Rapid turnaround, home-based saliva testing for COVID-19

-Study Design-

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Study Design Summary

Study Type:	*Experimental*
Estimated Enrollment:	*3060 participants*
Observational Model:	*Cohort*
Time Perspective:	*Prospective*
Official Title:	
Estimated Study Start Date:	*September 2020*
Estimated Primary Completion Date:	*December 2021*
Estimated Study Completion Date:	*January 2022*

Study Population:

The study population consists of a diverse group of Stanford faculty, staff, and students, and the local Santa Clara County community and specific external sources with known positive saliva samples and DUA/MTA in place. More specifically, there are three sub-populations to address our aims:

1) (Positive population) Hospitalized patients (N=15)
2) (High risk and positive population) People from Bay area community and external sites with known positive saliva samples and DUA/MTA in place; Those with a suspected COVID infection seeking testing within the Stanford Health Care system (N=1500)
3) (Low risk population) People from Bay area community; Students on the Stanford campus and employees at Stanford Health Care (N=1500)

Criteria for Hospitalized Patients

Inclusion Criteria

- >=18 years
- Hospitalized at Stanford Health Care for COVID-related complications and tested positive for SARS-CoV-2 with a PCR-based test
- Able to understand and consent to study and with a clinical trajectory likely to be consistent with multi-day participation

Exclusion Criteria:

- Participants with salivary gland dysfunction (including patients with Sjogren's disease or those with xerostomia associated with lupus or rheumatoid arthritis)
- Participants will not be eligible if they identify any reason they are unable to participate in the study

Criteria for High Risk and Positive Population

Inclusion Criteria:

- >=18 years
- Seeking testing for suspected COVID or a participant in a study of COVID-positive outpatients or from external sites with known positive saliva samples and DUA/MTA in place
- Own an internet-enabled phone or device capable of loading web pages, receiving text messages, and taking/uploading photos.
- Willing to participate in the study for one month

Exclusion Criteria:

- Participants who have color blindness
- Participants unable to operate the SnapDx device
- Participants with salivary gland dysfunction (including patients with Sjogren's disease or those with xerostomia associated with lupus or rheumatoid arthritis)
- Participants will not be eligible if they identify any reason they are unable to participate in the study

Criteria for Low Risk Population

Inclusion Criteria:

- >=2 years
- Have access to an internet-enabled phone or device capable of loading web pages, receiving text messages, and taking/uploading photos.
- Willing to participate in the study for one month, or permission and willingness from legal guardian for participants under 18 years old.

Exclusion Criteria:

- Participants with prior confirmed SARS-CoV-2 infection
- Participants who have color blindness
- Participants unable to operate the SnapDx device
- Participants with salivary gland dysfunction (including patients with Sjogren's disease or those with xerostomia associated with lupus or rheumatoid arthritis)
- Participants will not be eligible if they identify any reason they are unable to participate in the study

Study Location: Stanford University Campus, Stanford Health Care, Stanford affiliated COVID-19 testing sites (including drive through testing sites). We will accept deidentified and inactivated saliva samples from external sources with DUA/ MTA in place and IRB approval.

Funding: Stanford Medicine Catalyst

DUA , MTA OTL: We have DUA in place with a site in Guatemala and will have DUA/ MTA in place for the sharing of saliva samples from any/all sources as they become available.

Mobile Medical Website/App: REDCap
DRA: N/A or previously approved for Pattern Health

IND Exempt: Yes, observational diagnostic tool

Manufacturer: N/A

Rationale

Clinical testing for SARS-CoV-2 falls broadly into two categories: molecular testing to detect the genomic sequence of the virus and serology testing for the antibody response of the host. While CLIA based testing in both categories has proved valuable for individual care during the pandemic, the cost (up to $100 in some cases) and time (a minimum of 12 hours in most cases) required for these approaches mean they have had little public health impact on the pandemic. A home based surveillance test for the presence of SARS-CoV-2 in saliva, that is cheap (a few dollars) and fast (under 40 minutes) would be transformative in the global management of COVID-19. It would allow nothing less than the reopening of schools, businesses, and the safe return of many aspects of society to normal. Individuals could test themselves every morning before heading to work, for example, to demonstrate they were not currently infected. In this protocol, we aim to demonstrate the viability of such an approach in a target population of Stanford employees and students who would test themselves at our research facility and upload the results via a mobile website/app. The protocol includes enrichment for positive samples through a cohort recruited through the Stanford Emergency Department (that would allow calculations of sensitivity required by the US FDA) and CLIA validation of these samples, a collection of known Sars-Cov-2 samples from external sources (including from Guatemala) as well a random selection of negative home tests to allow estimation of specificity The overall aim is to demonstrate the feasibility, usability, and real-world validity of home surveillance testing at scale to provide the foundation for scaling up to potentially millions of tests around the world.

Detailed Description of the Technology

The loop-mediated isothermal amplification (LAMP) assay has proven to be a reliable and simple protocol that can detect small amounts of viral RNA in patient samples. This LAMP protocol has the benefits of being simple, requiring no specialized equipment; rapid, requiring less than an hour from sample collection to readout; and cheap, costing around $1 per reaction using commercial reagents. Heating to separate the reaction inhibitors from inactivated samples was shown to be an effective way to ensure reliable LAMP amplification. In this protocol, we take advantage of an open hardware solution invented by one of the PIs (SnapDx) that can be assembled with readily available components for the cost of <$5 dollars a unit and could be used together with the LAMP assay for point of care detection of COVID-19 RNA from saliva. We further utilize a novel substrate - cellulose - for RNA extraction to further simplify the protocol for LAMP. Finally, we utilize a novel approach in construction of the assay to run the

home tests in a manner to eliminate any chances for reaction cross-contamination; common in laboratory molecular tests.

Instruments used with the Test:

The SnapDx is designed to be a low-cost at-home device for detecting SARS-CoV-2 in saliva samples. The process utilizes LAMP (isothermal amplification) reagents to detect SARS-CoV-2 in saliva. With COVID-19 cases increasing globally, diagnostics need to be accessible to everyone, including resource-limited regions.

SnapDx test hardware: $3-5 dollars per unit (estimate)

Saliva tube and solutions: $1 dollar per unit (estimate)

Reagents and Materials:

Cost breakdown for reagents:

- TCEP - 1G (Pierce 20490) >3000 reactions - $80
- EDTA - 100mL (Sigma 03690) >10,000 reactions - $40
- Primers - (IDT) 1.2ml of 10X >500 reactions - $30
- NEB master mix (E1700L) >500 reactions - $650

Primers: It is good practice to make 20µL 100µM aliquots once they are resuspended. In case of contamination a fresh stock can be used. For a 10X stock- 16 µl FIP, 16 µl BIP, 2 µl F3, 2 µl B3, 4 µl LF, 4 µl LB, and brought to 100 µl with water.

Ordered from IDT, sequences from Rabe 2020.

As1_F3

CGGTGGACAAATTGTCAC

As1_B3

CTTCTCTGGATTTAACACACTT

As1_LF

TTACAAGCTTAAAGAATGTCTGAACACT

As1_LB

TTGAATTTAGGTGAAACATTTGTCACG

As1e_FIP

TCAGCACACAAAGCCAAAAATTTATTTTTCTGTGCAAAGGAAATTAAGGAG

As1e_BIP

TATTGGTGGAGCTAAACTTAAAGCCTTTTCTGTACAATCCCTTTGAGTG

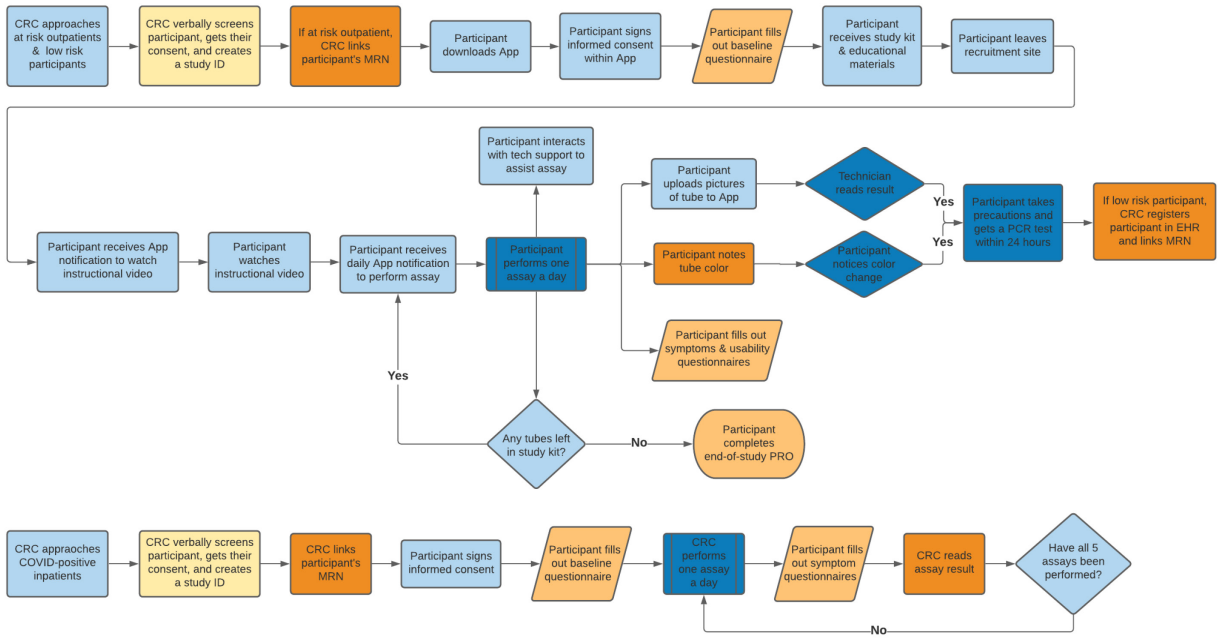
Materials included in SnapDx Kit:

Item	**Manufacturer**	**Order Number**
Spit tube	**BIC**	
SnapDx	**BIC**	
Instructions for Use	**PrakashLab**	

Materials included in the COVID-19 LAMP diagnostic assay (Gold Standard - clinically indicated):

Same as above.

Item	**Manufacturer**	**Order Number**
LAMP master mix	**NEB**	**M1804**
primers	**IDT**	**Standard Desalting**



Study Design

Figure 1
Study flowchart for recruitment, assay performance, and follow-up. Blue and yellow icons indicate processes and data collection respectively, in which key steps are colored with darker shades. CRC = Clinical Research Coordinator, PRO = patient reported outcome, MRN = medical record number.

Usability/Feasibility

All study procedures involving contact with individuals of any infectious disease risk status (low, moderate, high) will be performed by trained clinical research personnel with all the necessary personal protective equipment. All of these personnel have also been vaccinated against COVID-19. Nonetheless they will use standard precautions, including getting tested weekly and staying away from others including study personnel and potential participants, in any case of symptoms or illness.

A cohort of 120 participants with diverse demographic backgrounds will be recruited and each of them will take one SnapDx test at our study site. Additional saliva samples will be collected to be used for laboratory testing for clinical evaluation purposes. Participants will complete a Usability Survey towards the end of taking the SnapDx on our study site to answer questions on satisfaction, ease, information, and use. These Usability questions include the following with multiple choice answers (strongly disagree, disagree, somewhat disagree, neither agree nor disagree, somewhat agree, agree, strongly agree):

- (Satisfaction) Overall, I am satisfied with how easy it was to use the test.
- (Ease) It was easy to learn to use this test.
- (Information) It was easy to find the information I needed.
- (Use) If the SnapDx test were available to me today, I would use this on a regular basis.
- We also ask If they would be willing to retake the test

Answers to these questions will be summarized and reported to the FDA as the results of our Human Usability Study.

Validity

Saliva samples are to be collected in several patient populations, including from both inpatient and outpatient clinics, and COVID positive or negative patients determined by nasal swab PCR tests from several different locations. We will make a concerted effort to reach out to the Stanford and bay area communities and collect positive saliva samples from those who have tested positive through a standard PCR test. Five intended users will be given basic training to conduct SnapDx tests on these saliva samples as if they were taking the test themselves. A small portion of each saliva sample will also be tested for SARS-COV-2 using saliva direct PCR in the laboratory. SnapDx test results will be compared against saliva direct PCR results to determine the positive percent agreement (PPA) and negative percent agreement (NPA).

Aims

The aim of the study is to demonstrate the feasibility and validity of a saliva based home surveillance monitoring test for SARS-CoV-2 infection. These will be addressed in the following sub-aims.

1) To assess whether there is sufficient concordance between the technician and patient in interpretation of findings (Primary Feasibility Aim)
2) To assess sensitivity and specificity of the home saliva test (Primary Validity Aim)

Primary Feasibility Outcome:

An indicator for discrepancy in interpretation of findings by participant versus by technician via photo (interrater reliability)

Secondary Feasibility Outcomes:

There are 4 secondary outcomes involved in assessing feasibility. These include

1) An indicator for ambiguous findings as measured by percent confidence (if < 80% confident) in interpretation of results by participant and by technician (ease of interpretation)
2) An indicator that the participant appropriately followed instructions for using the kit (≥ 80% confident) (ease of use)
3) An indicator for whether participant called technical support and by reason (e.g., device leaked or there was a possible broken part) (ease of use)
4) An indicator by participant that the sample was sufficient and reached the indicated line for sample integrity (sample integrity)

Primary Validity Outcome:

An indicator for whether the home saliva test is positive (to assess sensitivity and specificity)

Statistical Considerations:

General Considerations:

We will use the Agresti-Coull method (Agresti and Coull 1998) to construct confidence intervals. Unless otherwise noted, all confidence intervals constructed will be 95% confidence intervals.

Analysis Sets:

There are two key analysis sets that are subsets of the study population. The first subset consists of outpatients. The second subset consists of the patients hospitalized for COVID-related complications who will be tested daily for 5 days.

Analysis of the Feasibility Outcomes

Analyses of all feasibility outcomes will be performed in the outpatient analysis set.

The concordance of the test results will be compared to the results of PCR testing. We will calculate the proportion in agreement (# of tests where SnapDx and PCR testing agree/# total tests), the proportion correctly identified as positive (# of tests SnapDx-positive/# PCR-positive), and the proportion correctly identified as negative (# of tests SnapDx-negative/# PCR-negative). A threshold of 80% overall agreement and 85% agreement in the positive tests will indicate the home test is feasible.

Percent confidence in interpretation of the results and that instructions were followed correctly will be summarized as 1) percent of respondents stating that they are at least 80% confident in their interpretation and 2) median and interquartile range of reported confidence levels. Results will be reported separately for participants and technicians.

The proportion of participants who call technical support will be presented and the frequency of the reason for the call will be presented. The proportion of tests that have a sufficient amount of saliva to reach the indicator line will be presented.

The feasibility outcomes will be presented separately by demographic characteristics in a subgroup. For example, we will evaluate whether the concordance is comparable across different age groups, between men and women, etc.

Analysis of the Validity Outcomes

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the test will be estimated using the results collected in outpatients. The results of the PCR-based test will be the gold standard used to estimate the test's diagnostic accuracy. Home test results will only be included in this analysis if the test is performed within 24 hours of the time the swab used in the PCR tests was collected.

Handling of Missing Data

Although we have designed our study to minimize the amount of missing data, we expect to have a small amount of missing data. For example, hospitalized patients who are very sick or are discharged prior to day 5 may not provide data for days 1-5; participants may not complete the surveys each time they provide results from their home tests; or some PCR test results may not be able to be linked to the home test results. Analyses in the outpatient population will assume that data is missing at random and will use multiple imputation methods to account for the missing data. Given the small number of hospitalized patients expected, we will perform a complete case analysis in the analysis set consisting of hospitalized patients. Sensitivity analyses will be performed to evaluate the robustness of our findings to assumptions about missing data.

Recruitment:

The study will recruit up to 3060 participants including 60 hospitalized patients from multiple locations. These will include SHC COVID testing sites, Stanford Health Care

300 and 500P locations (the Emergency Department and other in-patient wards particularly M7, the COVID ward), as well as the front entrance of Stanford hospital and several locations on Campus. We will also be recruiting folks from the bay area community for our study. Participants will fall into two categories: i) those receiving a CLIA certified nasal swab COVID-19 test at SHC at the moment of recruitment, and ii) those not.

We will be recruiting a high-risk population because we will be recruiting from a pool that includes people who are seeking medical attention and/or participating in COVID-related research studies. Feasibility and validity of the tests will be evaluated in the high-risk population. We will be additionally recruiting a low-risk population to assess feasibility in the general population. Hospitalized patients will provide information on concordance between the CLIA COVID test and the home saliva test in patients over a five day period when we expect to see a mix of positive and negative tests.

To increase the number of SARS-CoV-2 positive saliva samples, we will accept deidentified and deactivated saliva samples from external sources, including Guatemala. All of these external sources will have DUA/MTA in place and will have institutional approval and have received written informed consent from all participants for the local trials, including the sharing of these samples with Stanford University for the current trial.

Inclusion and Exclusion criteria:
(as noted above)

Consent

Potential participants will be approached by dedicated clinical research coordinators and study volunteers. They will be asked if they have an interest in participating and if so, whether they have an internet data-enabled cell phone capable of uploading high resolution photographs. Interested participants will then be directed to the digital e-consent implemented in REDCap on the mobile web browser. The CRC will be available to answer questions. The participants will also be provided an email and contact phone information for questions.

For the pediatric population, informed consent will be given by the legal guardian after they have been given the opportunity to answer any and all of the questions they may have and assent will be provided for children able to understand and complete the assent form (generally at least 7 years old).

Consent and Assent form will include a request to share access to their medical records for review of the results of any CLIA certified COVID test and any medical condition that might affect the veracity of testing. It will also include a request to observe the participants in-person or via video conferencing.

After consent, participants will receive a bag with materials for multiple home based tests as well as instructions on their phone via REDCap on how to use the test.

Test Protocol

The REDCap survey will include a video demonstration of the test procedure. In addition, written instructions for use (IFU) will be available.

Participants will be asked to carry out as many tests as are included in the bag (most likely between one and two) on a daily basis until they are used up.

Test description

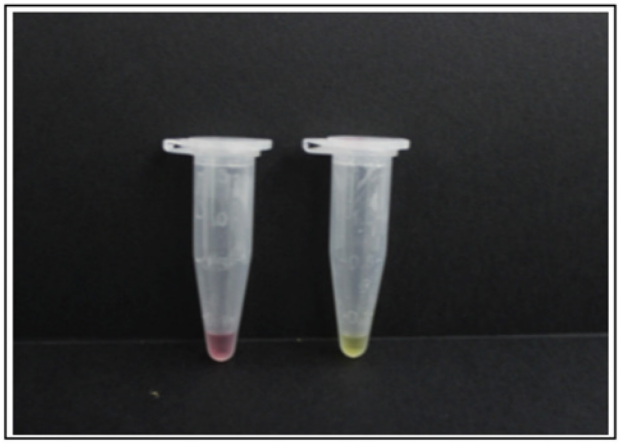
Test materials: saliva collection tube, small handheld centrifuge, test kit (plasticware) The test kit should be stored in a regular fridge (4C) at the location of the participant - prior to use.

The test materials include a cartridge containing a tube with closed reagents into which the participant will provide the saliva sample. Participants will be asked to provide 1ml of saliva into the collection tube, after which they will close the cap of the cartridge. They will be asked to pour boiling water into a mug and place the tube into the boiling water for 3 minutes. At the end of 3 minutes, they will then place the tube back in the mug of water for 40 minutes. At the end of 40 minutes, they remove the tube and note the color (pink/ orange = negative, yellow = positive). They will take a photograph of the tube against a given white photocard (provided in the kit) and upload it to the website. After filling in the short (<1 minute) symptom questionnaire and the short usability (<20 seconds) questionnaire they will dispose of the tube in the trash.

At that point, **if the test result is negative** (no evidence of infection) then they will be asked to do nothing other than fill in a survey and upload the image of the tube via study app. **If the test result is positive** they will be instructed to behave as if they might be infectious in their home and outside of their home (maximizing the use of masks and distancing) and asked to take a CLIA nasal swab test at their nearest SHC testing site at their earliest opportunity.

Our study provides the option for the participants to take a PCR nasal swab test at Stanford Health Care when their SnapDx tests result or the saliva direct PCR test done by the lab shows a positive SARS-CoV-2 result. Our study will cover the cost of the PCR nasal swab test for the participants.

Figure: Color change in tubes. Yellow color is a positive result



Left sample spiked with random plasmid DNA, right sample spiked with COVID-19 control RNA, at 50 genomes/µL.

Data Acquired

Data acquired on all participants in this study will include

1) A baseline demographics survey collecting age, sex, and race/ethnicity, education levels, socio-economic background and COVID-19 vaccination status
2) A symptom survey filled in by the participant (one per test)

Additional data acquired on the positive/at-risk and the low-risk participants includes:

3) A survey to collect confidence in interpretation and in following the instructions (one per test)
4) An associated image of the at-home LAMP assay test result (one per test)
5) A follow-up survey for the usability comments (once)

Data collected on participants who have a CLIA COVID-19 test performed within 24 hours of the home saliva test will additionally include:

1. A medical chart review for the results of any CLIA COVID-19

2. Technicians will additionally provide an evaluation of their confidence in interpreting the participant-provided images using a survey identical to the one shown to participants
3. Also included will be the additional documentation for the participant to follow the local healthcare authorities and CDC guidelines for COVID-19 related process.

Payments:
Participants will not be paid for participation. The study team will cover the costs of any follow-up CLIA certified testing required.