Rapid, onsite COVID-19 detection

Protocol Number: 2020-0855
Principal Investigator: David O'Connor

Funding Sources

Wisconsin National Primate Research Center Support - P51, Yr 59
PI: Steve Ackerman
Sponsor: WARF
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Protocol Version History

Protocol Version	Version Date	Summary of Revisions Made	Rationale
1.0	06/29/2020	Initial version	
2.0	8/05/2020	Updated for minors	
3.0	8/30/2020	Single consent, repeat testing	

Table of Contents

1.0 STATEMENT OF COMPLIANCE

I confirm that I have read this protocol. I will comply with the IRB-approved protocol, and applicable regulations, guidelines, laws, and institutional policies.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitment.

Name	Signature	Date
_____	_____	_____
Principal investigator		
_____	_____	_____
Sponsor		

2.0 LIST OF ABBREVIATIONS

AE	Adverse Event
AVRL	AIDS Vaccine Research Laboratory
BSC	Biological Safety Cabinet
CCC	Clinical Coordinating Center
CFR	Code of Federal Regulations
CRF	Case Report Form
CTMS	Clinical Trial Management Software
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DMC	Data Monitoring Committee
EtOH	Ethyl alcohol
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IDE	Investigational Device Exemption
ICTR	Institute for Clinical and Translational Research
IRB	Institutional Review Board
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PHI	Protected Health Information
PI	Principal Investigator
POC	Point of Contact
RT-LAMP	Reverse transcription loop-mediated isothermal amplification
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SMP	Study Monitoring Plan
SMS	Study Monitoring Service
UP	Unanticipated Problem
UWCCC	University of Wisconsin Carbone Cancer Center

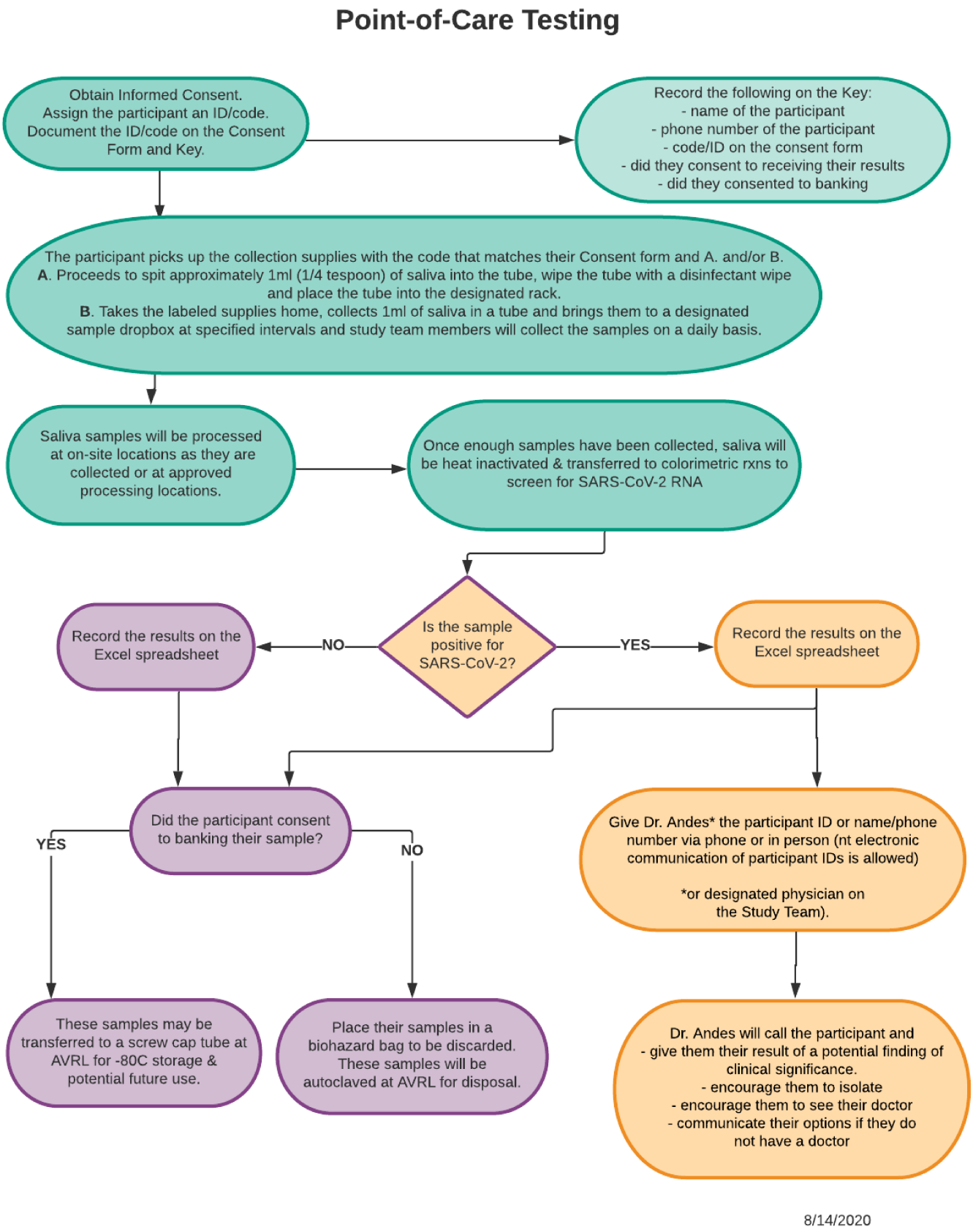
3.0 STUDY SUMMARY

3.1 Synopsis

Full Title	Rapid, onsite COVID-19 detection
Protocol Number	HS IRB 2020-0855
ClinicalTrials.gov Identifier & Summary	The purpose of this research study is to evaluate and improve a rapid COVID-19 test. The test is designed to identify people who are most contagious and likely to spread the virus to others. This test will be performed at various locations in the Madison area using a mobile laboratory or standard lab space for processing. Saliva samples can be collected and processed at these locations or participants can self-collect at home and drop their samples off at designated locations for same day processing. Results of potential findings of clinical significance will be communicated to the participants by a physician with appropriate expertise on the study team. Individuals with a potential finding of clinical significance will be encouraged to self-isolate and obtain a diagnostic test at their earliest convenience. No results will be given if the test is negative. If the participant consents, advanced molecular testing such as PCR or viral sequencing can be done and results can be shared via online databases, presentations and publications along with the date, site and county of collection to help facilitate tracking the spread of the virus. CT.gov Registration ID is NCT04460690
Number of Site(s)	This study will be conducted at multiple locations in the Madison area. Sample collection can occur on-site or off-site using sample drop off boxes in designated areas. Processing of samples can occur using both mobile laboratories and standard lab spaces.
Main Inclusion Criteria	• Must be at least 18 years old or be between 5-17yrs old and have a parent or legal guardian present to consent • Willing to provide informed consent and spit into a vessel (i.e cup or tube). • Adult subjects must have decision-making capacity to provide consent on their own behalf. • Subjects must be able to speak English • Subjects must not have visual or hearing impairments, or low literacy, that would prevent them from reading the consent form and interacting with a member of the research team to ask questions and receive responses during the consent process.
Main Exclusion Criteria	• Under 18 years old with no parent or legal guardian present • Under 5 yrs of age
Objective(s)	To show we can safely, consistently, and accurately detect high levels of SARS-CoV-2 RNA in non-invasive saliva samples using a mobile laboratory.
Endpoints	When we have tested at least 10000 samples and consistently shown that the assay can detect SARS-CoV-2 safely, consistently and accurately at multiple locations using the mobile laboratory and can be organized to test participants repeatedly over time.
Study Design	This is a non-randomized study where every participant will undergo the same procedure of spitting into a tube.
IND or IDE Number	Requesting an IDE nonsignificant risk (NSR) determination from the IRB.
Study Intervention	The RT-LAMP assay detects SARS-CoV-2 RNA in saliva samples using Salus Discovery LLC's RNA extraction technology.
Total Number of Subjects	A total of 10000 subjects could be recruited from the study sites.

Study Population	Anyone over the age of 5yrs old with consent to provide a saliva sample will be eligible to participate.
Estimated Subject Duration	The duration of the study for each subject is approximately 20 minutes each visit.
Estimated Enrollment Period & Study Duration	Study enrollment will occur over a 12 month period.

3.2 Schematic of Study Design



4.0 KEY ROLES

The following is a list of all key personnel and roles:

Principal Investigator	David O'Connor, Ph.D. Professor University of Wisconsin - Madison 585 Science Drive 608-890-0845 dhoconno@wisc.edu
Participating Site(s)	AVRL 585 Science Drive Madison, WI 53711 WNPRC 1220 Capitol Court Madison, WI 53715 EAGLE School 5454 Gunflint Trail Fitchburg, WI 53711 Athletic Department Camp Randall Madison, WI 53715 Others as requested
Laboratory Services	Mobile Laboratories, WNPRC with AVRL as the home base lab
Medical Monitoring	Dr. David Andes University of Wisconsin – Madison 5211 UW Medical Foundation Centennial Bldg 1685 Highland Ave Madison, WI 53705 dra@medicine.wisc.edu

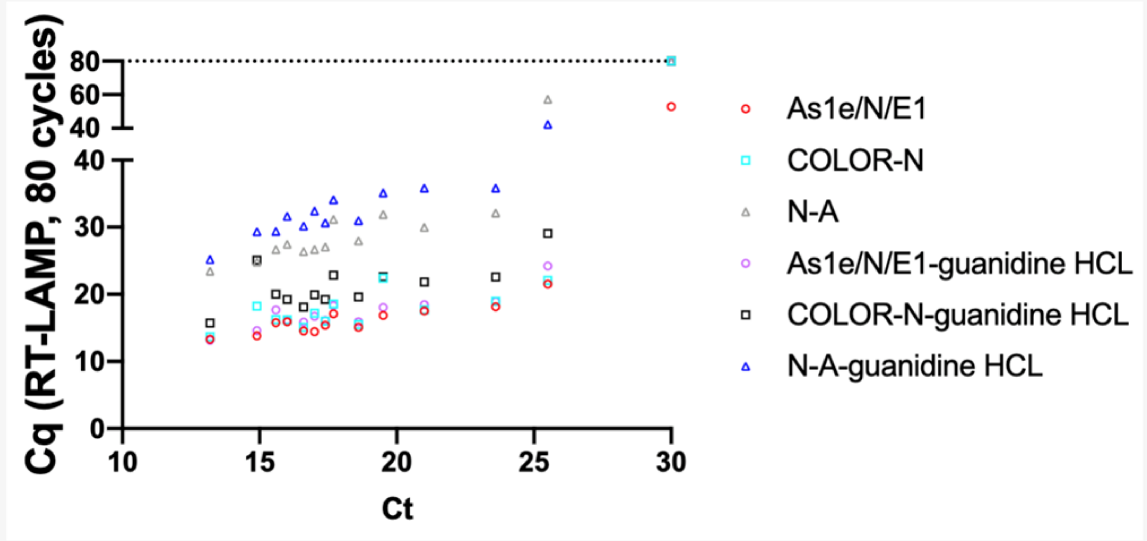
5.0 INTRODUCTION

5.1 Disease Background

Virtually all COVID-19 RNA assays require centralized laboratories. Samples collected in locations including public testing sites, workplaces, nursing homes, and residential housing are transported to centralized laboratories for testing, leading to lengthy delays in results reporting. The window of maximal contagiousness is thought to be only a few days, so these delays create the risk of excess transmission. Current testing methods are painful, which encourages testing hesitancy. Rapid, onsite detection of SARS-CoV-2 RNA from non-invasive saliva could overcome these issues and provide a pathway to high-throughput detection of people at the greatest risk of SARS-CoV-2 transmission but the logistics of such a testing program require real-world prototyping. That is the purpose of this project.

Shown below are RT-LAMP results using residual specimens from known SARS-CoV-2+ cases. Each vertical series of points is a different sample, the datapoints for each sample indicate performance with a different primer set (a variable that we were optimizing in this experiment). You'll note that all of these samples were detected with our RT-LAMP assay. There was one sample that didn't perform well with every

primer set (the one with the least amount of virus, Ct ~ 30), but this sample had the lowest amount of input virus.



False positives are rare to non-existent. Early in our RT-LAMP testing, we received blinded samples from the State Lab of Hygiene. 15/15 SARS-CoV-2-negative samples were properly classified. 14/15 SARS-CoV-2+ samples were properly classified. This was an early embodiment of the technology and the one sample that was a false negative also had a Ct ~ 30, corresponding to the low end of what we reasonably expect to detect right now:

Sample ID	Approx Ct	Expected Result	RT-LAMP concordance with CDC assay
nCoV-1	21	SARS-CoV-2	
nCoV-2		Negative	
nCoV-3	20	SARS-CoV-2	
nCoV-4	27	SARS-CoV-2	
nCoV-5		Negative	
nCoV-6	20	SARS-CoV-2	
nCoV-7		Negative	
nCoV-8	28	SARS-CoV-2	
nCoV-9		Negative	
nCoV-10		Negative	
nCoV-11	21	SARS-CoV-2	
nCoV-12	29	SARS-CoV-2	
nCoV-13		Negative	
nCoV-14	22	SARS-CoV-2	
nCoV-15		Negative	
nCoV-16		Negative	
nCoV-17	23	SARS-CoV-2	
nCoV-18		Negative	
nCoV-19		Negative	
nCoV-20		Negative	
nCoV-21	26	SARS-CoV-2	
nCoV-22		Negative	
nCoV-23		Negative	
nCoV-24	23	SARS-CoV-2	
nCoV-25		Negative	
nCoV-26	24	SARS-CoV-2	
nCoV-27	23	SARS-CoV-2	
nCoV-28		Negative	
nCoV-29	20	SARS-CoV-2	
nCoV-30	29	SARS-CoV-2	

Will this assay generate a lot of false positives? No. Could it generate some false negatives, especially for people who have low amounts of virus and are not thought to be contagious? Yes, and that is explicitly what we aim to learn as we bring this test into the "real-world". Can a test that identifies people who are contagious be useful even if it isn't as sensitive as a diagnostic test?

6.0 STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To show we can safely, consistently, and accurately detect high levels of SARS-CoV-2 RNA in non-invasive saliva samples using a mobile laboratory.	When we have tested at least 10000 samples and consistently shown that the assay can detect SARS-CoV-2 safely, consistently and accurately at multiple locations using the mobile laboratory.

7.0 STUDY DESIGN

7.1 General Design

We have developed a simple test to detect high concentrations of SARS-CoV-2 in saliva with assays that require no specialized equipment and can be completed in one hour. This assay is not diagnostic for SARS-CoV-2; instead, it specifically identifies saliva samples that have high amounts of viral RNA that are correlated with live virus shedding. Many samples that would be identified as SARS-CoV-2 by diagnostic qRT-PCR would be negative with our saliva assay. Conversely, detection of RNA in saliva is a potential finding of clinical significance, so individuals with that result will be contacted by a physician with appropriate expertise (Dr. David Andes) to discuss potential follow-up with their own health care provider, including a possible independent diagnostic testing.

Because the assay requires no specialized equipment, it can be performed at the site of sample collection using a portable laboratory that fits in the backseat of a car. We intend to prototype testing in a variety of settings that may include parking lots, workplaces, nursing homes and residential housing. Participants will be asked to consent to provide a saliva sample that will be tested for SARS-CoV-2 RNA levels consistent with live virus shedding. The consent document will explicitly state that this is not a diagnostic test for SARS-CoV-2 but that a potential finding of clinical significance should be discussed with a medical professional. Participants can opt-out of receiving any results at the time of consent and can provide samples for the sole purpose of contributing to community surveillance and assay development. Paper consent forms will be reviewed and signed and held by team members in a secure location (locked box at the collection site) until taken back to the AIDS Vaccine Research Laboratory for storage. Participants will then spit into a coded vessel (e.g., cup or tube). Samples should be at least 1ml (~¼ teaspoon) and will be assayed for the presence of SARS-CoV-2 RNA using the aforementioned detection assay; the presence of high concentrations of SARS-CoV-2 RNA is indicated by a colorimetric change from pink to yellow/orange.

Results of all testing will be documented. The codes of the samples that indicate the presence of SARS-CoV-2 RNA will be given to Dr. David Andes who will use the key to identify those participants. He will then contact them via phone with the finding of potential significance, encourage them to isolate and contact their healthcare provider for a possible diagnostic test. No results will be communicated to the employer of the participants. Parents will be contacted directly when giving results for all minors under 18 yrs of age.

The consent form will ask for name, date, county of sample collection and phone number. Samples may be banked for future use. Participants will have the opportunity to opt out of allowing their sample to be banked. As part of the study, participants must agree to have any virus found in their sample be sequenced. Virus that is sequenced will have the date and county of collection linked to it. This information will be shared with the scientific community via publications and presentations and online databases such as GISAID that is being used to collect all HCoV19/SARS-CoV-2 sequences and track the spread of the virus globally by the research community using the sequences of the viruses and when they are appearing in communities. By consenting, the participant will also be agreeing to allow us to perform advanced molecular testing on their sample to help answer critical questions and optimize the assay. We may also share their data and results of those additional tests with the scientific community and the public through publications, presentations, open research portals or other online databases.

7.2 End of Study Definition

The study will end when we have shown that the assay can consistently detect SARS-CoV-2 at multiple locations using the mobile laboratory.

8.0 SUBJECT SELECTION

8.1 Inclusion & Exclusion Criteria

Inclusion Criteria
1. Willing to provide informed consent.
2. Willing to provide informed consent and spit into a vessel (i.e cup or tube).

3. Individuals at least 18 years old or between 5-17 years old and have a parent or legal guardian present to consent.
4. Adult subjects must have decision-making capacity to provide consent on their own behalf.
5. Subjects must be able to speak English
6. Subjects must not have visual or hearing impairments, or low literacy, that would prevent them from reading the consent form and interacting with a member of the research team to ask questions and receive responses during the consent process.

Exclusion Criteria
1. Under 18 years of age with no parent or legal guardian present or under the age of 5 yrs
2. Cannot speak English

8.2 Vulnerable Populations

This study may enroll individuals with a potential status relationship with members of the study team, because detecting SARS-CoV-2 is important for all individuals, no matter the relationship to the study team members. To minimize risks to these individuals and limit the potential for undue influence, individuals who may have a status relationship with the study team will not be directly approached to participate in this study by their supervisor. Instead, other members of the research team will contact and interact with potential participants.

Any study team member who may have a supervisory relationship or the ability to provide input regarding that individual's performance will not be involved in the informed consent process and will not have access to any directly identifiable information collected for the study about that individual.

This study will enroll children because the research is critical to show that it can be used in a school setting to help schools open safely. This presents minimal risk to the children and will help inform an important scientific question that may benefit children in the future. To further minimize risks to children, permission will be obtained from a single parent/guardian and minors between the ages of 5-17 yrs old will be given the opportunity to read and sign an assent form after having the study described to them in an age appropriate manner and given the opportunity to ask questions. Dissent from any minor will be respected.

In the first phase of testing we will focus on testing at schools before they are in session. Parents/legal guardians and students will be asked to come to the school for testing. All aspects of the testing (consent/assent, sample collection and processing) will be done on school grounds such as next to the building or in the parking lot. As schools determine their plans for reopening, we will add approval letters from the school along with their plans addressing the consenting process and students leaving the building for testing.

8.3 Subject Identification

8.3.1 Self-Identification

Potential subjects may self-identify by responding to IRB-approved recruitment efforts, such as email blasts. These email blasts may be sent to administration of local businesses and forwarded to their employees or sent directly to employees by the study team once given approval to do so by the business administration. IRB-approved screening scripts and email response templates will be utilized when communicating with potential subjects who respond to these recruitment methods. Information

collected from the potential participant is limited to protect the potential participant's privacy. Indirect recruitment materials and response communications will not contain subject health information.

8.3.2 Workplace/School Identification

Local businesses will be contacted to see if they would be interested in having their employees tested at their location. IRB-approved recruitment efforts will be used to communicate with those employees to coordinate testing.
Schools or departments of schools will be contacted to see if they would be interested in having their employees, students or family and friends of employees/students tested at their location. IRB-approved recruitment efforts will be used to communicate with the locations to coordinate testing.

8.4 Subject Recruitment

A total of up to 10000 subjects will be recruited from at least 3 sites in the Madison, Wisconsin area. The specific recruitment will be email.

1.1.1 Email

An email blast may be sent to local businesses or schools via their administration. Email blasts may also be sent to UW faculty, staff and students directly or by using the UW DoIT department process. A description of the study's purpose and participation requirements will be reviewed with potential participants via email. This includes a statement that participation is voluntary. After all questions have been answered, the study team member will ask the site administrators if there are enough individuals interested in proceeding to the next step in recruitment for the study (e.g., determining the date to review and sign the consent form and provide the saliva sample).

8.5 Early Termination and Withdrawal

Residual saliva samples not used for RNA detection may be saved and used for advanced molecular analyses (e.g., viral genome sequencing and PCR testing) with consent. Participants will be able to opt out of advanced molecular testing at the time of consent.

If participants decide to not take part in the study, or if they choose to not allow their or their child's sample to be banked for future research, the choice will not affect any treatment relationship they have with healthcare providers at UW-Madison, UW Health, their employment or class standing at the University of Wisconsin-Madison or any affiliated organizations, or any services they receive from them. No matter what decision they make, and even if the decision changes, there will be no penalty to them. They will not lose medical care or any legal rights.

The authorization for researchers to use the protected health information (PHI) does not have an end date. However:

- The participant or parent/legal guardian can choose to take back their authorization for researchers to use their health information. They can do this at any time before or during their participation in the research.
- To take back their authorization, they will need to tell the researchers by contacting the Lead Researcher, David O'Connor, at dhoconno@wisc.edu or by a written letter to UW AIDS Vaccine Research Laboratory, ATTN: David O'Connor, Science Drive, Madison, WI 53711.

9.0 STUDY AGENT (STUDY DEVICE) AND/OR PROCEDURAL INTERVENTION

9.1 Study Agent and Control Description

Investigational Assay

RT-LAMP Assay to detect SARS-C-V-2 RNA

We are requesting an IDE nonsignificant risk (NSR) determination from the IRB.

10.0 STUDY VISITS AND PROCEDURES

10.1 Consent and Enrollment

10.1.1 Informed Consent

Eligible subjects will be invited to the testing site on a designated date and time for informed consent (initial visit only) and sample collection. The informed consent process will be conducted during the first visit following all federal and institutional regulations relating to informed consent. Informed consent will be obtained prior to conducting any study-related activities.

The informed consent process will be performed as follows:

- Collection sites are being asked to provide the following: a relatively secluded approximately 10 foot x 10 foot workspace where sample processing can be performed, as well as a secondary location at least 20 feet away where volunteers can review the consent form, provide informed consent, and self-collect saliva samples. The study team will adhere to current public health guidelines when setting up testing stations.
- A study team member will review the informed consent form and discuss the study in detail with the potential research subject including any minors with their parents/legal guardians.
- A study team member will explain the study, its risks and benefits, what would be required of the research subject, and alternatives to participation.
- The research subject will be given the opportunity to read the informed consent/assent form and ask questions and have all questions answered by the study team member.
- The informed consent/assent document must be signed and dated by the research subject.
- The study team member will review the informed consent/assent document to ensure that all fields that require a response are complete (i.e., checkbox marked yes or no, etc.) as applicable. The research subject will be given the opportunity to take a picture of their signed and dated informed consent/assent form for their records. They will be given the opportunity to take a blank paper copy of the consent form as well. The original signed informed consent form is kept in a locked box while the study team is at the sample collection site and then transported to the AIDS Vaccine Research Laboratory where it will be stored in a locked filing cabinet within the locked building that has limited and controlled access
- A study team member will explain the study to minors at an age appropriate level and:
  - Minors between the ages of 15-17 years old must sign a Consent Form AND have a parent/legal guardian sign a Parental Consent Form.
  - Minors between the ages of 8-14 years must sign an Assent Form AND have a parent/legal guardian sign a Parental Consent Form.
  - Minors between the ages of 5-7 years must have the study explained to them in age appropriate language AND have a parent/legal guardian sign a Parental Consent Form.

Adults 18 yrs and older	Must sign a Consent Form.
Minors 15 yrs to 17 yrs	Must sign a Consent after having the study explained to them AND have a parent/legal guardian sign a Parental Consent Form.
Minors 8 yrs to 14 yrs	Must sign an Assent Form after having the study explained to them AND have a parent/legal guardian sign a Parental Consent Form.
Minors 5 yrs to 7 yrs	Must have the study explained to them AND have a parent/legal guardian sign a Parental Consent Form.

10.1.2 Enrollment

A research subject will be defined as "enrolled" in the study when they meet the following criteria:

- The subject has been consented by a study team member.
- The subject has provided at least one saliva sample.

Participants may provide a saliva sample as many times as they want for the duration of the study. Informed consent will only be performed once.

11.0 CORRELATIVE | SPECIAL STUDIES

11.1 Biospecimen Collection Guidelines.

We will communicate to the participants that we recommend they not eat or chew gum at least 30 minutes prior to donating the saliva sample and when possible, limit what they drink to only water in the 30 minutes prior to donating the saliva.

After consent, approximately 1ml (~¼ teaspoon) of saliva will be self-collected at room/ambient temperature in a vessel (i.e tube or cup) labeled with a code linked to the consent form/participant. Between collection and testing, the samples will be kept at room temperature. Samples will be tested within 24 hours. All samples will be transported to AVRL for proper storage and/or disposal. Samples may be banked/stored for future use if the participant has given consent to do so. If the sample will not be banked, it will be decontaminated and discarded appropriately.

The dropboxes will be lockable and portable so the entire box can be transported from the drop off locations to the processing locations if needed. We will designate specific times on specific days when the box for that location will be available for the participants to drop off samples. (i.e. Tuesdays from 8am to 11am ay WNPRC) We will work with each testing site to determine the best place to put the dropboxes so that security but also availability to the participants is ideal. For example, WNPRC's dropbox will be inside the building thereby restricting access from the public but making it available to participants from that location. The boxes for AVRL and EAGLE school will potentially be placed just outside the front doors and supervised during the drop off time. The dropboxes will remain inside behind locked doors at all other times to prevent the box from being tampered with. The dropboxes for the Athletic Department may be available in different locations on different days depending on the athletes that will be dropping off samples. The drop box will be supervised during drop off times and stored in a secure location at all other times to prevent being tampered with.

Advanced molecular testing

Advanced molecular testing, such as PCR, viral loads, viral sequencing (sequencing the virus, not any human elements in the sample), or any testing we feel will help us optimize the assay, may be performed on any samples. The following will be used by the study team and can be shared with the scientific

community and the public through modes such as scientific publications and presentations as well as open research portals and online databases, such as GISAID:

- results of the sequencing, PCR or other molecular tests
- sample collection date and location
- sample collection county

GISAID is being used to collect all SARS-CoV-2 sequences and track the spread of the virus globally by the research community using the sequences of the viruses and when they are appearing in communities.

- This activity will not help the participant directly. The results will help researchers track the spread of the virus globally.
- Results of this activity will not be relayed back to the participant or put into their medical record.

Results of this study will be shared with the public via open research portals and online dashboards.

11.2 Assay Methodology

Direct SARS-CoV-2 colorimetric RT-LAMP assays with heat-inactivated saliva samples
(Variations of this methodology and assay may be used in the optimization experiments and on-site/mobile testing.)

1) Collection of ~1ml saliva samples after informed consent
    a. An individual will spit into a pipet tip that is placed inside a 1.5ml Eppendorf tube containing a label showing the participant's assigned ID.. The saliva will drain into the Eppendorf tube.
    b. The participant will close the tube, dispose of the tip (in biohazard waste when available) and then wipe the outside of the tube with a disinfectant wipe.
    c. If the participant is at a testing site, they will place the tube containing saliva in a rack inside a cooler that contains a cold pack. If they are collecting the sample at home or another location, they will store the tube at room temperature until taken to the drop box or testing site.
    d. The participant can dispose of the disinfectant wipe in a biohazard bin (if available) or in the trash and clean their hands with sanitizer or soap and water.
    e. Samples will be collected from the cooler or designated drop off site by study team members wearing proper PPE and transported to the testing location using approved UW transport methods.

2) **Behind Plexiglass or in a biological safety cabinet (BSC):** (Wipe tubes with 10% bleach between movements)
    a. Place the tubes containing saliva in a thermomixer or dry bath at 65^{o}C and shake for 30 minutes to inactivate the virus.
    b. Transfer samples to a thermomixer or dry bath at 98^{o}C and shake if needed for 3 min to complete inactivation of virus particles and saliva nucleases.
    c. Spin the samples for 2 minutes in a benchtop centrifuge at 300 rpm.
    d. Dilute saliva into 1X PBS 1:1 and mix prior to adding to the LAMP reaction, or add saliva directly to the LAMP reaction with an equal volume of 1X PBS..

3) Once these heating steps are complete, transfer the tubes with diluted, inactivated saliva to a cooler or refrigerator prior to setting up the colorimetric RT-LAMP reactions.

a. **Beyond this point, any potential SARS-CoV-2 particles in the saliva can be considered inactivated and could be handled without the plexiglass shield or outside of a BSC.**

4) While saliva samples are being heat inactivated, prepare the appropriate volume of the following Master Mix for the colorimetric RT-LAMP reactions:

Reagent	**1X Rxn**	**9X Rxn**	**25X Rxn**	**100X Rxn**
WarmStart Colorimetric LAMP 2X Master Mix (NEB, M1800L)	10 ul	90 ul	250 ul	1000 ul
dH2O	5 ul	45 ul	125 ul	500 ul
10X LAMP primer mix (ColorLAMP N)	2 ul	18 ul	50 ul	200 ul

5) Aliquot 17 ul of colorimetric RT-LAMP reaction mix into a fresh set(s) of PCR strip-cap tubes. Prepare samples in duplicate for each saliva sample. Hold these LAMP reaction tubes in a cooler prior to adding heat-inactivated saliva samples.

6) Add 3ul of heat-inactivated saliva (that is already diluted 1:1 in 1X PB) or add 1.5 ul of heat-inactivated saliva and 1.5ul of 1x PBS to the LAMP reaction. The final four positions in each batch of LAMP reaction strip-cap tubes should be reserved for two negative and two positive controls, respectively.
   - The **negative control** reactions should contain 3 ul water/PBS.
   - The **positive contro**l reactions should include 1000 copies of SARS-CoV-2 virus that has been inactivated by heat or UV irradiation. The vRNA is prepared in a 50:50 mix of water and 1X PBS to a concentration of 333 copies/ul so that we can add 3 ul to the positive control. With 1000 copies of vRNA in the positive control, this would equate to a saliva sample concentration of 666,666 copies of SARS-CoV-2 per ml of saliva. Thus, our assay will readily detect saliva containing a concentration of 10^6 copies of SARS-CoV-2 per ml.

7) Take a picture of the strip tubes before incubating the RT-LAMP reaction.

8) Incubate the colorimetric RT-LAMP reactions in a thermomixer or dry bath at 65℃ for 30 minutes while shaking if needed.

9) Document any color changes at a 30-minute timepoint by digital photography of the RT-LAMP reactions against a prelabeled template on a white background. Color change from pink to yellow in both replicates is indicative of a sample with SARS-CoV-2 RNA present. If the color change to yellow is only clear in one of two replicate saliva samples, the sample will be scored as a potential finding of clinical significance.

10) Once a session of RT-LAMP testing is completed, disinfect all lab equipment with 10% bleach wipes followed by 70% EtOH spray. Collect all used pipet tips, plasticware, wipes, PPE, etc. in a biohazard

container and transport them back to AVRL to autoclave (if no autoclave is available at the site where samples are being processed) noting precautions for potential sharps on the autoclave bag. Saliva samples with evidence of SARS-CoV-2 RNA may be banked at AVRL at -80^{o}C if the study participant has given appropriate consent to do so.

12.0 DATA COLLECTION, HANDLING, SHARING, AND RECORD KEEPING

12.1 Data Collection

12.1.1 Data Collection Forms

Data collection is the responsibility of study team members under the supervision of the Principal Investigator (PI). The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the recorded and reported data.

Data being collected consists of the name of the person, the date and the county the sample was collected in. This information will be collected on the Consent form which will already have a participant ID associated with it. The ID will be assigned to that participant for the duration of the study. All forms must be completed in a legible manner; any missing data will be explained. Data entry errors will be corrected with a single line through the incorrect entry and the correct data is entered above/near the correction. All changes will be initialed and dated.

Data collection/consent forms are maintained in the subject files and retained as described in Section 12.3: Records Retention.

12.1.2 Data Management

The date and county of collection along with the viral sequence will be entered into spreadsheets for use in presentations, manuscripts and publications and online databases such as GISAID. The participant's name will not be put into spreadsheets or stored on any devices.

12.2 Confidentiality and Privacy

Subject confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released without consent of the participant. Participants may give consent to have the date and county of collection linked to viral sequencing done on their sample. This data will be shared through online databases, publications and presentations but because it will only be the sample collection date and county that is shared with the sequence of the virus found in their sample, there is a very minimal risk of being identified associated with the sharing of this data.

All research activities will be conducted in as private a setting as possible.

All study staff engaged in the conduct of this project have completed training on the protection of human subjects and the Health Insurance Portability and Accountability (HIPAA) Privacy Rule. In addition, all key personnel (i.e.., Principal Investigator, individuals involved in identifying/recruiting subjects, obtaining informed consent, or interacting and intervening with subjects) have undergone Good Clinical Practice (GCP) training.

Information about study subjects will be kept confidential and managed according to HIPAA requirements. All subjects will sign a combined informed consent and HIPAA authorization form that includes specific

privacy and confidentiality rights. Study data will be maintained per federal, state, and institutional data policies.

The investigator(s) will ensure that the identities of subjects are protected by using coded subject information. The key where participant's identifying information is linked to their code on the consent form will be maintained by the investigator with a copy also being held by Dr. David Andes or a physician with appropriate expertise on the study team. The key and all study records maintained at AVRL will be in a locked building with restricted access within a locked cabinet. A copy of the key will be kept by Dr. David Andes or a physician with appropriate expertise on the study team and will be kept in a locked room or cabinet when not in use. The key will remain a hard copy vs electronic. Electronic study files containing the participant's code, date and county of collection and the viral sequences will be stored on the School of Medicine and Public Health, Department of Pathology and Laboratory Medicine servers and accessed via networked computers that are password-protected with access provided only to authorized study personnel. Consent forms will remain in paper form only.

Authorized representatives of the following groups may need to review this research as part of their responsibilities to protect research subjects: representatives of the IRB, regulatory agencies and federal oversight agencies, such as the Food and Drug Administration (FDA). The study team will permit access to such records.

Study staff may use email to communicate with site administrators, research subjects or their parents/legal guardians prior to consenting but information contained in the emails will be limited to answers to general questions about the study or confirm testing dates and times. All emails to subjects will be sent from UW/wisc.edu accounts; personal, home or Gmail email accounts will not be used.

To further protect the privacy of study subjects, a Certificate of Confidentiality has been obtained. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research subjects, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping to assure confidentiality and privacy to subjects.

12.3 Records Retention

It is the investigator's responsibility to retain study essential documents for a minimum period of 7 years following completion of the study per UW-Madison institutional policy, or at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product, whichever comes last.

12.4 Retention for Future Research: Data & Biospecimen Banking

12.4.1 Purpose of Storage

Samples, linked data (including only date, location and county of collection) and viral sequences will be stored for future research projects potentially related to saliva sample and viral sequencing techniques.

12.4.2 Data and/or Biospecimens Being Stored

Data and viral sequence will be retained and linked to the sample being stored.

12.4.3 Location of Storage

The samples will be stored at approx -80°C at AVRL or a designated departmental storage site on the UW campus for biospecimens of this nature.

12.4.4 Duration of Storage

The samples will be stored for the duration of the study or up to 7 years. Data associated with the samples and viral sequencing will be kept indefinitely.

12.4.5 Access to Data or Biospecimens and Security Measures

All members of the study team at the UW-Madison will have access to the consent forms, data, samples and viral sequences.

12.4.6 Procedures to Release Data or Biospecimens

Participants will consent to having their data (not their name) and viral sequences shared with the public via manuscripts, presentations and online databases therefore the study team will share that with other researchers when needed. Samples can be shared with UW researchers via the usual routes and non-UW researchers through the Material Transfer Agreement process.

12.4.7 Process for Returning Results

The study team will contact Dr. David Andes and let him know the code of the samples that had a potential finding of clinical significance and he will use the key to contact the corresponding participant. If Dr. Andes has not received the key yet for the testing done that day, a member of the study team (that is not in a supervisory role to the participants) will give him the names and phone numbers of the individuals (or their parent/legal guardian) that had a potential finding of clinical significance over the phone. Dr. Andes will contact the participant with the results and reiterate that the test is investigational and the results are not diagnostic. He will strongly recommend that the participant obtain a clinical diagnostic test for COVID-19 and practice self-isolation until the results of the test are received. Dr. Andes will also provide information about testing options.

12.4.8 Process for Tracking Subject Consent and Authorization

The study team will create a spreadsheet to document which samples and data are allowed to be shared.

12.5 Protocol Deviations

A protocol deviation is any noncompliance with the protocol. The noncompliance may be either on the part of the subject or the investigator. As a result of deviations, corrective actions may be implemented.

It is the responsibility of the Principal Investigator/study team to use continuous vigilance to identify and report deviations. The Principal Investigator is responsible for assessing whether the deviation constitutes noncompliance as defined by the reviewing IRB and if so, reporting it in accordance with posted guidance. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

12.6 Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central (https://www.ncbi.nlm.nih.gov/pmc/) upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

Data from this study may be requested from other researchers 7 years after the completion of the primary endpoint by contacting David O'Connor at the University of Wisconsin - Madison.

13.0 STUDY ANALYSIS

13.1 Statistical Hypotheses

- **Primary Efficacy Endpoint(s):**
  This is not a comparative study so there is not a formal, testable null hypothesis, per se. The purpose of the study is to determine if rapid, onsite testing for SARS-CoV-2 can be performed. Tests with potential findings of clinical significance using the RT-LAMP assay will be referred to clinicians for follow-up, but we are not privy to the results of follow-up diagnostic tests that participants may undertake in consultation with their healthcare provider.

13.2 Sample Size Justification

The key question of whether we can establish rapid, on-site testing and identify issues that would challenge scale-up and expansion to additional sites will benefit from testing as many individuals as possible. We anticipate that a single test site will initially be able to process up to 200 samples a day, growing to up to 1,000 samples a day as workflows are optimized and improved.

13.3 Subject Population(s) for Analysis

We will analyze data from all individuals who consent to receive RT-LAMP testing. All results will be used in analyses, unless they are excluded due to technical or quality control issues (e.g., samples are incorrectly pipetted or there is evidence of cross-contamination).

13.4 Statistical Methods

As noted above, our intention is to deploy and scale testing, so statistical methods aren't germane; there is no comparator group.

14.0 RISK/BENEFIT ASSESSMENT

14.1 Potential Benefits to the Subjects

There will be no direct health benefit to participants.

Conventional SARS-CoV-2 testing is uncomfortable and has a long lag time between testing and results reporting. The benefits to society are that if we can show that onsite testing is possible then more people can be tested, more conveniently, and with more rapid results dissemination than is possible today.

If more places such as assisted living residences, dorms and work places have access to this type of testing then more people who have COVID-19 are able to self-isolate and fewer people will come in contact with them and become infected themselves.

14.2 Known Potential Risks

There are no risks associated with sample collection. There could be some psychological stress associated with receiving a potential finding of clinical significance. Individuals with a potential finding of clinical

significance may be asked by medical professionals to self-isolate while diagnostic testing is performed and processed which could create additional psychological stress.

The consent documentation clarifies that the rapid COVID-19 test is experimental and not allowed to be used by a healthcare provider. The participants are encouraged to get a clinical diagnostic test to confirm any results of potential findings of clinical significance because we do not know yet if having that result means people are immune or protected from getting COVID-19 again. Additionally, a negative result does not mean that the participant does not have COVID-19 or the infection that causes it therefore they are being told to continue to adhere to local guidelines.

14.3 Risk/Benefit Analysis

The benefit of getting this test and impacting the spread of COVID-19 outweighs the potential short-term stress associated with getting a result of a potential finding of clinical significance and talking to a physician with appropriate expertise about getting a diagnostic test.

15.0 DATA AND SAFETY MONITORING

15.1 Unanticipated Problems

An unanticipated problem (UP), as defined by the DHHS Office for Human Research Protection (OHRP), is any incident, experience, or outcome that meets all of the following criteria:

- The incidence, experience, or outcome is unexpected given the research procedures described in protocol-related documents (e.g., the study protocol, the informed consent documents) and the characteristics of the subject population being studied.
- An event may be considered unexpected if it exceeds the nature, severity, or frequency described in the study-related documents.
- The incidence, experience, or outcome is related or probably related to participation in the research study. "Probably related" means the incidence, experience, or outcome is more likely than not to be caused by the research study procedures.
- The occurrence of the incidence, experience, or outcome suggests that the research places subjects or others at a greater risk of harm (physical, psychological, economic, or social) than was previously known or recognized.

The investigator will report UPs to the reviewing IRB. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol, informed consent documents, or other corrective actions that have been taken or are proposed in response to the UP.

UPs will be reported within 14 days in accordance with posted guidance.

15.2 Protocol Deviations

Refer to section 12.5.

15.3 Study Stopping Rules

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided promptly by the PI to the IRB and will include the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:
- Determination of unexpected, significant, or unacceptable risk to subjects
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns are addressed. All data and samples collected to that point will be managed as described in previous sections.

16.0 STUDY FEASIBILITY

16.1 Facilities and Locations

The goal is to secure multiple sites in the Madison area to test the assay. We are asking these sites to provide a relatively secluded approximately 10 foot x 10 foot workspace where testing can be performed, as well as a secondary location at least 20 feet away where study team members and participants can review consent paperwork, provide written consent, and self-collect saliva samples. We will have a team of 2-4 people to set up the mobile laboratory, perform testing, and then clean up.

We will also have locked sample drop boxes at designated locations for study participants to drop their labeled samples. Only study team members will have access to the drop box keys/code. A study team member will pick up samples on a daily basis. Samples will be transported using approved biological transport methods to AVRL or other approved sample processing locations.

16.2 Feasibility of Recruiting the Required Number of Subjects

Several sites both on and off the University campus have shown interest in participating in this study on a regular weekly or daily basis. Each site has significant numbers of people that want to participate. The goal of testing 200 to 1000 samples a day is feasible based on this information.

16.3 Principal Investigator Considerations

16.3.1 Time Devoted to Conducting the Research

The Principal Investigators have assembled a trained study team that will assist with this study so that they do not need to be present during all testing.

16.3.2 Process for Informing Study Teams

All study team members are required to read the IRB approval, Study Protocol, Consent forms and any other documentation or institutional required training prior to beginning the study.

16.4 Availability of Medical or Psychological Resources

All participants with a potential finding of clinical significance will have access to Dr. David Andes' expertise and knowledge of additional medical and psychological resources.

17.0 APPENDICES

Rapid, onsite COVID-19 detection

Protocol Number: 2020-0855
Principal Investigator: David O'Connor
NCT04460690

There was no statistical Analysis plan for this study.