CLINICAL STUDY PROTOCOL

Investigational Device

Validation of a rapid quantitative test for loss of smell in COVID-19 subjects

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Confidentiality Statement:

Confidential - Not to be circulated outside the context of the IRB. Unique attributes of the device are considered confidential.

REVISION HISTORY

Revision #	Version Date
2 (pre-IRB panel)	May 20th, 2020
3 (pre-IRB panel: transfer of PI)	June 1st, 2020
4	June 5, 2000
5	June 8, 2020
6	July 1, 2020

Synopsis

Primary Objective The primary objective of this study is to validate the utility (sensitivity, specificity and accuracy) of a quantitative non-biased olfactory device for the rapid identification of SARS-CoV-2 infected subjects.
Secondary Objectives: The secondary objective is to test if SARS-CoV-2 positive 'asymptomatic' COVID-19 subjects may actually present with a mild defect in smell (hyposmia), which is revealed through our quantitative olfactory smell test.
Study Duration: The expected duration of this study is 3 to 6 weeks.
Study Design: This is a non-therapeutic correlative trial. Loss of smell in test subjects will be compared to the presence of SARS-CoV-2 coronavirus, as determined by RT-PCR.
Study Population: This study is oriented towards the average US adult population and is limited to anyone over 18 years old and excludes pregnant women. The study will focus on two cohorts: 'drive-thru' outpatients and people at high-risk of being infected with coronavirus (healthcare workers that work in a nursing home or at the Yale Hospital COVID-19 ICU).

Number of Participants: ~100 SARS-CoV-2 positive outpatient subjects and ~250 total asymptomatic Health Care Workers. An equal or greater age and gender matching group of SARS-CoV-2 negative subjects will be included as a comparison group.

Number of Study Sites

The proposed study sites would be:

Outpatients:

- Drive Up Coronavirus PCR testing
  - Greenwich Hospital , Greenwich, CT
  - Central Louisiana Department of Public Health

Asymptomatics:

- Waveny LifeCare Nursing Home in New Canaan, CT
  - Point Pleasant Nursing Home, Bradeton, Florida
  - Westminster Winter Park/Baldwin Park Nursing Home

Primary Outcome Variables

The subject's score on using the olfactory device will be compared to the PCR results (**SARS-CoV-2 negative or positive)** on COVID19 outpatients. As the smell test has a variable scale (0-5) the ideal cutoff will be determined to maximize these factors:

- Sensitivity
- Specificity
- Accuracy

The score with the olfactory device will also be compared to subjects' response to the question "Do you have a new loss of smell or taste?" (current standard). Repeatability will also be examined in a test-retest score conducted within 24 hours with a second device in which the sequence of the odorants is altered.

Secondary and Exploratory Outcome Variables (if applicable)

We will determine if 'asymptomatic' SARS-CoV-2 positive subjects experience a partial loss of smell (hyposmia) and if so determine the fraction of subjects in which this occurs. Sensitivity, specificity and accuracy will be measured.

Visit Schedule Table (Optional). N.A.

Study Flow Chart (optional). N.A.

Abbreviations

Abbreviation	Explanation
HCW	Health Care Worker
UPSIT	University of Pennsylvania Smell Identification Test
COVID-19- or COVID-19+	Negative for COVID-19 virus (as determined by RT-PCR) or Positive for COVID-19 virus (as determined by RT-PCR)
ICU	Intensive Care Unit
IFRA	International Fragrance Association
CPSIA	Consumer Product Safety Information Act

Glossary of Terms

Glossary	Explanation
Anosmia	Full loss of ability to smell odors
Hyposmia (aka. Microsmia)	Partial loss of ability to smell odors
Normosmia	Normal olfactory function and ability to smell odors (most people)
COVID-19	Disease associated with the SARS-CoV-2 coronavirus

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1 Introduction

1.1 Introductory Statement

This document is a protocol to evaluate in a non-therapeutic correlative human research study the performance of a non-invasive olfactory device as an indicator of loss of smell, and an initial indicator of COVID-19 positive subjects. The purpose of this protocol is to ensure that this study is to be conducted according to ICH GCP guidelines, CFR 21 Part 812, applicable government regulations and Institutional research policies and procedures.

2 Background

2.1.1 Device Preclinical Experience

A common assay to detect anosmia (loss of smell) is the use of 'scratch-n-sniff'™ type of scent cards, as in the long establish University of Pennsylvania SIT (UPSIT) olfactory test (Doty et al, Laryngoscope, 1984, 94, 176-8) . Here microencapsulated non-toxic scents are scratched by the test subject, sniffed and in a discriminatory forced-choice manner the subject chooses the best matching scent choice as part of a multiple choice exam. Typically this is a long format taking 10-15 minutes and is optimized to minimize malingering. There are also shorter format tests. This UPSIT type of test is recognized by the FDA for general anosmia and in the field of use of neurological diseases (e.g. Parkinson's and Alzheimer's Disease). However, the UPSIT test is long, expensive and non-variable, neither of which makes it suitable for widescale or repeated testing. Dr. Toomre designed and had manufactured a new design of olfactory test card (*u-Smell-it*™) that could be deployed widescale. These 'scratch and sniff' ™ type devices are deemed safe by the FDA as they are non-invasive and use non-toxic fragrance. The device card proposed here uses the same fragrance and microencapsulation process as used in the NIH Toolbox® tests for loss of smell. Further the fragrances used in the *u-Smell-it*™ test kit are compliant with IFRA and its components do not contain oils derived from nuts, wheat or glutens and do not use any phthalates or their derivatives. These formulations are in widespread use in children's books and cards for over 40 years, as well as other consumer markets. Further, the FDA has approved of use of a 'scratch-n-sniff'™ for anosmia. As described below there is now strong mounting evidence for the association of anosmia and COVID-19, albeit largely through patient surveys (see Background 2.2).

2.1.2 Device Clinical Experience

A similar style 'scratch and sniff' assay that use 40 odorants (as a 40 card booklet) was recently shown in a study in Iran to identify smell dysfunction in COVID-19 positive inpatients (Moein et al, Int Forum Allergy Rhinol, 2020; PMID: 32301284). Our quick 4-5 single card odorant '*u-Smell-it'* device uses similar microencapsulated scents that are manufactured in an identical process on a single card. It is optimized for speed, cost, and maximizing signal-to-noise in the analysis, and ease of use. The device is very quick to use, taking <60 seconds and our forced choice test has four to five odor options and a "no scent" option that serves to optimize the signal/noise of the assay in a small fast format, as both points are essential for widespread testing. Thus, there is evidence that quantitative testing has value and the Moein study and numerous reports suggest that acute loss of smell (hyposmia) cannot be reliably self-reported and instead needs a quantitative blinded test, as proposed here.

2.2 Background/prevalence of research topic

There is an urgent need for rapid testing on a massive scale (millions of COVID-19 tests/week) and longitudinal testing of high risk people (e.g. HCWs) to identify

COVID-19 positive people. PCR based testing is the gold-standard, but it is estimated that up to 50-times more tests per week will be needed than is currently achieved. The key hypothesis underlying this clinical trial is that loss of smell (anosmia) may analogous to a 'canary-in-a-coalmine' and serve as an indicator of being infected with the COVID-19 coronavirus.

In support of this hypothesis emerging evidence has come from a number of different countries and includes the following studies (only some are fully peer reviewed):

- Anecdotal evidence suggests loss of smell (and taste) in COVID-19+ patients and may be an early onset factor (*1*)
- A paper from 12 European hospitals in 6 countries showed that 85% of COVID-19+ people reported loss of smell (anosmia); this paper was based on patient surveys and self-reports of smell loss. (*2*)
- In an European self-report survey of COVID-19 positive patients 70% reported anosmia and 45% reported fever. Thus anosmia is a better single indicator than fever for COVID-19. (*3*)
- In contrast, in a Korean a self-report survey of 3,191 COVID-19 positive patients only 15% reported anosmia, with a mean recovery of 7 days (*4*).
- A US study showed that smell loss was 10x more likely to be from COVID-19 than other causes; this was based on patient surveys and self-reports of smell loss. (*5, 6*)
- A study in Iran using olfactory testing showed that 58% of COVID-19+ inpatient had anosmia/severe microsmia and 27% had moderate microsmia. 98% of COVID-19+ inpatients had some type of olfactory dysfunction (*7*).
- A paper showed that loss-of-smell was a good indicator of being positive for COVID-19 if one eliminates loss of smell associated with a stuffy or runny nose. Unlike other viruses such as influenza and the common coronavirus that also present with loss of smell (*8*), COVID-19+ patients have loss of smell with no sinus blockage. This supports that loss of smell in COVID-19 patient is a different mechanism that in other viral diseases and may be a good diagnostic indicator.
- SARS-CoV-2 entry factors (ACE2 and TMPRSS2) are highly expressed in nasal cavity and localize to multiciliated cells.
- New loss of taste or smell was recently recognized by the CDC (but not yet WHO) to be a symptom associated with COVID-19.
- Olfactory Dysfunction (OD) is present in ~20-86% of COVID-19 patients and is frequently an early symptom. (Speth, Thirza et al, In Press)
- Most studies of an association of COVID-19 and anosmia were retrospective and few addressed smell reduction (hyposmia) and mainly considered hospitalized patients, which can have selection bias (*9*).

In summary, although there is strong support of an association of loss of smell with COVID-19, the degree to which this occurs is variable and may in a large part reflect that nearly all of the studies were retrospective patient surveys, without any direct

olfactory testing. Direct olfactory tests may be especially important as evidence supports that acute loss of function is not noticeable by the subject (7), but we predict may be detected with our device. Further an open important question is do asymptomatic COVID-19+ (PCR) people actually have a mild symptom of olfactory loss that could be detected? If so they could self-isolate and avoid virus transmission to others and potentially receive earlier treatment with anti-viral drugs.

References:

1. J. F. Gautier, Y. Ravussin, A New Symptom of COVID-19: Loss of Taste and Smell. *Obesity (Silver Spring)* **28**, 848 (2020).
2. J. R. Lechien *et al.*, Olfactory and gustatory dysfunctions as a clinical presentation of mild-to-moderate forms of the coronavirus disease (COVID-19): a multicenter European study. *Eur Arch Otorhinolaryngol*, (2020).
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7. S. T. Moein *et al.*, Smell dysfunction: a biomarker for COVID-19. *Int Forum Allergy Rhinol*, (2020).
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3 Rationale/Significance

3.1 Problem Statement

A major problem is the loss of olfaction has been identified as a key symptom of COVID-19, yet the degree to which this really occurs is unclear as most reports are potentially biased, not well controlled, and based on surveys. Here we seek to validate this olfactory device and robustly determine its usefulness in terms of sensitivity, specificity, accuracy and repeatability. Of particular relevance to its sensitivity we seek to identify if COVID-19+ asymptomatic individuals in fact have a mild defect in smell, that can only be identified in a sensitive and objective test, as proposed here.

3.2 Purpose of Study/Potential Impact

We posit that most (or even perhaps nearly all) COVID-19+ individuals have a transitory (~7 day) early loss of smell. If this is true and could be robustly and rapidly measured in a device that could be deployed at large scale repeatedly then this could be ultimately used for a massive pre-testing for COVID-19. If this holds true then this olfactory device could outperform PCR tests in the following areas:

Area	PCR	*'u-Smell-it'* Device
Cost per test	~$35 (with labor)	<$1
Time per test	10 min to 5 days	<1 min
Danger to HCW	Considerable for Nasal Swabs (less for saliva)	None; self-performed
Deployment: #/day in USA	~300,000 currently	> ~1-3 million
False Negative Rate	~15-30%	TBD here
False Positive Rate	<5%	TBD here

If the device performs well it ultimately would be broadly deployed as an initial indicator test to identify people likely infected with SARS-CoV-2 that would be confirmed or refuted by secondary PCR screening. It could also serve as a tool to identify local outbreaks of COVID-19.

3.2.1 Potential Risks

This proposed study poses virtually no risks to the test subjects as it is a non-invasive test with materials that have been used clinical extensively. Firstly, these 'scratch-n-sniff'™ style scent cards use the same odorants have been heavily used in FDA approved studies of anosmia and are part of the NIH toolkit© and meet fragrance safety compliance. A very minor risk of a potential adverse reaction to scents may exist for asthmatics and people with scent allergic and thus these people will be excluded. Their condition will be identified by patient self-report. This study will not prescribe any action to the participant so there is no risk of interpretation.

The only other risk is that the test subject if COVID+ could contaminate the answer form with live virus, and this should be treated as a biohazard. To avoid this risk the consent and answer form will be photographed while the subject is in the car through the closed window (they will merely hold it up) but not showing the full face of the subject so as to hide their identity. Only information needed to interpret the results of the study will be transmitted. Using these digital/electronic consent measures eliminates potential contamination of HCW during the reporting due to the COVID-19 virus. As kindly noted in discussions with Alan Teller the YCCI have a new coronavirus guidance allowing informed consent electronically. In the consent document to the participating subject the addition of an electronic consent in

lieu of a printed document is now explicitly discussed and in so signing the subject would opt in for electronic/digital mediated consent.

3.2.2 Potential Benefits

The major benefit to society would be to potentially provide a robust indicator test for COVID-19 that could be rapidly employed at a large scale at an order of magnitude greater daily testing than currently possible - so as to curtail viral transmission and rapidly identify new outbreaks. While the scaling of the device is feasible, the key open question is its performance characteristics, which this study seeks to address. It also seeks to identify if seemingly asymptomatic may have a minor or transitory symptom of loss of smell, that the test would reveal.

4 Study Objectives

4.1 Hypothesis

The major hypothesis is that a quantitative and unbiased smell test will be a useful tool to identify COVID-19 positive individuals.

The study will address what fraction of outpatients truly have a loss-of-smell (including a partial loss) and is expected to outperform the current question that is used to identify COVID-19 related anosmia “Do you have a new loss of smell or taste?” (yes/no) in terms of sensitivity and specificity.

The study will address if high-risk asymptomatic people whom are SARS-CoV-2 positive have a partial (or perhaps transitory) loss of smell.

4.2 Primary Objective

The primary objective of this study is to validate the utility (sensitivity, specificity and accuracy) of a quantitative non-biased olfactory device for the rapid identification of SARS-CoV-2 infected subjects (as identified by PCR). The performance of the device will also be compared to the standard CDC patient query for ‘new loss of smell or taste’.

Secondary Objectives

The secondary objective is to test if SARS-CoV-2 positive ‘asymptomatic’ COVID-19 subjects may actually present with a mild or transitory defect in smell (hyposmia), which is revealed through our quantitative olfactory smell test.

5 Study Design

5.1 General Design Description

This is a non-therapeutic correlative trial. Loss of smell in test subjects will be compared to the presence of SARS-CoV-2 coronavirus and statistically measured.

The study will focus on two cohorts: 'drive-thru' outpatients within the Yale New Haven Health System and in Central Louisiana and people at high-risk of being infected with coronavirus (New Canaan and Florida nursing homes) These sites are chosen as they have a significant incidence of COVID-19, they have on-going PCR testing, and they will provide support staff with experts in ENT to implement these studies.

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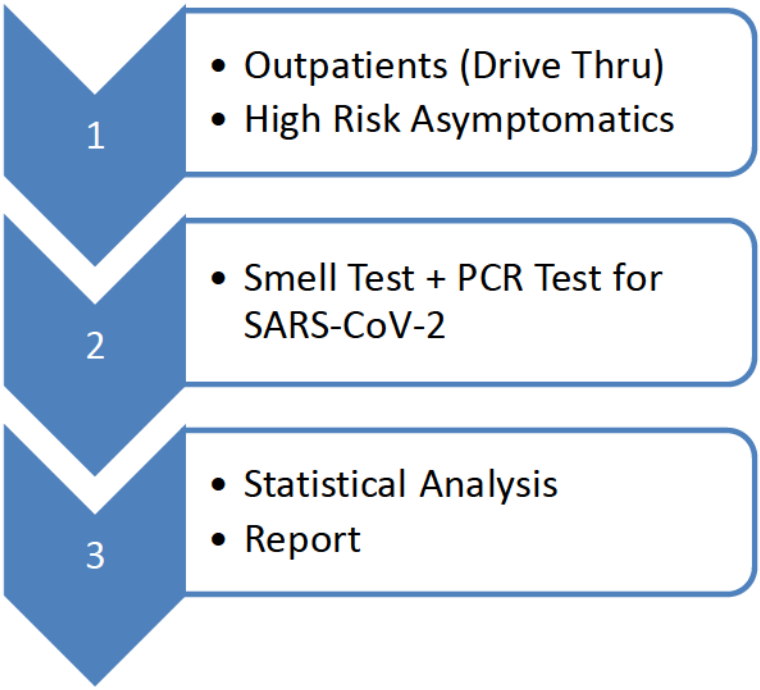
The tests are double blinded as neither the subject or administering staff/doctor will know the results. The COVID-19 outpatients will be randomly recruited with the only preselection being the elimination of pregnant women, those with pre-existing anosmia, head injury, scent allergies, asthma or a self-reported stuffy/runny nose. These conditions will be identified by patient self-report.

The key features of the study design are:



5.1.1 Study Date Range and Duration

There is a pressing need to begin this study as soon as possible due to the US health and economic crisis. We are ready to begin the clinical trial immediately after the IRB is approved as all the material needed for the test is in hand.

Namely, all the initial materials to begin the study are in hand, including the smell device card, bags, and golf pencils. In discussion with CT **Senator Kasser** whom represents Fairfield County (Greenwich & North Stamford) Greenwich Hospital (a Yale New Haven Health System affiliate) was identified as a good site for the drive through outpatient testing. Yale New Haven Hospital is also an ideal site given the volume of testing. The Nursing home of Waveny Life Care in New Canaan as an appropriate site for asymptomatic subjects, for they have done widescale PCR screening and they have at least 30 asymptomatic COVID+ people and staff. The President and CEO of Waveny (**Russell R. Barksdale, Jr., MPA/MHA, FACHE)** is eager to begin as soon as possible and will complete PCR tests of all subjects and provide nurses to assist with administration of the test. They also have ~250 untested employees and thus have a young high-risk cohort and would be eager to test asymptomatics. We expect that the initial testing will be done in < 2 weeks (depending on the local incidence rate), with all completed studies taking as little as 3 weeks, with a maximum estimated duration of ~6 weeks. These studies could begin almost immediately after approval from Yale's IRB.

5.1.2 Number of Study Sites

The planned study sites for the drive-up testing would be Greenwich Hospital, Yale-New Haven Hospital and the Central Louisiana Department of Public Health. The asymptomatic study would occur at the Florida based nursing homes and Waveny Life Care nursing home in New Canaan (the President and CEO of this center, **Russell R. Barksdale**, strongly supports this study and will provide corresponding PCR testing and support staff). These are the principle sites, outside sites would be considered as long as any testing would be confirmed by PCR and meet all conditions prescribed herein.

5.2 Outcome Variables

5.2.1 Primary Outcome Variables

The subject's score on using the olfactory device will be compared to the PCR results (**SARS-CoV-2 negative or positive)** on COVID19 outpatients. As the '*u-Smell it'* test has a variable scale (0-5) the ideal cutoff will be determined to maximize any one (or combination) of these factors:

- Sensitivity

- Specificity
- Accuracy

We will compare the performance of the smell test to the current CDC metric of self-described assessment of smell loss (the de facto standard – e.g. “I have a new loss of smell or taste”) in terms of sensitivity, specificity and accuracy. Repeatability will also be examined in a test-retest score conducted within 24 hours in a random subset of 25 asymptomatic HCW with a second device in which the order of the odorants is altered.

5.2.2 Secondary and Exploratory Outcome Variables (if applicable)

We will determine if ‘asymptomatic’ SARS-CoV-2 positive subjects experience a partial or transitory loss of smell (hyposmia) and if so determine the fraction of subjects in which this occurs. Sensitivity, specificity and accuracy will be measured.

5.3 Study Population

This study is oriented towards the average US adult population and is limited to anyone over 18 years old (and excludes pregnant women). The goal is to be wide reaching and minimize selection bias of choosing outpatients. The study will focus on two cohorts: ‘drive-thru’ outpatients with minor COVID-19 symptoms (enough to merit a test) and healthy volunteer asymptomatic adults at high-risk of being infected with coronavirus. We will exclude pregnant women, those with pre-existing anosmia, head injury, scent allergies, asthma or a self-reported stuffy/runny nose. These conditions will be identified by patient self-report.

We will also exclude vulnerable patients with dementia as they are less likely to understand the test. They will be identified by asking if they usually make their own decisions about their medical care and sign for themselves; if not, then they would be excluded.

5.3.1 Number of Participants

Upon consultation with Yale biostatisticians Profs. James Dziura and Denise Esserman, based on our sampling they calculated the following table to show the precision of sensitivity/specificity measurements for different sample sizes (**Table 1**). For example, the 95% confidence interval for 90% sensitivity with 100 subjects ranges from 82 to 95% , but if we have 400 subjects, the confidence interval ranges from 87 to 93% (about +/- 3%). Thus the number of participants will be guided by statistical analysis of the data. A minimal estimate is ~100 outpatients that are SARS-Co-V2 positive so as to have good confidence

intervals. Here we presume to have at least 100 age/gender matched SARS-Co-V2 negative subjects. If there are 3:1 SARS-Co-V2 negative to SARS-Co-V2 positive then ~400 outpatients (300 +100) will be tested. For the more exploratory testing with asymptomatics

	Sample Size for Denominator*						
Sens/Spec	**100**	**150**	**200**	**250**	**300**	**350**	**400**
0.50	0.20	0.17	0.14	0.13	0.12	0.11	0.10
0.60	0.20	0.16	0.14	0.13	0.11	0.11	0.10
0.70	0.19	0.15	0.13	0.12	0.11	0.10	0.09
0.80	0.17	0.13	0.12	0.10	0.09	0.09	0.08
0.90	0.13	0.10	0.09	0.08	0.07	0.07	0.06

we will limit the study to ~100 COVID-19 positive subjects and a similar number of SARS-Co-V2 negative subjects.

Table 1: Sample Size Calculations (Width of a 95% confidence interval around the proportion).

*Denominator is those that are COVID+ for sensitivity and those that are COVID- for specificity

5.3.2 Eligibility Criteria/Vulnerable Populations

Inclusion: In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or female, aged 18 or greater
4. Have a corresponding PCR test for SARS-CoV-2 on the same day.

Exclusion: An individual who meets any of the following criteria in either study population will be excluded from participation in this study (as this may cause unnecessary risk to the test subject or confound the results if the subject has pre-existing anosmia).

1. Adults unable to consent
2. Individuals who are not yet adults (infants, children, teenagers)
3. Pregnant women
4. Individuals with allergic to fragrances
5. Asthmatics

6. Patients with known neurocognitive disorders: dementia, Alzheimer's disease, Parkinson's disease
7. Adults with Acute or Chronic rhinosinusitis (They will be asked if they have a ‘stuffy or runny nose’; they may be included in some tests, but their analysis would be segregated as they may be false positives due to allergies or a common cold or flu)

6 Methods

6.1 Treatment – Device

6.1.1 Intended Use for Device

The intended use of the 'u-Smell-it'™ olfactory test device is to in a quantitative, fast and repeated manner rapidly determine if an individual has a loss of smell and if so, based on the score and the precision of the device determine the likelihood that s/he is at risk of being positive for SARS-CoV-2. This result would in turn serve as a quantitative indicator to guide judicious action (analogous to how temperature is used to truly identify a fever vs. patient self-identification) – e.g. a follow up PCR test. The comparative gold standard (most used) is the question of if the person believes that s/he has "a new loss of smell or taste", as currently stipulated by the CDC (whether performed in a written or electronic questionnaire). While there are other smell based test kits they are either very long (10-15 min) and tedious (UPSIT) and will not be suitable for wide-scale rapid testing, or for the rapid 3-item SIT are non-variable, and as such will not be a blinded test, especially for repeated testing. Thus, we will compare the performance of our *u-Smell-it* test to the user-identified stated "new loss of smell/taste" – and anticipate a real unbiased test to greatly outperform a highly subjective assessment. A related underlying hypothesis that we seek to test in this trial is that COVID19+ people perform poorly in self-assessments and grossly underestimate their loss of smell. If this premise is validated AND the performance of the device is statistically high (especially in terms of sensitivity) then one would seek FDA approval under emergency authorization use (EUA). If this eventually clears the FDA then it would be used in wide scale US testing at the state and federal level.

6.1.2 Device Administration and Schedule

The device requires minimal preparation. The only key aspect is to not inadvertently damage it and as such would be given in a sealed pouch with a 'golf pencil', consent form, instructions and multiple choice answer sheet.

Patients who are in queue for drive-through COVID-19 testing will be approached by a research team member. No prior medical information will be provided to the study team regarding a patient's eligibility.

The device in a pouch would be given to drive up outpatients that are undergoing PCR testing for COVID. Typically, the device packet would be placed on the test subject's windshield just as they drive in and are waiting. If the subject consents to take the test, it will only take a minute. They will have the smell test and consent form digitally recorded (by camera) as they hold the test answers against the closed windows while inside the car. They will then give a nasal or oral sample for PCR testing, which is not conducted as part of the study. Of note, the subjects are already undergoing PCR testing and the smell study merely piggybacks on this other scheduled test. Thus there is practically no extra burden.

For the asymptomatic test the same protocol will be used, with the only minor difference is that the test can be replicated multiple times with different variants. As these tests are done in presence of a HCW there is little issue of compliance.

6.1.3 Method of Assignment/Randomization (if applicable)

All drive-through subjects that meet the inclusion/exclusion requirements will be tested.

6.1.4 Device Calibration

The is no gold standard for calibration as every individual responds to odors somewhat differently. But in a healthy population (COVID-19 negative) we expect to get a statistical distribution of results. We will ensure that over time for the control group that the performance of the device is similar and repeat test a random subset of individuals.

6.1.5 Storage Conditions

The device can be stored at ambient temperature (5-40 deg. C) for up to 6 months with negligible loss of performance. Attention should be given to not accidently scratch the device as this could release some scent. The device should also not get wet. Thus individual devices or bundles thereof will be stored in sealed pouches.

6.1.6 Concomitant therapy

No restrictions at this stage.

6.1.7 Restrictions

No restrictions other than the patient selection criteria.

6.2 Assessments

No guidance available.

6.2.1 Efficacy

The olfactory test enabled from the device will be compared to:

- Patient self-identification for loss of smell: “Do you have any new loss of smell or taste?” YES or NO.
- PCR results for the presence of SARS-CoV-2

The methods for the assessment will be statistical, as described above.

6.2.2 Safety/Pregnancy-related policy

There is minimal safety issues regarding the device EXCEPT the consideration that it may be contaminated by SARS-CoV2 and thus should be treated as hazardous wasted. This will be mitigated by having no direct contact with the test material as it will be photographed.

Pregnancy status will be sepf reported and any pregnant patients will be excluded from the study.

6.2.3 Adverse Events Definition and Reporting

Based on extensive use with scratch-and-sniff™ cards without adverse effects such events would be considered unlikely, nevertheless should they occur they will be immediately reported to the P. I. (Dr. Manes). Also the test is fast and non-invasive – highly limiting adverse events.

6.2.4 Pharmacokinetics (if applicable)

None

6.2.5 Biomarkers (if applicable)

The outpatients are already scheduled for RT-PCR testing of SARS-CoV2 which will be typically collected by a swab to the oral/nasal cavity and performed under conditions as mandated by the CDC and PCR test's manufacture directions.

6.3 Study Procedures

For the outpatient drive-up studies the following protocol steps will be performed.

1. As patients drive-up and are still in the car with the windows closed a HCW with suitable PPE will place a ziplock pouch under the wiper of the driver's side windshield. The bag will contain the following materials:
    a. A consent form
    b. A pre-sharpened 'golf-pencil'
    c. The 'u-Smell-it' olfactory device test card
    d. A multiple choice answer form
2. While in the car, if the patient consents, they will sign the consent form, read the simple instructions (which are also shown as pictograms to make it very easy to follow), take the test, and fill in the answers. This process will take about 1 minute.
    a. Description of the test: The test card device has 5 windows each printed with a different scent (or no scent) and marked #1, 2, 3 ,4, and 5. The test subject merely needs to use their pencil to scratch back and forth 3 times the first window, sniff the window, and looking at the answer score sheet pick the best choice of five choices and fill in the multiple choice circle ("no scent", and scent A, B, C or D). For instance this could be: no scent / apple / orange /

cherry /banana / rose. This procedure will be repeated for each of the 5 windows.

b. The subject will also be asked on the test if:
    i. "Do you have any new loss of taste or smell?" (Yes/No)
    ii. "Does anything smell different in the last year?" (Yes/No)
    iii. Do you have a stuffy or runny nose? (Yes/No)

c. If the test site is not part of EPIC the patient will be asked their gender and age band (18-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80+)

3. After the patient completes the test they will pull out the **Consent and Multiple choice questionnaire form**, and with the window still CLOSED hold it up to the driver's side window and it will then be photographed by the attending HCW (likely an ENT resident doctor) whom will add the unique identifier number and add it to the EPIC system.
    a. [Alternative should there be any issues with the camera or the patient must get out of the car]: The patient will take the completed test packet, place it back in the pouch and under the windshield. The HCW will take the packet and remove the **Consent Form** and **Multiple choice questionnaire form**, check if it is completed (if ask the patient), and place all remaining materials in a biohazard box for destruction. The Consent form and the Questionnaire will be digitalized (scan or photographed) and linked with EPIC so as to be compared to the PCR test results.
4. Information from the uploaded and **Multiple choice questionnaire form** will be entered by a person authorized to access the information into a table that includes:
    a. Unique identifier
    b. Gender
    c. Age band (10 year)
    d. Y/N to patient self-identified new loss of smell or taste
    e. Y/N to patient response to "things in the last year smell different"
    f. Y/N to patient self-identified blocked or stuff nose
    g. Individual answers on the 5 window smell test
    h. RT-PCR results for SARS-CoV-2 (+, - or Inconclusive)
    i. Any other tests for COVID19 (CT or Serological) if given
5. The data will be analyzed in the following fashion:
    a. Patients that report a blocked or stuffy nose will be excluded from the main analysis (as COVID-19 loss of smell is not due to nasal congestion); but will be analyzed separately to see how this would confound any interpretation.
    b. Patients that are denoted SARS-CoV-2 "inconclusive" will be excluded from the main analysis; but will be separately analyzed to see if they colocalize with normosmia or anosmia.
    c. Patients will be split into SARS-CoV-2 positive and negative. As the negative pool is likely to be much larger it will be statistically matched by gender and age profile (potentially with larger bands – e.g. 20 year, depending on the sample size)

d. The + and - SARS-CoV-2 groups individual performance on the smell test (score 0-5) will be plotted as a histogram. A cutoff will best be chosen to segregate people with normosmia and anosmia (e.g. score = 2 or 3).
e. False Positives (FP) and False Negatives (FN) will be identified and used to measure the selectivity and sensitivity, respectfully.
f. Based on the statistical performance (see Table 1) we will determine when we have enough patients to complete the study with a given value of precision.
g. If a subject fails the smell test (hyposmia or anosmia) but is SARS-CoV-2 negative, yet has other symptoms suggesting being positive for COVID19, we will consider re-testing the subject in 2-3 days by the PCR or serological assay in case the loss of smell precedes a PCR result, or the loss of smell extends past the point in which the viral titer is low and no longer picked up by PCR and would instead be detected by an antibody.

6. The treatment of asymptomatic people will follow an identical protocol with the following differences:
    a. The study group would consist of high risk subjects that are determined to be positive to SARS-CoV-2 (yet asymptomatic) and the control group would be SARS-CoV-2 negative subject of matching age and gender.
    b. The smell kit will be directly given to the subject and for HCW the test can be performed at their home.
    c. There will be a variation of the test in which the order of the scents is altered so that the HCW subject can be re-tested.
    d. The initial sample size is smaller as this study would firstly see if there was a significant increase of the fraction of anosmia or hyposmia subjects in COVID19+ asymptomatic individuals.

6.3.1 Study Schedule

- The outpatient study visit would only consist of the testing that is done in the car.
- Asymptomatics would have one or two (limited repeat) tests and this would be self-performed at Waveny.

6.3.2 Informed Consent

I confirm that provisions are in place for seeking IRB-approved informed consent of participants and that the process will minimize undue influence or coercion and offer sufficient time for review.

6.3.3 Screening

The initial screening of outpatients would involve patients who are in queue for drive-through COVID-19 testing will be approached by a research team member. No prior medical

information will be provided to the study team regarding a patient's eligibility. No other screening is needed. For asymptomatic COVID19+ these would already be identified by the facility.

6.3.4 Enrollment

There is no need for active recruitment of patients as they can easily opt-in and take the test during the drive thru visit. Only patients that consent and meet eligibility criteria will be considered and may partake in the simple tests.

6.3.5 On Study Visits

The one-minute olfactory test is very simple and the only procedure is to collect the consent, test results and confirm that they will be examined by PCR for COVID19.

6.3.6 End of Study and Follow-up

Most of the study is a single timepoint test. If there are any adverse events these will be noted and sent to the lead investigator. As it is largely a single timepoint withdrawing early would have a minimal effect.

6.3.7 Removal of subjects

All outpatients are a single timepoint so the early withdrawal concern is moot. Asymptomatics that can only complete a single timepoint will only have that one timepoint considered.

6.4 Statistical Method

6.4.1 Statistical Design

This is a non-therapeutic correlative trial. Loss of smell in test subjects will be compared to the presence of SARS-CoV-2 coronavirus, as determined by RT-PCR. The true olfactory results will also be compared with the subjects self-assessment for perceived new loss of smell or taste.

6.4.2 Sample Size Considerations

Upon consultation with Yale biostatisticians Profs. James Dziura and Denise Esserman, based on our sampling they calculated the following table to shows the precision of sensitivity/specificity measurements for different sample sizes (Table 1). For example, the 95% confidence interval for 90% sensitivity with 100 subjects ranges from 82 to 95% , but if we have 400 subjects, the confidence interval ranges from 87 to 93% (about +/- 3%). Thus the number of participants will be guided by statistical analysis of the data. A minimal estimate is ~100 outpatients that are SARS-Co-V2 positive so as to have good confidence intervals. Here we presume to have at least 100 age/gender matched SARS-Co-V2 negative subjects. If there are 3:1 SARS-Co-V2 negative to SARS-Co-V2 positive then ~400 outpatients (300 +100) will be tested. For the more exploratory testing with asymptomatics we will limit the study to up to 250 HCW. If the results are encouraging with a loss of smell in asymptomatic the sample size could be increased to increase the precision, as done in the outpatient cohort.

Table 1 (replicated from before): Sample Size Calculations (Width of a 95% confidence interval around the proportion).

*Denominator is those that are COVID+ for sensitivity and those that are COVID- for specificity

	Sample Size for Denominator*						
Sens/Spec	**100**	**150**	**200**	**250**	**300**	**350**	**400**
0.50	0.20	0.17	0.14	0.13	0.12	0.11	0.10
0.60	0.20	0.16	0.14	0.13	0.11	0.11	0.10
0.70	0.19	0.15	0.13	0.12	0.11	0.10	0.09
0.80	0.17	0.13	0.12	0.10	0.09	0.09	0.08
0.90	0.13	0.10	0.09	0.08	0.07	0.07	0.06

6.4.3 Planned Analysis

6.4.3.1 Primary Analyses

Loss of smell in outpatients will be compared to the presence of SARS-CoV-2 coronavirus, as determined by RT-PCR. Key metrics that will be statically evaluated and done with support of YCCI and Yale biostatisticians Profs. James Dziura and/or Denise Esserman Sensitivity are:

- Sensitivity
- Specificity
- Accuracy
- Repeatability

- Other parameters that will be considered are the Area under the Curve (AUC)

6.4.3.2 Secondary Objectives Analyses

Potential loss of smell in asympotomatics will be compared to the presence of SARS-CoV-2 coronavirus, as determined by RT-PCR. For the more exploratory testing with asymptomatics we will limit the study to ~250 HCW, with focus on the sensitivity and specificity and if in short longitudinal testing there is an acute loss of smell. If the results are encouraging with a loss of smell in asymptomatic subjects the sample size may be increased to improve the precision, as done in the outpatient cohort.

Safety

No safety issues to report as this is an observational trial.

6.4.3.3 Analysis of Subject Characteristics

Specify descriptive analysis to define subject population(s).

6.4.3.4 Interim Analysis

Not applicable

6.4.3.5 Health economic evaluation

We will analyze how much the projected cost would be to test every American in the US with different cost models of the device (e.g. $1 Unit) as compared to PCR based tests and current costs. We will then estimate based on the sensitivity and projected prevalence of Coronavirus, and current testing with PCR, the number of new cases that might be identified if taken to full scale, and the number that would be missed. This would provide a generalized estimated range given the underlying assumptions of the cost/benefit of wide-level screening with the olfactory device. Importantly unlike PCR tests this test could be rapidly scaled up by orders of magnitude. Also the test can be performed at home with only the test device and a cell phone. Because of this potential huge upside, we believe that it is critical to validate or invalidate the device as soon as possible

6.4.3.6 Other

Specify any additional analysis that will be done.

6.4.4 Subsets and Covariates

One confounding variable is related to the assumption that the PCR gold standard represents the ground truth, as it has ~5% false positives and 15-30% false negatives. This is the best current standard, but would be improved if combined with a CT scan, but this is not widely done. Thus, it is conceivable the performance of the smell test may be underestimated. Ideally there would be a true ground truth or a PCR assay with higher sensitivity, however this is not currently an option.

6.4.5 Handling of Missing Data

Tests with missing data of the olfactory performance will be excluded.

7 Trial Administration

7.1 Ethical Considerations: Informed Consent/Assent and HIPAA Authorization

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. No deception will be involved and there is no payment to participants. Only the minimal data necessary to complete the study will be obtained and age information will be binned help blind it. No direct subject indicators will be listed.

It is conceivable that potential new observation of anosmia in SARS-CoV-2 negative patients, may suggest a possible underlying condition, such as Alzheimer's or Parkinson's Disease (or head trauma), which may merit further testing by more in-depth tests. If so the olfactory test may be added to the record. The data will be flagged in EPIC for potential follow up and consideration by the patient's primary physician. The PI of the clinical trial team is **Dr. Peter Manes, MD who is a Yale Associate Professor of Rhinology and Endoscopic Skull Base Surgery** and is Yale's expert in olfaction and he could be consulted about the most appropriate follow up.

7.2 Institutional Review Board (IRB) Review

The protocol will be submitted to the IRB for review and approval. Approval of the protocol must be obtained before initiating any research activity. Any significant change to the protocol or study team will require an approved IRB amendment before implementation. The IRB will determine whether informed consent and HIPAA authorization are required. In addition:

- The IRB will conduct continuing review at intervals appropriate to the degree of risk, but not less than once per year.
- A study closure report will be submitted to the IRB after all research activities have been completed.
- Other study events (e.g. data breaches, protocol deviations) will be submitted per Yale IRB's policies.

7.3 Subject Confidentiality

Subject confidentiality is held in strict trust by the research team. Subject medical record review will be limited to the just the elements needed to complete the study, which includes age, gender and results of the COVID test. Only authorized HIPAA and CITI trained (if applicable) study team members will be allowed to extract research data from medical records and enter it into our research database. No direct subject identifiers will be entered into the research database.

Each subject will be assigned a unique study number. A master list linking the unique study number to the human subject will be maintained in a locked drawer in Dr. Manes' office.

7.4 Deviations/Unanticipated Problems

If the study team becomes aware of an anticipated problem (e.g. data breach, protocol deviation), the event will be reported to the IRB by an email from Dr. Manes.

7.5 Data Collection

Data will be collected for outpatients during the drive up test. It will be digitalize (by camera) and manually entered into a spreadsheet. Asymptomatic test results will be collected in a similar fashion. Physical records will be destroyed at the end of the study. All data will be de-identified and de-linked.

7.6 Data Quality Assurance

Good Clinical Practice is followed and will be further overseen by Dr. Ben Judson, MD (Chief, Yale Section of Otolaryngology). Should there be any deviations in the PCR data we will use the Connecticut State Testing Laboratory and we have already spoken at length to **Jafar H Razeq, Ph.D., HCLD (ABB), Laboratory Director Connecticut Department of Public Health** and he would be delighted to do any secondary testing and insure that our testing and data quality meets the highest bar.

7.7 Study Records

The key documents considered in the study records include the protocols, consents forms, case report forms, subject medical records (EPIC), and surveys.

7.8 Access to Source

The source document (Consent from and multiple choice answers) will be entered into EPIC. The PI will have access to the source documents.

7.9 Data or Specimen Storage/Security

Outpatient data will be collected, by hard copy, digitalized and stored and maintained in a secure manner by password protection.

7.10 Retention of Records

The study records will be retained for at least 3 years after the study is closed, and up to 6 years if any personal health information is connected to the records. If permission is needed to move or destroy the records, we will identify the person who will need to be contacted (investigator, sponsor, etc.).

7.11 Study Monitoring

Dr. Ben Judson, MD (Chief, Yale Section of Otolaryngology) will monitor the study.

7.12 Data Safety Monitoring Plan

There are minimal risks associated with this non-invasive olfactory device with no risk to subject. No children will be involved.

7.13 Study Modification

If there are any significant study modifications either due to poor performance or a special opportunity the IRB will be contacted. Changes in the protocol will be highlighted and if approved they would be acted upon. We note that as this is a non-invasive device any risk to the patient will be minimal, thus this may not require review of the entire IRB panel, rather a subset.

7.14 Study Discontinuation

The asymptomatic element of study may be discontinued if no differences are seen in olfactory performance of COVID-19+ asymptomatics compared to their COVID-19- counterparts.

7.15 Study Completion

We anticipate completion by ~3-6 weeks after the IRB is approved, and when done the IRB will be notified. The exact timing will depend on how quickly the data is collected and the incident rate of COVID-19 in the population.

7.16 Conflict of Interest Policy

There is no link of this study to the pharmaceutical industry. We disclose that Dr. Toomre founded and has related intellectual property in 'u-Smell-it, LLC', the company that has developed the u-Smell-it ™ olfactory testing device that is being evaluated in this COVID-19-related clinical trial. Due to these COI he is not be an investigator on the IRB protocol and is not participating in the study apart from supplying the test devices to Dr. Manes. For transparency the PI, Dr. Manes, has no financial ties or intellectual property associated with 'u-Smell-it, LLC'

7.17 Funding Source

There is no funding required for this very brief study as the olfactory test cards have already been obtained and will be given at no charge from 'u-Smell-it LLC'.

7.18 Publication Plan

We anticipate publication of these findings in a prominent journal. Dr. Manes will hold the primary responsibility for publishing the study results.

8 Appendices

Appendix #	Title	Section	Topic