benefit from further optimization and improved performance through higher sensitivity nanoparticles, etc. This test was designed with a buffer compatible with anterior nares swabs. Future work should confirm its suitability in other sample matrices, including saliva.

Significant validation work is still required for this assay, including clinical validation in relevant settings. This work is ongoing, and this preprint will be updated with clinical data as it becomes available.

Conclusion

In this paper we present an LFA designed for the detection of the nucleocapsid protein of SARS-CoV-2. Significantly, it meets the stated analytical sensitivity requirements proposed by WHO.[16] We utilize only commercially available reagents and conventional protocols, to allow the straightforward duplication and modification of this LFA. The LOD of this assay meets the requirements stated by the WHO for use as a POC test. Further work includes validation of the assay in a clinical setting and its performance using different specimen types.

Acknowledgements

References

(1)   Zhu, N.; Zhang, D.; Wang, W.; Li, X.; Yang, B.; Song, J.; Zhao, X.; Huang, B.; Shi, W.; Lu, R.; Niu, P.; Zhan, F.; Ma, X.; Wang, D.; Xu, W.; Wu, G.; Gao, G. F.; Tan, W. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N. Engl. J. Med. 2020, 382 (8), 727–733. https://doi.org/10.1056/NEJMoa2001017.

(2)   Coronavirus disease (COVID-19) Situation Report https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200723-covid-19-sitrep-185.pdf?sfvrsn=9395b7bf_2 (accessed Jul 23, 2020).

(3)   Rahimi, F.; Bezmin Abadi, A. T. Case-Finding: Fast, Available, and Efficient Font-Line Diagnostics for SARS-CoV-2. Arch. Med. Res. 2020. https://doi.org/10.1016/j.arcmed.2020.04.008.

(4)   Wang, C. J.; Ng, C. Y.; Brook, R. H. Response to COVID-19 in Taiwan: Big Data Analytics, New Technology, and Proactive Testing. JAMA - Journal of the American Medical Association. American Medical Association April 14, 2020. https://doi.org/10.1001/jama.2020.3151.

(5)   Lazer, D.; Baum, M. A.; Quintana, A.; Druckman, J.; Della, J.; Simonson, M. THE STATE OF THE NATION: A 50-STATE COVID-19 SURVEY.

(6)   Larremore, D. B.; Wilder, B.; Lester, E.; Shehata, S.; Burke, J. M.; Hay, J. A.; Tambe, M.; Mina, M. J.; Parker, R. Test Sensitivity Is Secondary to Frequency and Turnaround Time for COVID-19 Surveillance. medRxiv 2020, 2020.06.22.20136309. https://doi.org/10.1101/2020.06.22.20136309.

(7)   He, X.; Lau, E. H. Y.; Wu, P.; Deng, X.; Wang, J.; Hao, X.; Lau, Y. C.; Wong, J. Y.; Guan, Y.; Tan, X.; Mo, X.; Chen, Y.; Liao, B.; Chen, W.; Hu, F.; Zhang, Q.; Zhong, M.; Wu, Y.; Zhao, L.; Zhang, F.; Cowling, B. J.; Li, F.; Leung, G. M. Temporal Dynamics in Viral Shedding and Transmissibility of COVID-19. Nat. Med. 2020, 26 (5), 672–675. https://doi.org/10.1038/s41591-020-0869-5.

(8)   Alexandersen, S.; Chamings, A.; Bhatta, T. R. SARS-CoV-2 Genomic and Subgenomic RNAs in Diagnostic Samples Are Not an Indicator of Active Replication. medRxiv 2020, 2020.06.01.20119750. https://doi.org/10.1101/2020.06.01.20119750.

(9)   Sofia SARS Antigen FIA https://www.fda.gov/media/137885/download (accessed Jul 23, 2020).

(10)   Veritor System For Rapid Detection of SARS-CoV-2 https://www.fda.gov/media/139755/download (accessed Jul 23, 2020).

(11)   Expression of interest - FIND https://www.finddx.org/eoi-covid19-ag-rdt/ (accessed Jul 23, 2020).

(12)   David, C.; Helen, H.; Veronika, G.; Joshua D, B.; H Gleda, H.; Brianda, B.-L.; Ben D, G.; Caitlin E, A.; Ethan, S.; Samantha, K.; Ryan, G.; Rafael, R.; Crissa, B.; Sam A, B.; John T, C.; Puneet K, D.; David, S. B.; Bernhard H, W.; Kevin P, N. Antibody Screening Results for Anti-Nucleocapsid Antibodies Towards the Development of a SARS-CoV-2 Nucleocapsid Protein Antigen Detecting Lateral Flow Assay. chemRxiv 2020. https://doi.org/10.26434/chemrxiv.12709538.v1.

(13)   Grant, B.; Anderson, C.; Williford, J.; Alonzo, L.; Glukhova, V.; S. Boyle, D.; Weigl, B.; Nichols, K. A SARS-CoV-2 Coronavirus Antigen-Detecting Half-Strip Lateral Flow Assay Towards the Development of Point of Care Tests Using Commercially Available Reagents. Anal. Chem. 2020. https://doi.org/10.1021/acs.analchem.0c01975.

(14)   Ritz, C.; Baty, F.; Streibig, J.; Gerhard, D. Dose-Response Analysis Using R. PLoS One 2015, 10 (12), e0146021. https://doi.org/10.1371/journal.pone.0146021.t001.

(15)   Armbruster, D. A.; Pry, T. Limit of Blank, Limit of Detection and Limit of Quantitation. Clin. Biochem. Rev. 2008, 29 Suppl 1 (Suppl 1), S49-52.

(16)   COVID-19 Target product profiles for priority diagnostics to support response to the COVID-19 pandemic v.0.1 https://www.who.int/publications/m/item/covid-19-target-product-profiles-for-priority-diagnostics-to-support-response-to-the-covid-19-pandemic-v.0.1 (accessed Aug 8, 2020).

Supplemental Information

Table S1 – Raw data for dose response curve shown in Figure 2 for the LFA as run in pooled nasal matrix with 2% IGEPAL CA-630

TCID50	copies	LFA Intensity (arb)
0	0	230
0	0	180
0	0	130
31250	5.15	250
31250	5.15	220
31250	5.15	280
62500	10.29	290
62500	10.29	240
62500	10.29	260
125000	20.59	240
125000	20.59	400
125000	20.59	450
250000	41.18	870
250000	41.18	530
250000	41.18	710
500000	82.35	810
500000	82.35	940
500000	82.35	1630
1000000	164.71	3800
1000000	164.71	3590
1000000	164.71	2270
2000000	329.41	6620
2000000	329.41	2700
2000000	329.41	2570