PROTOCOL TITLE

Clinical Performance Evaluation of the Bio-Self™ COVID-19 Antigen Home Test

SPONSOR

BioTeke USA, LLC.
900 Brickell Key Blvd
Suite 2304
Miami, FL 33131

PROTOCOL NUMBER

BTK-01-1002

NCT NUMBER

NCT05334758

VERSION NUMBER

2.0

VERSION DATE

05 May 2022

Table of Contents

1. List of Abbreviations

Term	Definition
AE	Adverse Event
AN	Anterior Nares
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
°C	Degrees Celsius
CRF	Case Report Form
CRO	Contract Research Organization
Ct	Cycle Threshold
EUA	Emergency Use Authorization
FDA	U.S. Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IFU	Instructions for Use
IRB	Institutional Review Board
IVD	In Vitro Diagnostic
LIVD	Laboratory In Vitro Diagnostics
LOINC	Logical Observation Identifiers Names and Codes
NPA	Negative Percent Agreement
NSR	Non-Significant Risk
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PPA	Positive Percent Agreement
QRI	Quick Reference Instructions
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SNOMED	Systematized Nomenclature of Medicine
SOC	Standard of Care
SOP	Standard Operating Procedure
U.S.	United States

2. Key Roles and Responsibilities

2.1. Sponsor Study Staff Contract Information

Sponsor: Sponsor Address: Sponsor Contact: Sponsor Telephone: Sponsor E-mail:	BioTeke USA, LLC. 900 Brickell Key Blvd. Suite 2304 Miami, FL 33131 Chang Oh Turkmani 202-679-2112 sales@bioteke-USA.com
CRO: CRO Address: CRO Contact: CRO Telephone: CRO E-mail:	CSSi LifeSciences 1099 Winterson Road Suite 136 Linthicum Heights, MD 21090 Janice Cattano 443-308-5837 jcattano@cssilifesciences.com

2.2. Site Investigator Contact

A list of the names and titles of clinical investigators and the addresses and telephone numbers of study sites will be maintained in the sponsor master files.

3. Protocol Approval Page

Study Title:	Clinical Performance Evaluation of the Bio-Self™ COVID-19 Antigen Home Test
Protocol Number	BTK-01-1002
Version	2.0
Date of Issue	05 May 2022
Sponsor Name and Address:	BioTeke USA, LLC. 900 Brickell Key Blvd. Suite 2304 Miami, FL 33131

I, the undersigned have read and approve this protocol and agree on its content. It is confirmed that the information and guidance given in this protocol complies with scientific principles.

	Name and Title	Signature and Date
Reviewed and Approved by:	Chang Oh Turkmani Title: President	

4. Investigator Protocol Agreement

Protocol Title: Clinical Performance Evaluation of the Bio-Self™ COVID-19 Antigen Home Test

Protocol Number: BTK-01-1002

Version: 2.0

By my signature, I

1. Agree to conduct the study in accordance with the relevant, current protocol and will only deviate from the protocol when necessary to protect the safety, rights, or welfare of the subject.
2. Agree to personally conduct or supervise the described investigation.
3. Agree to inform any patients, or any persons used as controls, that the study product is being used for investigational purposes and I will ensure that the requirements relating to obtaining informed consent in 21 CFR Part 50 and institutional review board (IRB) review and approval in 21 CFR Part 56 are met.
4. Agree to report to the Sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64. I have read and understand potential risks and side effects of the study product.
5. Agree to ensure all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Agree to maintain adequate and accurate records.
7. Ensure that an IRB that complies with the requirements of 21 CFR Part 56 will be responsible for the initial and continuing review and approval of the clinical investigation. I agree to promptly report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects and others. I will not make any changes in the research protocol without consent from the Sponsor and will not institute those changes in the research protocol until after approved by the Sponsor and IRB, except where necessary to eliminate apparent immediate hazards to human subjects.
8. Agree to comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements in 21 CFR Part 312.

_____ _____

Investigator's Signature Date

_____

Print Name

5. Study Synopsis

Full Title	Clinical Performance Evaluation of the Bio-Self COVID-19 Antigen Home Test
Short Title	Bio-Self Clinical Performance Home Use Study
Protocol Number	BTK-01-1002
Protocol Version	2.0
Study Sponsor	BioTeke USA, LLC.
Study Purpose	The purpose of this study is to evaluate the performance of the Bio-Self COVID-19 Antigen Home Test. The study will evaluate the accuracy (sensitivity and specificity) in a simulated home use environment of the Bio-Self COVID-19 Antigen Home Test when compared to the PerkinElmer New Coronavirus Nucleic Acid detection kit, a high-sensitivity Emergency Use Authorization (EUA) SARS-CoV-2 RT-PCR assay.
Investigational Material/Product	The Bio-Self COVID-19 Antigen Home Test is immunochromatographic and uses double-antibody sandwich method to detect SARS-CoV-2 antigen from individuals with or without symptoms or other epidemiological reasons to suspect a COVID-19 infection. The test is intended for non-prescription home use with self-collected direct anterior nares swab samples from individuals ages 14 years and older or adult collected anterior nares swab samples from individuals ages 2 to 13.
Study Objectives	The primary objective of this study is to determine the accuracy of the Bio-Self COVID-19 Antigen Home Test when compared to a high-sensitivity Emergency Use Authorization (EUA) SARS-CoV-2 RT-PCR assay.
Study Design	This is an open label, prospective study to evaluate the sensitivity and specificity of the Bio-Self COVID-19 Antigen Home Test when a lay person conducts the test on themselves or another study participant. Potential subjects will be those presenting for COVID-19 testing. The study will evaluate the performance of the Bio-Self COVID-19 Antigen Home Test by comparing it to a high sensitivity EUA SARS-CoV-2 RT-PCR assay to calculate both the positive percent agreement [(PPA) sensitivity] and negative percent agreement [(NPA) specificity]. The order of testing will be 1) standard of care (SOC) collection by a healthcare practitioner, 2) layperson conducts the test on themselves or another study participant using the Bio-Self Test, and 3) comparator test collection by the healthcare practitioner.
Inclusion Criteria	Subjects are eligible for inclusion only if <u>all</u> of the following criteria are met: 1. An Institutional Review Board (IRB) approved informed consent and assent, if applicable, is signed and dated prior to any study-related activities. 2. Male and female Subjects 2 years of age and older. 3. Subject is willing to provide a self-collected nasal swab sample. (If under the age of 14, the sample will be collected by an adult.) 4. Subject is willing to have a nasal swab collected by a healthcare professional. 5. Subject agrees to complete all aspects of the study.
Exclusion Criteria	A Subject is <u>not</u> eligible for inclusion if <u>any</u> of the following criteria apply:

	1. Subject has a visual impairment that cannot be restored with glasses or contact lenses. 2. Subject has prior medical or laboratory training. 3. Subject had a positive COVID-19 test in past three (3) months. 4. Subject uses home diagnostics, e.g., HIV Tests, glucose meters, etc.

Target Population/ Sample Size	The first 50 subjects will be consecutively enrolled (i.e., in an "all comers" style) including those who are either asymptomatic or symptomatic for COVID-19. Following the enrollment of the first 50 subjects, enroll only subjects who are symptomatic for COVID-19. When at least 30 RT-PCR confirmed positive specimens have been collected from symptomatic subjects, enrollment will continue with all comers, i.e., symptomatic and asymptomatic for COVID-19 until at least 10 RT-PCR confirmed positive specimens have been collected from asymptomatic subjects.
	
	Enrollment population must include:
	• At least 10 RT-PCR confirmed positive specimens collected from subjects that are asymptomatic for COVID-19.
	• At least 30 RT-PCR confirmed positive specimens collected from subjects that are symptomatic for COVID-19.
	• At least 30 children between 2 – 13 years of age.
	• Representation in the following age groups:
	  • <14 years of age, where the Parent or legal guardian collects a sample from their child (e.g., ages 2-13) and performs the test.
	  • 14-24 years of age
	  • 25-64 years of age
	  • ≥65 years of age
	• At least 10-20% of the clinical samples are low positive.
	
	Current estimates of positive cases are approximately 8 % of all-comers. Up to 600 subjects may be enrolled in this study to obtain a minimum of 30 RT-PCR confirmed positive specimens from subjects who are symptomatic, and 10 RT-PCR confirmed positive specimens from subjects who are asymptomatic for COVID-19 with at least 10-20% being low positive.

Site Requirements	Up to three (3) sites, with a minimum of 2 different testing locations.
Study Duration	The overall study participation for each subject will be approximately one hour.
Study Endpoint	Primary Endpoint 1. Establish the performance characteristics of the Bio-Self COVID-19 Antigen Home Test with lay users' self-collection and testing or adult collection and testing for those ages 2-14. Clinical accuracy will be compared to RT-PCR test results. Accuracy will refer to positive percent agreement [(PPA) sensitivity] and negative percent agreement [(NPA) specificity].

6. Introduction

6.1. Background

6.1.1. COVID-19 is a contagious respiratory illness, caused by infection with the novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This is a communicable disease easily transmitted through aerosolized droplets containing the infectious virus. Infections with SARS-CoV-2 can have varying degrees of signs and symptoms, ranging from asymptomatic to severe and believed to be contagious from as early as 2 days prior to symptom onset to at least 10 days after onset of symptoms. Rapid detection of infections and contacts and the implementation of infection control measures are critical for mitigation of this virus.

6.1.2. Bio-Self COVID-19 Antigen Home Test is immunochromatographic and uses double-antibody sandwich method for the detection of SARS-CoV-2 nucleocapsid protein antigens from individuals with or without symptoms or other epidemiological reasons to suspect a COVID-19 infection.

6.2. Study Purpose

6.2.1. The purpose of this study is to evaluate the performance of the Bio-Self COVID-19 Antigen Home Test in a simulated Home Use environment.

6.2.2. The study will evaluate the accuracy in the home use environment of the Bio-Self COVID-19 Antigen Home Test when compared to a high-sensitivity Emergency Use Authorization (EUA) SARS-CoV-2 RT-PCR assay.

6.3. Risk and Benefit Assessment

6.3.1. Potential Risks

3.3.1.1 The only study-related procedures that could impact Subject safety is in the collection of nasal swabs. The risks of collecting a nasal swab may include symptoms associated with standard collection practices such as slight pain and/or a small amount of bleeding in the nose.

3.3.1.2 Subject(s) will be de-identified using a study-specific number at the time of enrollment. All study forms, Case Report Forms (CRFs) and study samples will be labeled with the de-identified subject ID number. No identifiable patient information will be provided to BioTeke USA, LLC. No patient treatment or management decisions will be based on any results generated from the investigational kit during this study.

3.3.1.2 Information collected for this study is confidential. However, officials of FDA or other regulatory agencies may research records during the ordinary course of carrying out their functions. A sponsor representative may also inspect research records to make certain the study data is accurate. The Investigator, institution, regulatory agencies and sponsor will protect the confidentiality of the records.

6.3.2. Potential Benefits

3.3.2.1 The information obtained in this study will not benefit the study subjects directly but may aid future patients by helping to rapidly and accurately detect infection caused by SARS-CoV-2.

6.4. Study Device Description

The Bio-Self COVID-19 Antigen Home Test is immunochromatographic and uses double-antibody sandwich method to detect SARS-CoV-2 antigen. The Bio-Self COVID-19 Antigen Home Test is designed to detect antigen of the SARS-CoV-2 from the anterior nares (Nasal) specimens from patients with or without symptoms or other epidemiological reasons to suspect a COVID-19 infection.

6.4.1.Mechanism of Action

Bio-Self COVID-19 Antigen Home Test is immunochromatographic and uses double-antibody sandwich method to detect nucleocapsid protein from SARS-CoV-2 virus. During detection, the treated samples are added to the sample well of the test cartridge. When the concentration of SARS-CoV-2 antigen in the specimen is higher than the minimum detection limit, the viral antigen will form complexes with labeled antibodies first. Under chromatography, the complexes move forward along the nitrocellulose membrane until captured by pre-coated monoclonal antibodies of SARS-CoV-2 in the detection zone on the nitrocellulose film (Test line – T) to form a pink/purple reaction line on the detection zone, indicating the result is positive. Conversely, if there is no viral antigen or the concentration of antigen in the specimen is below the minimum detection limit, no pink/purple reaction line appears in the detection zone and the result is considered negative. Regardless of whether or not the sample contains viral antigens, the pink/purple reaction line that appears in the quality control zone (Control line – C) is the criterion for determining if the chromatography process is performing properly.

6.4.2.Device Output

Bio-Self COVID-19 Antigen Home Test is designed to be visually read by detecting a visible color band.

1. A test line (T) and control line (C) indicate a positive result.
2. A single control line (C) indicates a negative result.
3. A test line (T) only, or the absence of the control line (C) and test line (T), or blurred lines indicate an invalid test.

Positve Result

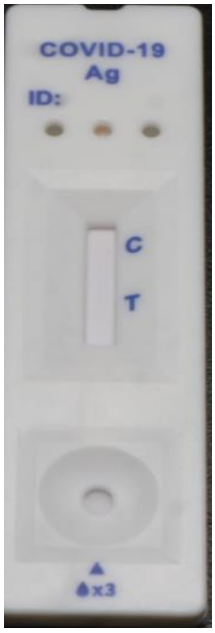
C-Line	**T-Line**	**Result**
Pink/purple	Pink/purple	Positive

Negative Result

C-Line	T-Line	Result
Pink/purple	Colorless	Negative

Invalid Result

C-Line	T-Line	Result
Colorless	Colorless or pink/purple	Invalid, retest



6.4.3. Intended Use

The Bio-Self COVID-19 Antigen Home Test is an immunochromatographic double-antibody sandwich assay intended for the qualitative detection of SARS-CoV-2 nucleocapsid protein antigens from individuals with or without symptoms or other epidemiological reasons to suspect a COVID-19 infection. This test is authorized for non- prescription home use with self-collected direct anterior nasal (nares) swab samples from individuals aged 14 years or older or adult collected anterior nasal swab samples from individuals aged 2 – 13 years of age.

Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen. The antigen is generally detectable in anterior nasal swab specimens during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with past medical history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses and the agent detected may not be the definite cause of disease. Individuals who test positive with the Bio-Self COVID-19 Antigen Home Test should self- isolate and seek follow-up care with their physician or healthcare provider as additional testing may be necessary.

Negative results should be treated as presumptive and confirmation with a molecular assay for patient management, may be performed if necessary. Negative results do not rule out SARS-CoV-2 infection and should not be used as the sole basis for treatment or patient management decisions, including infection control decisions. Negative results should be considered in the context of an individual's recent exposures, history, and the presence of clinical signs and symptoms consistent with COVID-19. Additional confirmatory testing with a molecular test for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposures to COVID-19 or residing in communities with low prevalence of infection. Individuals who test negative and continue to experience COVID-like symptoms of fever, cough and/or shortness of breath may still have SARS-CoV-2 infection and should seek follow up care from their healthcare provider. Individuals should provide all results obtained with this product to their healthcare provider for public health reporting. All healthcare providers will report all test results they receive from individuals who use the authorized product to relevant public health authorities in accordance with local, state, and federal requirements using appropriate LOINC and SNOMED codes, as defined by the Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests provided by CDC.

6.5. For Investigational Use Only

6.5.1. The Bio-Self COVID-19 Antigen Home Test is an immunochromatographic double-antibody sandwich assay intended for the qualitative detection of the SARS-CoV-2 nucleocapsid protein antigen in human direct anterior nares (nasal) specimens directly collected from subjects suspected of COVID-19 infection. The performance characteristics of the test using nasal swabs have not been established. The results obtained from the Bio-Self COVID-19 Antigen Home Test used in this study will not be used for diagnostic purposes or patient management.

6.5.2. Results are for the identification of SARS-CoV-2 nucleocapsid antigen. Antigen is generally detectable in nasal samples during the acute phase of infection. Positive results indicate the presence of viral antigens, but clinical correlation with patient history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

6.6. Study Sites

6.6.1. Study enrollment for this study will be conducted at up to three (3) clinical sites with at least two testing locations. The testing locations have a diverse demographic patient population with varying educational backgrounds.

6.6.2. The RT-PCR comparator test will be conducted at a minimum of one (1) CLIA certified laboratory within the U.S.

6.6.3. The applicable IRB/EC will review and approve the study protocol, informed consent and assent documents, Quick Reference Instructions (QRI) and any other patient facing materials prior to enrolling Subjects into this study.

6.7. Study Objectives

6.7.1. The primary objective of this study is to determine the accuracy (in terms of sensitivity and specificity) of the Bio-Self COVID-19 Antigen Home Test when compared to the PerkinElmer New Coronavirus Nucleic Acid detection kit, a high-sensitivity Emergency Use Authorization (EUA) SARS-CoV-2 RT-PCR assay.

6.8. Study Design

This is an open label, prospective study to evaluate the sensitivity and specificity of the Bio-Self COVID-19 Antigen Home Test when a lay person conducts the test on themselves or on another study participant. Potential subjects will be those presenting for COVID-19 testing.

Subjects that present for COVID-19 testing (both symptomatic and asymptomatic) that meet the eligibility criteria will be enrolled into this study. The subject population may include English and Spanish speaking individuals across all ages 2 – 65+ years. The following age groups must be represented:

- <14 years of age, where the Parent or legal guardian collects a sample from their child (e.g., age 2-13) and performs the test
- 14-24 years of age
- 25-64 years of age
- ≥65 years of age

The subjects will first have the standard of care (SOC) test performed by a healthcare practitioner. To mitigate any potential bias which may affect the subject's visual interpretation of the Bio-Self COVID-19 Antigen Home Test result, the healthcare practitioner will not provide the SOC test results to the subject until after the results of the Bio-Self kit are read by the subject.

After a minimum of 15 minutes after the SOC sample is collected the subject will be provided the Bio-Self COVID-19 Antigen Home Test and the Quick Reference Instructions. Once the test is completed, the results will be recorded.

After a minimum of 15 minutes after the Subject self-collection (or adult tester) the healthcare practitioner will collect the nasal sample for the RT-PCR comparator.

Comparator:
The subjects will have a healthcare practitioner collect two nasal samples, one for the SOC testing and the second for the comparator test. The SOC sample will be used for patient management. Again, the results of the SOC will not be shared with the subject until the subject has interpreted the results from the Bio-Self COVID-19 Antigen Home Test. A minimum of 15 minutes will be required between each sample collection. The subject should be instructed to blow their nose prior to each sample collection. The comparator sample will be tested at a CLIA lab using the PerkinElmer New Coronavirus Nucleic Acid detection kit. Positive samples (RT-PCR and Bio-Self COVID-19 Antigen Home Test) will be sequenced by the CLIA lab. Once 30 sequence-confirmed omicron positive clinical samples have been identified, no further sequencing will be performed. Additionally, to ensure 10-20% of the samples are low positive, a cycle threshold (Ct) count will be performed for all RT-PCR positive samples. Low positives are defined as samples in which any gene target is within 3 Ct's of the mean Ct count of the RT-PCR comparator test's LoD. The following information will be collected and provided in the EUA application.

- specimen collection device
- specimen volume,
- eluate volume,

- specific extraction kit,
- extraction equipment,
- consumables
- platform.

Line data collection:
The original information and data for each subject will be collected in an excel spreadsheet including the following data elements for each specimen tested:

- Specimen type for the antigen test
- Specimen collection date and time for the antigen test
- Specimen testing date and time for the antigen test
- VTM type, as applicable, for the PCR
- Antigen test result with the reader
- Specimen type for RT-PCR
- Specimen collection date and time for RT-PCR test
- Specimen testing date and time for RT-PCR test
- Name of RT-PCR used as the comparator for each specimen
- RT-PCR test results (+/-)
- RT-PCR test value results (Ct values) for positive samples
- RT-PCR and Bio-Self COVID-19 Antigen Home Test positive samples sequencing, for the first 30 samples.
- Indicate if subject is asymptomatic or symptomatic. If symptomatic which of the following symptoms are they experiencing: Fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, other-specify.
- Number of days post-onset of patient symptoms
- Subject age, sex, race and ethnicity, if available.

7. Study Enrollment and Withdrawal

7.1. Study Population

7.1.1. Enrollment population should represent different socioeconomic and educational backgrounds.

7.1.2. High risk individuals should not be excluded from the study.

7.1.3. Both symptomatic and asymptomatic individuals should be enrolled in the study.

7.1.4. At least thirty (30) children ages of 2 – 13 should be enrolled in the study.

7.1.5. Enrollment population must include subjects in the following age groups:

- <14 years of age, where the Parent or legal guardian collects a sample from their child (e.g., ages 2-13) and performs the test
- 14-24 years of age
- 25-64 years of age
- ≥65 years of age

7.1.6. The first 50 subjects should be consecutively enrolled (i.e., in "all comers" style), including those who are either asymptomatic or symptomatic for COVID-19. Following the enrollment of the first 50 subjects, enroll only subjects who are symptomatic for COVID-19. When at least 30 RT-PCR confirmed positive specimens have been collected from symptomatic subjects, enrollment will continue with all comers, i.e., symptomatic and asymptomatic for COVID-19 until at least 10 RT-PCR confirmed positive specimens have been collected from asymptomatic subjects.

7.1.7. A minimum of 30 symptomatic subjects testing positive and 30 subjects testing negative, confirmed by RT-PCR must be enrolled in the study.

7.1.8. A minimum of 3 low positive samples.

7.1.9. A minimum of 10 asymptomatic subjects who test positive, confirmed by RT-PCR must be enrolled in the study.

7.1.10. Enrollment population will include any subject that presents for COVID-19 testing (both symptomatic and asymptomatic) that meets the eligibility criteria.

7.2. Subject Inclusion Criteria

To be enrolled in the study, the subject must meet all of the following inclusion criteria:

1. An Institutional Review Board (IRB) approved informed consent and assent, if applicable, is signed and dated prior to any study-related activities.
2. Male and female Subjects 2 years of age and older.
3. Subject is willing to provide a self-collected nasal swab sample. (If under the age of 14, the sample will be collected by an adult.)
4. Subject is willing to have nasal swabs collected by a healthcare professional.
5. Subject agrees to complete all aspects of the study.

7.3. Subject Exclusion Criteria

To be enrolled in the study, the subject must not meet any of the following exclusion criteria:

1. Subject has a visual impairment that cannot be restored with glasses or contact lenses.
2. Subject has prior medical or laboratory training.
3. Subject had positive COVID-19 test in past three months.
4. Subject uses home diagnostics, i.e., HIV tests, glucose meters, etc.

7.4. Study Duration

7.4.1. Each subject's involvement in the study will last for approximately one (1) hour. No follow-up or subsequent subject involvement is required.

7.5. Subject Withdrawal

7.5.1. Subjects may discontinue their participation in the study at any time. A subject may be withdrawn from the study at any time at the discretion of the Investigator.

7.6. Reason for Withdrawal

A subject may be considered withdrawn for the purpose of this study if:

- Subject or parent of subject withdraws consent for study participation
- Subject non-compliance
- Investigator decision to withdraw subject as study participation is no longer in the subject's best interest

8. Study Procedures

8.1. Screening

8.1.1. Informed consent

8.1.1.1 Study staff will be notified upon presentation of eligible subjects to the clinical site. Study staff trained in basic human research subject's protection will explain the study protocol, procedures and objectives to the patient and obtain written informed consent and assent (if applicable) for participation in the study from each subject prior to performing any study procedures in accordance with requirements outlined in the Code of Federal Regulations (CFR) Title 45, Part 46 and Title 21, Part 50. This will be done by reviewing all information contained within the Consent /Assent form (if applicable), allowing the subject ample time

to review the form(s), ask questions and have their questions answered to the satisfaction of the Subject.

8.1.1.2 Subjects wishing to participate will be asked to sign the Consent/Assent form (if applicable) and will be provided with a copy for their records; the original will be maintained with the Subject's study records. The Consent/Assent Form will be presented to the eligible Subjects in a language that is fully comprehensible to the Subject.

8.1.2. Vulnerable Populations

8.1.2.1 Studies will be conducted in accordance with the relevant articles of the Declaration of Helsinki. The Declaration of Helsinki defines vulnerable individuals as having "An increased likelihood of being wronged or of incurring additional harm." For the purpose of this study, we define vulnerable individuals as those whose capacity to protect or exercise their rights is restricted due to their mental, social, economic or ethnical status, age or health condition. Subjects deemed vulnerable by the IRB and/or the Principal Investigator will not be eligible for this study.

8.1.2.2 This study involves children who have not attained the legal age for consent to treatment or procedures involved in research and are a vulnerable population. Children are a group already burdened by COVID-19 and while there is no benefit to study participants, the nares swabs for this study do not pose greater than a minimum risk to children.

8.1.3. Subject Eligibility

8.1.3.1 Once written informed consent has been obtained from the subject, a Case Report Form (CRF) will be completed to document adherence to the inclusion and exclusion criteria.

8.1.3.2 Where a subject fails to fulfill any element of the inclusion and exclusion criteria, this will be documented and the signed consent form and completed inclusion/exclusion criteria retained by the Principal Investigator. The subject will not be advanced any further into this clinical investigation.

8.2. Study Enrollment

8.2.1. Subject Identification

8.2.1.1 When a subject is considered eligible for entry into this clinical study, the subject will be allocated a study-specific number (Subject ID number). This number will be a unique identifier to the subject and written on each page of the CRF, and all other documentation relating to that subject.

8.2.1.2 The Subject ID number will consist of the study site testing location number and a sequential enrollment number at that location. For example, the third subject enrolled at site 1 would have a Subject ID 01-003.

8.2.1.3 The Principal Investigator (PI) or designee at each site will maintain a master log that will correlate each Subject ID number to the subject's name. This log will remain at the study site and will be confidential.

8.2.2. Subject Information

8.2.2.1 The following information will be obtained and documented on the appropriate CRF for all subjects enrolled in the study:

- Age (age of the subject and of the person performing the test)
- Sex
- Race
- Ethnicity
- Level of education (of the person performing the test)

- Occupation(of the person performing the test)
- Exposure to someone with COVID-19 and when the exposure took place.
- Presence or absence of one or more COVID-19 symptoms, specifically: fever over 100° F or chills; cough; shortness of breath; difficulty breathing; muscle or body aches; fatigue; sore throat; headache; congestion or runny nose; new loss of taste or smell. Subjects will be asked how many days prior to the test the symptoms appeared.
- COVID-19 Vaccine Status including which vaccine, number of doses and date of last dose.

8.3. Group Assignment

8.3.1. Subjects will be assigned to one of two groups: Self-Test or Pediatric Test.

8.3.1.1 The Self-Test group will include the subject only. 8.3.1.2 The Pediatric group will include an adult tester and a pediatric subject (age 2 – 13).

8.3.2. The healthcare practitioner will first collect the SOC sample. To mitigate any potential bias which may affect the subject's visual interpretation of the Bio-Self COVID-19 Antigen Home Test result, the healthcare practitioner will not provide the SOC test results to the subject until after the results of the Bio-Self COVID-19 Antigen Home Test are read by the subject.

8.3.3. Subjects will be taken to an area where they cannot observe other subjects that are participating in this study and that simulates a home use environment.

8.3.4. The subject (or adult tester) will be provided with the Bio-Self COVID-19 Antigen Home Test and the Quick Reference Instructions (QRI).

8.3.5. After a minimum of 15 minutes after the SOC sample collection, the subject will be asked to conduct the test using the QRI. This includes collection of the sample, conducting the test, reading and interpreting the test results.

8.3.6. After a minimum of 15 minutes after the subject (or adult tester) sample collection, the healthcare practitioner will collect the nasal sample from the subject, that will be used for the Comparator.

8.3.7. A minimum of 15 minutes is required between each sample collection. The subject will be asked to blow their nose prior to collecting each sample.

8.3.5.1 The comparator sample will be labeled with the subject's study identification number.

8.3.5.2 The comparator sample will be shipped to the CLIA laboratory conducting the RT-PCR test. A Ct value will be obtained for all RT-PCR positive samples. A minimum of 30 positive samples will be sequenced.

8.4. COVID-19 RT-PCR Inconclusive Result

8.4.1. If the result from the COVID-19 RT-PCR Test is inconclusive, the laboratory should repeat the COVID-19 RT-PCR test. If the test result is inconclusive a second time, the results should be reported as inconclusive, and the subject should be re-tested.

8.4.2. Contact the subject and request that they return for a retest. Confirm a time and date for an unscheduled visit.

8.4.3. Follow the steps under Section 8.3.2. – 8.3.5.

9. Problem Reporting

If problems occur during the conduct of the study, the clinical monitor should be notified within five (5) days of incident.

10. Premature Study Termination or Suspension

10.1. This study may be suspended or prematurely terminated by the sponsor or the investigator immediately upon notice for any cause. Written notification via email or post, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party. If the study is prematurely terminated or

suspended, the Principal Investigator will promptly inform the IRB and will provide the reason(s) for suspension or termination.

10.2. Circumstances that may warrant termination include, but are not limited to:

- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Determination of futility

11. Safety Considerations

11.1. Safety Warnings and Precautions

Handle all samples as potentially infectious according to universal precautions and good laboratory practices (GLP). Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

11.2. Definition of Adverse Events

11.2.1. An Adverse Events (AE) is any untoward medical event that occurs to the subject during the course of a study (with onset after the first study-specific procedure), whether or not that event is considered study-related.

11.2.2. An adverse event may be mild, moderate or severe and are usually unexpected.

11.2.3. All adverse events observed by the Principal Investigator or reported by the subject, must be reported to the Sponsor within 24 hours. To report an adverse event, the Principal Investigator will complete an adverse event form or relevant form.

11.2.4. Adverse events will be considered study-related if the event follows a reasonable temporal sequence from a study related procedure and could readily have been produced by that study procedure. For the purpose of this study, only the collection of nasal nares swabs is considered to be the study procedures.

11.3. Reporting of Serious Adverse Events

11.3.1. A Serious Adverse Event **(SAE)** is any adverse event that:

- led to death
- led to a serious deterioration in the health of the subject that either resulted in a life-threatening illness or injury, or a permanent impairment of a body structure or a body function, or in-patient hospitalization or prolongation of existing hospitalization, or in medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function
- led to foetal distress, foetal death or a congenital abnormality or birth defect

11.3.2. Hospitalization is defined as greater than 24 hours in the hospital.

11.3.3. All serious adverse events must be reported to the Sponsor by telephone within 24 hours of the Principal Investigator becoming aware of it.

11.3.4. The Sponsor will be contacted on the following numbers:

Contact Name: Chang Oh Turkmani

Telephone Number: 202-679-2112

11.3.5. The Principal Investigator should institute appropriate therapeutic and follow-up measures in accordance with good medical practice but should notify the monitor of such actions and record them in the Subject's CRF. Each telephone reported reaction must be reported in writing to the monitor within 10 working days of the event.

11.3.6. It is also the responsibility of the Principal Investigator to inform the representative of the appropriate IRB in a timely manner.

11.4. Possible Risks Associated with Study Procedures

11.4.1. The only study-related procedures that could impact subject safety is in the collection of a direct anterior nares (nasal) swab. The risks of collecting a nasal nares swab may include symptoms associated with standard collection practices such as slight pain, minor bleeding. Subjects will be identified using a study specific number at the time of enrollment. All study forms and any associated laboratory reports will be labeled with the subject number. No identifiable patient information will be provided to the Sponsor. No patient treatment or management decisions will be based on any results generated from this study, with the exception of the Standard of Care sample.

11.4.2. Information collected for this study is confidential. However, officials of FDA or other regulatory agencies may research records during ordinary course of carrying out their functions. A sponsor representative may also inspect research records to make certain the study data is accurate. The Investigator, institution, regulatory agencies and Sponsor will protect the confidentiality of the records.

11.4.3. No medical decisions will be based on investigational assay results generated as part of the study.

12. Regulatory and Administrative Requirements

12.1. FDA Guidance on EUA

FDA Guidance on EUA testing and data acknowledges these products are not subject to 21 CFR 812 noting all other human Subject protection regulations (45 CFR 46) apply. This study does not meet the definition of a clinical investigation as defined by 21 CFR 812 and is not subject to IRB surrogate determination of risk. This study is, however, a non-significant risk (NSR) study in that nasal sample collection is not considered invasive and the test results will not be used to manage clinical care. Implementation of this study will include waiver of otherwise-applicable GCPs to accommodate the emergency response COVID-19 diagnostic testing needs during the declaration time period.

12.2. Responsibility of Investigator

The Principal Investigator is responsible for the proper conduct of the study at their site(s). The investigator will ensure that the study is conducted in compliance with GCP, the protocol, the Clinical Study Agreement, and applicable regulations in 45 CFR 46, and any other applicable regulatory requirements. These requirements include:

- Obtaining IRB approval for the study and related documents before study initiation and enrolling Subjects.
- Obtaining informed consent from each subject in accordance with 21 CFR Part 50, as applicable.
- Protecting the rights, safety, and welfare of Subjects under their care.
- Reporting adverse events.
- Coordinating and providing reasonable medical care to subjects for an adverse event related to study participation.
- Reporting protocol deviations to the sponsor and IRB per IRB requirements.
- Maintaining traceability and secured storage of all test kits and study samples.
- Ensuring confidentiality of all study documents, procedures, and study samples.

12.3. Responsibility of Sponsor

12.3.1. The sponsor has the overall responsibility for the conduct of the study, including assurance that the study meets the FDA EUA regulations and other regulatory requirements as applicable.

The sponsor's study-related duties and functions may be transferred to a contract research organization (CRO).

12.4. Protocol Deviations

12.4.1. A protocol deviation is failure to comply with the requirements specified within the clinical study protocol or IRB requirements. Investigators are not allowed to deviate from the protocol

except when a deviation is necessary to protect Subject's rights and well-being, or the scientific integrity of the study.

12.5. Changes to Protocol

12.5.1. In the event the study protocol is amended, the monitor will provide the Principal Investigator with the protocol amendment(s) and a revised protocol.

12.5.2. Protocol amendments that affect the Subject rights, safety, or welfare must be submitted to and approved by the IRB. The Investigator must be notified of the approval, in writing, prior to implementation.

12.6. Reconciliation of Study Material, Supplies and Samples

The Sponsor will supply sufficient quantities of study materials and supplies to enable the site to complete the study and the site will return all unused materials and supplies to the Sponsor or dispose of according to provided instructions. The site is required to maintain the inventory of these materials provided and document the reconciliation of these materials. If materials are disposed at the study site, the Investigator must provide the Sponsor with a signed record of disposition.

13. Statistical Considerations

Except where otherwise specified, the following general principles apply to the planned statistical analyses. All statistical analysis will be conducted using a current version 9.4 (or later) of SAS (SAS Institute Inc., Cary, NC), Excel version 2010 or later, or other widely accepted statistical or graphical software as required. Continuous variables (e.g., age) will be summarized by the number of subjects; mean, standard deviation and median. Categorical variables (e.g., sex) will be summarized with frequency counts and percentages. Confidence intervals (CI) may be presented, where appropriate, using the exact method for categorical variables. In general, percentages will be displayed to 1 decimal place.

13.1. Sample Size Considerations

Enrollment will continue until at least 30 RT-PCR confirmed positive subjects and 30 RT-PCR confirmed negative subjects have completed the study, with at least 3 low positives. In addition, at least 10 RT-PCR positive results should be from asymptomatic subjects and at least 30 subjects less than 14 years of age shall be included in the subject population.

Enrollment population must include subjects in the following age groups:

- <14 years of age, where the Parent or legal guardian collects a sample from their child (e.g., age 2-13) and performs the test
- 14-24 years of age
- 25-64 years of age
- ≥65 years of age

Current estimates of positive cases are approximately 8% of all-comers. Up to 600 subjects may be enrolled in this study. However, enrollment will be considered complete when the requirements for the study population have been met.

13.2. Statistical Methods

13.2.1. Performance Study - Sensitivity and Specificity

A 2 x 2 contingency table will be constructed as shown in Table 1 to display the frequencies of the Bio-Self COVID-19 Antigen Home Test and EUA SARS-CoV-2 RT-PCR test results, based on the positive and negative outcomes.

Table 1. Concordance of Bio-Self COVID-19 Antigen Home Test and the EUA SARS-CoV-2 RT-PCR comparator assay			
Bio-Self COVID-19 Antigen Home Test	EUA SARS-CoV-2 RT-PCR Comparator Assay		
	Pos	**Neg**	**Total**
Pos	n_{11}	n_{12}	$n_{1.}$
Neg	n_{21}	n_{22}	$n_{2.}$
Total	$n_{.1}$	$n_{.2}$	$n_{..}$

Pos=positive, Neg=negative.

Based on the notation of Table 1, The sensitivity, specificity and 95% two-sided exact binomial confidence interval will be established.

The sensitivity is computed as the proportion of positive samples as called by the EUA SARS-CoV-2 RT-PCR assay, which are also positive as called by the Bio-Self COVID-19 Antigen Home Test; the sensitivity shall be computed as the ratio of n_{11} / (n_{11} + n_{21}).

The specificity is computed as the proportion of negative samples as called by the EUA SARS-CoV-2 RT-PCR assay, which are also negative as called by the Bio-Self COVID-19 Antigen Home Test; the specificity shall be computed as the ratio of n_{22} / (n_{12} + n_{22}).

13.2.2. Performance Study - Percent Agreement

A 2 x 2 contingency table will be constructed as shown in Table 1 to display the frequencies of the Bio-Self COVID-19 Antigen Home Test and EUA SARS-CoV-2 RT-PCR test results, based on the positive and negative outcomes.

Based on the notation of Table 1, The percent agreement and 95% two-sided exact binomial confidence interval will be established.

The overall percent agreement shall be computed as equal to 100% x $(n_{11} + n_{22})/(n_{11} + n_{12} + n_{21} + n_{22})$

The positive percent agreement (PPA) shall be computed as equal to 100% x $n_{11}/(n_{11} + n_{21})$

The negative percent agreement (NPA) shall be computed as equal to 100% x $n_{22}/(n_{12} + n_{22})$

Per FDA's Template for Developers of Molecular and Antigen Diagnostic COVID-19 Test for Home Use dated 11/09/2021, the following performance criteria will be used:

- NPA ≥98%
- PPA
  - For OTC single-use testing in all patient populations, including individuals with or without symptoms or other epidemiological reasons to suspect COVID-19: ≥ 80% PPA demonstrated in a clinical evaluation including both symptomatic and asymptomatic individuals;
  - For OTC testing in all patient populations, including individuals with or without symptoms or other epidemiological reasons to suspect COVID-19, with additional mitigations such as serial screening, as discussed in the "Supplemental Template for Developers of Molecular and Antigen Diagnostic COVID-19 Tests for Screening with Serial Testing"[1]: ≥ 80% PPA with a lower bound (LB) of the two sided 95% confidence interval (CI) ≥70%, demonstrated

[1] Available at https://www.fda.gov/media/146695/download.

in a clinical evaluation including symptomatic individuals only or both symptomatic and asymptomatic individuals;

- For prescription home use single-use testing in individuals suspected of COVID-19 by their healthcare provider: ≥ 80% PPA demonstrated in a clinical evaluation including symptomatic individuals only (note that, for antigen tests, the indication may be limited to symptomatic individuals within a certain number of days of symptom onset, depending on the data); or
- For OTC single use testing in symptomatic individuals: ≥80% PPA demonstrated in a clinical evaluation including symptomatic individuals only (note that, for antigen tests, the indication may be limited to symptomatic individuals within a certain number of days of symptom onset, depending on the data).

14. Ethical Considerations

The investigator and site study staff will be responsible for the ethical conduct of this study in accordance with 45 CFR 46, 21 CFR 50, 21 CFR 56 governing IRB review and informed consent.

14.1. Institutional Review Board

14.1.1. Each participating site must provide for review and approval of this protocol and associated informed consent by an appropriate IRB. Any amendments to the protocol or consent materials must also be reviewed and approved by the IRB prior to use.

14.1.2. Prior to study initiation at each site, the Institutional Review Board (IRB) responsible for the studies to be conducted at each site or a central IRB must provide written approval of the protocol and informed consent. The investigator will provide IRB documentation for all applicable IRB submissions to the Sponsor. If IRB approval is withdrawn, the investigator will immediately report this to the Sponsor.

14.2. Informed Consent Process

14.2.1. Informed consent is a process that is initiated prior to the Subject's agreement to participate in the study and continues throughout the study participation. The Principal Investigator must explain to each patient the nature of the clinical study, including any risks and benefits, its purpose and procedures, and expected duration of involvement in the clinical study. Each patient must be informed that participation in the clinical study is voluntary and non-participation will not affect his / her right to the most appropriate treatments or affect the doctor/clinician-patient relationship. Each patient must be given sufficient time to decide whether they wish to participate and have all their queries addressed prior to signing the IRB-approved consent form(s). A copy of the signed consent form(s) must be provided to the patient. Study specific procedures will not be initiated until the Subject has signed and dated the appropriate consent forms(s). Patients have full rights to withdraw from the clinical study at any time, irrespective of their initial consent.

14.2.2. Each Subject must also give their permission for representatives of the Sponsor, auditor and regulatory authorities to review their hospital records for the purposes of source data verification.

14.2.3. Written informed consent from the Subject must be obtained before any clinical investigation related procedures are performed.

14.3. Subject Confidentiality

14.3.1. Confidentiality of Subject data will be maintained at all times. Subject anonymity will be guaranteed and all documentation relating to a Subject will be kept in a secure location.

14.3.2. Study documents provided to the Sponsor will be stored in a secure location with access. None of the stored documents will contain any personal identifying information or direct identifiers.

14.3.3. Study files will be made available to the IRB, regulatory authorities, Sponsor (or designee) and/or clinical study staff should they request access for auditing purposes.

15. Data Management

The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All data collection should be completed in a neat, legible manner to ensure accurate interpretation of data.

15.1. Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. Data must be recorded using black ink and any changes or corrections made must be done by drawing a single line thru the original entry. The corrected entry must be initialed and dated by the individual making the corrections. **DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL**. The investigator or designee must review unanticipated problems.

15.2. Study Records Retention

The Principal Investigator will retain all copies of the records for a period of at least 2 years after the latter of the following two dates: the date on which the study is terminated or completed, or the date the declaration of emergency is rescinded. In all cases, the Principal Investigator must obtain written consent from the Sponsor prior to disposing of any records related to the clinical study.

16. Study Materials

- Bio-Self COVID-19 Antigen Home Test
- Bio-Self COVID-19 Antigen Home Test Quick Reference Instructions
- BioTeke Website

17. Literature References

1. Template for Developers of Molecular and Antigen Diagnostic COVID-19 Test for Home Use. Version November 9, 2021
2. Template for Developers of Antigen Tests. Version October 6, 2021
3. Supplemental Template for Developers of Molecular and Antigen Diagnostic COVID-19 Tests for Screening with Serial Testing. Version October 25, 2021

18. Protocol Amendment History

Version	Date	Section	Description of Change	Rationale
1.0	24 Mar 2022		Initial release	
2.0	05 May 2022	5. Synopsis; Study Design	Original: The order of testing will be 1) Subject self-collection, 2) standard of care (SOC) collection by a healthcare practitioner, and 3) comparator test collection by the healthcare practitioner. Revised to: The order of testing will be 1) standard of care (SOC) collection by a healthcare practitioner, 2) layperson conducts the test on themselves or another study participant using the Bio-Self Test, and 3) comparator test collection by the healthcare practitioner.	Additional input from FDA
2.0	05 May 2022	5. Synopsis; Target Population	Original: Subjects will be consecutively enrolled (i.e., in an "all comers" style) until at least 30 RT-PCR confirmed positive subjects and 30 RT-PCR confirmed negative subjects have completed the study. Revised to: The first 50 subjects will be consecutively enrolled (i.e., in an "all comers" style) including those who are either asymptomatic or symptomatic for COVID-19.	Clarification

			Following the enrollment of the first 50 subjects, enroll only subjects who are symptomatic for COVID-19. When at least 30 RT-PCR confirmed positive specimens have been collected from symptomatic subjects, enrollment will continue with all comers, i.e., symptomatic and asymptomatic for COVID-19 until at least 10 RT-PCR confirmed positive specimens have been collected from asymptomatic subjects.	
2.0	05 May 2022	5. Synopsis; Target Population	Clarified enrollment population: At least 30 RT-PCR confirmed positive specimens collected from subjects that are symptomatic for COVID-19.	clarification
2.0	05 May 2022	5. Synopsis; Target Population	Original: Up to 600 subjects may be enrolled in this study to obtain a minimum of 30 RT-PCR confirmed positive subjects of which 10 subjects are asymptomatic and 10-20% are low positive. Revised to: Up to 600 subjects may be enrolled in this study to obtain a minimum of 30 RT-PCR confirmed positive specimens from subjects who are symptomatic, and 10 RT-PCR confirmed positive specimens from subjects who are asymptomatic for COVID-19 with at least 10-20% being low positive.	Clarification
2.0	05 May 2022	5. Synopsis; Site Requirements	Revised the minimum of 3 testing locations to minimum of 2 testing locations	Administrative
2.0	05 May 2022	6.8 Study Design	Revised the order of sample collection and added: To mitigate any potential bias which may affect the subject's visual interpretation of the Bio-Self COVID-19 Antigen Home Test result, the healthcare practitioner will not provide the SOC test results to the subject until after the results of the Bio-Self kit are read by the subject.	Additional input from FDA; clarification
2.0	05 May 2022	6.8 Study Design Comparator	Revised the order of collection and added the results of the SOC will not be shared with the subject until the subject has interpreted the results from the Bio-Self COVID-19 Antigen Home Test.	Additional input from FDA; clarification
2.0	05 May 2022	Section 7.1.6	Revised enrollment from all comers to: The first 50 subjects should be consecutively enrolled (i.e., in "all comers" style), including those who are either asymptomatic or symptomatic for COVID-19. Following the enrollment of the first 50 subjects, enroll only subjects who are symptomatic for COVID-19. When at least 30 RT-PCR confirmed positive specimens have been collected from symptomatic subjects, enrollment will continue with all comers, i.e., symptomatic and asymptomatic for COVID-19 until at least 10 RT-PCR confirmed positive specimens	Clarification

			have been collected from asymptomatic subjects.	
2.0	05 May 2022	Section 7.1.7	Added: Symptomatic to the 30 positive samples	Administrative
2.0	05 May 2022	Section 8.3	Revised the order of sample collection	Additional input from FDA