Validation Protocol, H206

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Dispersed Clinical Validation of the Hyfe Cough Monitoring System

Date of Protocol: December 02, 2024

Protocol Number: ID206-2

Intended Registry: clinicaltrials.gov

Sponsor: Hyfe, Inc

Principal Investigator: Peter M. Small, MD

Affiliate Professor, University of Washington

Chief Medical Officer, Hyfe, Inc

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Age 21 or older
Have problematic cough defined as an expressed concern about their cough
Able to collect 24 hour auditory recording
Reside in location without unusually high noise
Willing to wear watches for 24 hours
Fluent in English
Access to a device for video calls
Have an iPhone for download of the Hyfe Companion App
Accept the Hyfe Companion App Terms of Use and Privacy Policy</td></tr>
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	Exclusion: Ongoing relationship with any company making a cough monitor Participation in a cough monitor device study in the past 6 months Have privacy concerns Inability to avoid unusually loud environments Significant antitussive therapy changes in preceding week
Study Dates	Begin Q4 2024 Complete Q4 2025

1. GENERAL INFORMATION

1.1. Identification of trial

Protocol Number: ID206-2

Title of trial: Dispersed Clinical Validation of the Hyfe Cough Monitoring System

1.2. Details of sponsor

Hyfe Inc.

1209 N Orange St.

Wilmington, DL 19801

1.3. Principal Investigator

Peter Small, MD.

Hyfe North East American Clinical Office.

3 Conant Rd, Hanover NH, 03755

(Hyfe)

1.4. Details of trial investigators

HYFE

PHONE: +1 (206) 261-2824

EMAIL: investigator@hyfe.ai

1.5. Centers where study takes place

Decentralized in USA

1.6. Expected duration of trial

The study will last up to 11 months.

2. BACKGROUND AND JUSTIFICATION OF THE STUDY

Cough is one of the most common reasons for which patients seek medical care in the US - accounting for more than 18 million visits per year (US NAMCS, 2018) and more than $12 billion for over the counter cough remedies alone in 2021. (Statista, 2021) The most common causes of chronic cough are postnasal drip, asthma and GERD. Because of the substantial overlap between these conditions and the cost/benefit of often invasive diagnostic tests, medical management is generally a series of “empiric trials” of over the counter (OTC) medications such as H2 blockers and antihistamines. However, the outcome of these trials is never formally measured, but just based on subjective assessment, which is insensitive, unvalidated, and biased. For about 10% of these individuals the cough becomes unexplained chronic cough (UCC), defined as cough persisting for longer than 2 months without an obvious etiology. In many patients the cough persists despite empiric trials (termed refractory chronic cough, RCC).

The standard of care for treating refractory and unexplained chronic cough is largely empiric and includes beta agonists, H2 blockers, decongestants, inhaled corticosteroids, neuromodulatory therapies and behavioral interventions. In general, these are provided with little objective measurement of efficacy, instead relying on either subjective reports by patients or (in clinical trials) manual counts of cough episodes recorded by specialized instruments. (Decalmer et al, 2007) This results in poor feedback between the treatment prescribed and the impact perceived. It is a clinical paradox that the management of a cough does not include an objective measurement of cough. This paradox does not arise from a lack of desire to have such data but from an inability to collect it as manifested by more than 25 years of unsuccessful product development efforts. (Leconte et al, 2011)

Despite two decades of product development, the currently available digital cough monitoring systems have serious shortcomings. Many depend on the use of external microphone arrays and dedicated systems to detect noises exceeding predefined thresholds and acoustic patterns associated with cough. (Matos et al, 2007; Barton et al, 2012; McGuinness et al, 2012) The use of human listeners to count coughs from 24 hour continuous sound files obtained from contact and ambient microphones that are semi autonomously extracted is often used in pharmaceutical research but is not FDA cleared for cough counting (VitaloJAK). (FDA Briefing Document, Pulmonary-Allergy Drugs Advisory Committee Meeting November 17, 2023)

The Hyfe Cough Monitor System is developed to be a privacy preserving and unobtrusive means of continually monitoring cough due to any etiology for protracted periods that can be shared with health care providers. The ability of a prior version of the HCMS to passively and continuously monitor coughing when used by 23 individuals with problematic cough, under common living conditions has been assessed in a multicenter observational study (see manuscript Appendix A). Twenty four hours of continuous sounds from subjects with a variety of cough causing conditions were recorded while they simultaneously wore the HCMS. The continuous sounds were labeled by multiple trained annotators using validated SOPs (see Appendix B). The timestamps of these human-detected coughs were compared to those of the HCMS to determine the system’s overall performance using hourly rate correlation and event-to-event analysis. Over the 546 hours monitored, 4,454 cough events were detected. The Bland Altman Plot of the concordance of Hyfe reported and human annotated hourly cough rates is shown in Figure 1; the overall bias was 0.23 (95% CI of -0.039 to 0.51), the lower limit of agreement was -3.7 (95% CI -5.2 to -3), and the upper limit of agreement was 4.8 (95% CI 4 to 6). The event by event analysis demonstrated a sensitivity of 90.4% (95% CI of 88.3% to 92.2%), a false positive rate of 1.03 false positives per hour (95% CI of 0.84 to 1.24) and a positive predictive value of 87.8% (95% CI of 81.9% to 91.6%). There were no adverse events reported in any of the subjects enrolled in the study. Although done with a prior version of the HCMS, basic similarities in the device and algorithms provide sufficient confidence to proceed with this validation study (Chaccour et al , 2024).

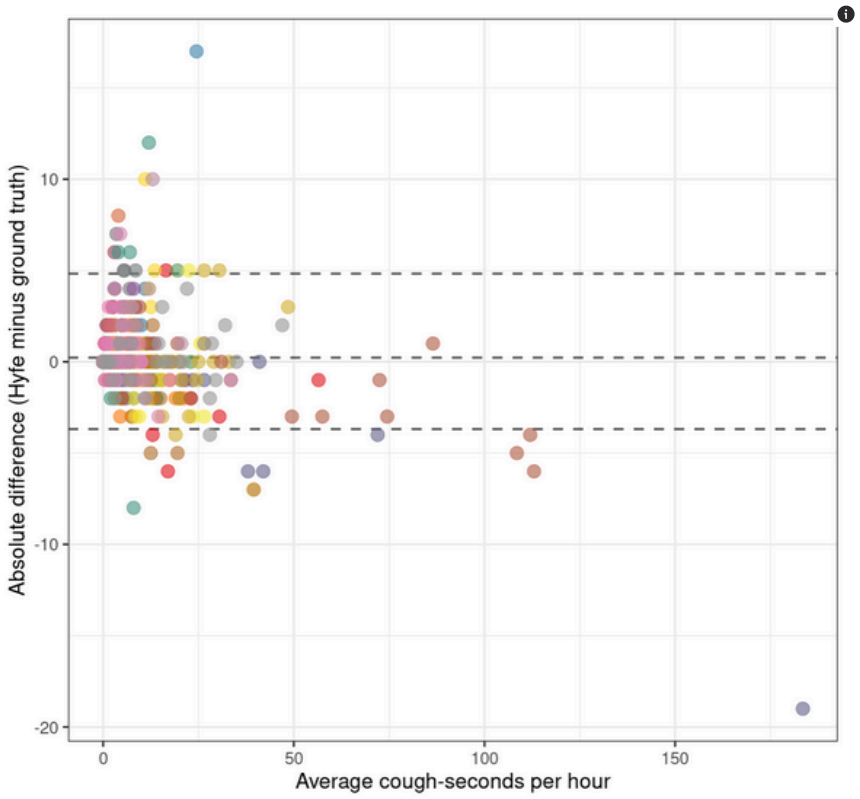

Figure 1: Concordance of Hyfe reported and human annotated hourly cough rates presented in a Bland Altman plot from a validation study (conducted with a prior version of the HCMS). Twenty three subjects were monitored (comprising a total of 546 hours monitored and 4,454 cough events). The overall bias was 0.23 (95% CI of -0.039 to 0.51), the lower limit of agreement was -3.7 (95% CI -5.2 to -3), and the upper limit of agreement was 4.8 (95% CI 4 to 6).

The HCMS that will be used in this current study has a lighter watch with improved battery life, cough detection algorithms have been further optimized and will report results via a companion app.

If the Hyfe Cough Monitor System is validated and cleared for marketing, its ability to quantify coughing could profoundly improve the lives of patients, the quality of care provided by practitioners, the efficiency of clinical studies and the efficiency of health care systems.

2.1. Investigational intervention

We will evaluate the accuracy of the modified Hyfe Cough Monitoring System's (HCMS) ability to recognize and timestamp coughs, and report a patient's hourly cough rate. The HCMS (described in detail below) is a dedicated smart watch running specialized cough recognition software (aka the "Hyfe Watch") connected via Bluetooth to a user interface on a companion iOS application.

Subjects will be recruited remotely through targeted outreach, and contacted by phone, at which time they will be screened for eligibility. Eligible subjects will be consented remotely. Study materials will be mailed to research subjects. On an initiation video call they will be instructed how to download the companion app on their phone, how to pair the Hyfe Watch with the app, and how to wear the Hyfe Watch and a second wrist worn device serving as a continuous sound recorder. These devices will be kept on the wrists during the day and placed on bedside chargers (within 3 feet of the mouth) at night. Participants will be asked to go about their day as usual while wearing these devices. Additionally, participants will be given a printed Hyfe diary to write down the exact time when the devices were turned on/off, when they went to and arose from bed, and any times the devices were not being worn.

Hyfe Cough Monitor

Continuous Sound Recorder

The subjects will be instructed to inform others in their environment that sound is being recorded. Additionally, they will be told that the device is water resistant and can be worn while showering or bathing, however it should remove it while diving, swimming, or while in a sauna. Participants will be instructed to charge the watches on a nightstand next to the bed while they sleep using the provided watch chargers. During charging the devices continue to collect data and combined with the daytime results comprise the 24 hour period of the study. At the end of the 24-hour period, participants will again be contacted and instructed to remove the watches. They will be asked to download the generated data file from the Hyfe companion app and to electronically send this file to the Investigator. The subjects will then mail the devices and study diary back to the researchers.

Continuous ambient sounds collected from each participant will be listened to by 2 different Hyfe trained cough annotators and each explosive sound will be marked as cough or not using proprietary audio annotation software, according to a detailed standard operating procedure. Sounds for which the two labelers disagreed will be adjudicated by a third expert physician annotator who makes the final decision as to how that sound should be labeled. These results will be compared with the coughs detected by the Hyfe Cough Monitoring System and presented according to the statistical plan detailed below.

2.2. Study population

This study will enroll individuals in a dispersed clinical trial with problematic cough recruited so as to reflect the diversity of the intended users of the device in terms of cough frequency, sex, age, race, ethnicity and use environments. We will use social media augmented by distribution of flyers to healthcare practitioners and facilities that see large numbers of respiratory patients. Screening will be conducted by telephone by study personnel and we will record the sex, age, race and ethnicity of participants. Given the breadth of these characteristics among the variety of etiologies (including, but not limited to refractory chronic cough, bronchiectasis, COPD, asthma and IPF), they will not be pre-specified but will be reported as part of our results.

2.3. Evaluation of benefit/risk ratio for the participants in the clinical trial

Being merely observational, the participants will derive no direct benefits from participating in this trial.

There is negligible risk for confidential audio based information being leaked from the Hyfe device given that the algorithms operate fully on the watch, no sounds are retained and only timestamps of coughs are reported, stored and analyzed. Efforts to ensure privacy and security of these data are described throughout this protocol. All data and metadata collected by Hyfe are secured and stored in safe, secure servers. Only anonymized data will be shared beyond the study site to the sponsor and investigators.

There is also a risk that participant confidentiality could be breached due to the continuous 24-hour audio recordings. However, the continuous recordings will be parsed into 30 second anonymized segments by Hyfe study personnel. These

recordings will be accessible by a limited number of Hyfe cough annotators trained for privacy, confidentiality and cough annotation according to the SOPs developed by Hyfe (Appendix B) on a password and ID-protected Hyfe web labeling application. Hyfe will be distributing the logins for the web labeling application only to qualified study personnel. The continuous recordings will be securely transferred to Hyfe's cloud storage and will only be accessible by Hyfe for analysis and annotation and to enrich Hyfe's database for future product development.

The Hyfe Watch utilizes a commercially available smartwatch, with custom firmware installed. The sound recorder watch is also a commercially available device. As these hardware devices have been available as consumer products for many years, there is minimal safety risk to participants from their use in this study. Similar to other smartwatches on the market, the materials used in these watches may contribute to skin irritation or allergic reaction in some users, and there is the possibility that the watch may overheat, becoming warm to touch, causing discomfort or injury. Participants will be advised during the consent process to remove the watches if they experience any irritation or if either watch becomes uncomfortably warm.

Based on the above, while there is no direct benefit to study participants, there are no unacceptable risks of harm as a result of participation in this study.

3. OBJECTIVES OF THE TRIAL

Primary objective: To assess the agreement in cough counting between the Hyfe Cough Monitoring System and human listeners on an hour by hour basis when used by individuals with problematic cough under common living conditions using a Bland-Altman analysis.

Secondary *objective #1:* To assess the agreement in cough counting between the Hyfe Cough Monitoring System and human listeners on an hour by hour basis when used by individuals with problematic cough under common living conditions using a linear analysis.

Secondary *objective #2:* To assess the overall performance of the Hyfe Cough Monitoring System on an event by event basis when used by individuals with problematic cough under common living conditions in terms of sensitivity, false positive rate, and positive predictive value.

Additional information that will be reported

- Adverse events
- Statistics of cough frequency, race, ethnicity, sex and age of participants
- The types and numbers of cough related diagnoses

Exploratory Analysis:

Using the same analytical approaches to:

- Assess the differential performance of the HCMS during daytime versus night time.
- Assess the performance of the HCMS individually for each study subject
- Assess the dependence of the HCMS's performance on subjects' cough rates

Table 1 . Objectives and Endpoints

Objective	Endpoints
Primary: To assess the agreement in cough counting between the Hyfe Cough	Concordance with human annotators using Bland Altman (with

Monitoring System and human listeners on an hour by hour basis using a Bland-Altman analysis	95% CI)
Secondary #1 : To assess the agreement in cough counting between the Hyfe Cough Monitoring System and human listeners on an hour by hour basis using a linear analysis.	Concordance with human annotators using linear analysis
Secondary #2 : To assess the performance of the Hyfe Cough Monitoring System on an event by event basis	Agreement with human annotators in terms of Sensitivity, False Positive Rate and Positive Predictive Value
Exploratory: Differential performance of Hyfe 1. Between day and night 2. Between individuals 3. Between high, middle and low cough rates	Bland Altman (with 95% CI) Linear analysis Sensitivity, False Positive Rate and Positive Predictive Value

4. DESIGN OF TRIAL

4.1. Global design

This is a dispersed observational study and will include individuals with a variety of cough related conditions including but not limited to refractory or unexplained cough, Bronchiectasis, COPD, COVID-19, non-tuberculous mycobacterial infection, tuberculosis, or upper respiratory tract infection.

4.2. Subject recruitment

The goal of recruitment is to identify 30 subjects residing in the United States who have problematic coughs due to a variety of etiologies who are willing and able to participate in a decentralized study. The justification for increasing this from the 20 subjects in the prior study is described below in section 9.3.4 (sample size calculation). This will be accomplished by targeted outreach via social media, email to users of our health and wellness app, members of cough support groups and select physicians and facilities who treat large numbers of respiratory patients. See Appendix C for recruitment materials.

Individuals expressing interest in participation will be contacted by phone by study personnel for eligibility screening, to ensure enrolling diversity in terms of diagnoses and demographics (See Appendix D for screening form). Statistical records will be kept of those excluded and included during enrollment.

4.3. Obtaining consent

IRB-approved Informed consent will be obtained remotely before carrying out any of the specific procedures of the study. The Informed Consent Forms (ICFs -see Appendix E) will be sent to eligible participants for discussion on a phone or video call and signed digitally for those who agree to participate.

Once consent is obtained a study package will be mailed to participants containing the watches, chargers and a printed diary to note the exact timing of beginning and ending wearing the watches, the time they got into and out of bed, when the watch

was charging, and any time they were away from the devices (see Study Diary Appendix F).

4.4. Monitoring period

Subjects will be contacted by study personnel at a predetermined time. On this call, basic clinical information will be collected which will include information on the patient's age, sex, race, ethnicity and cough related diagnosis (see Case Report Form Appendix G). The subjects will be instructed to download the Hyfe Companion App and to pair the Hyfe Watch with the app. They will be advised to place the chargers at their bedside, charge both watches before use, to put the two watches on opposite wrists (noting which side the HCMS is worn on the case report form), wear them continuously during the day and place them on the chargers at their bedside when they go to bed. The monitoring period will be 24 hours conducted under the participants standard living conditions as they go about their activities of daily living.

Not less than twenty four hours later the study personnel will again contact the participants via video call to ensure they have complied with the protocol and completed the diary. They will be asked to download the generated data file from the Hyfe Companion App and to electronically send this file to the Investigator, and they will be instructed to return the study materials by express mail. Study personnel will review the forms for completeness and enter all results in the Case Report Forms. Subjects contributing at least 20 hours of data on both watches will be included in the analysis.

4.5. Cough annotation

Continuous recordings from digital sound recorders will be used to define the cough rate ground truth. To obtain this, these recordings will be annotated by human annotators trained in cough recognition in continuous audio recordings using a detailed standard operating procedure (SOP). The SOP defines cough, describes the role of vocal cords in coughing, and explains the waveform of cough sounds. It outlines procedures for identifying coughs, throat clears, and sneezes, using visual and audio examples and distinguishing those that are from the monitored individual ('near') and from others ('far'). Annotators are trained in using Hyfe's web-based audio annotation software to accurately identify the start and end of coughs and similar sounds, ensuring the entire duration is captured. After training, a test questionnaire is administered for assessment. Annotators are then evaluated based on their performance on a sample audio set with independently verified ground truth data, and individual feedback is provided to them on their accuracy in annotation. Each annotator is assigned a unique ID for Hyfe's web-based dashboard, and the number of audio snippets processed by each ID is monitored.

These trained annotators will listen to the recordings and mark the precise start and stop of each cough using Hyfe's proprietary web-based audio annotation software, following the SOP developed by Hyfe for annotation of continuous audio recordings (Appendix B). An earlier version of this SOP (detailed in Sanchez-Olivieri et al, 2023) was used in evaluating the performance of trained cough annotators and showed excellent intralabeller (Pearson's correlation 0.98) and interlabeller agreement (Pearson's correlation 0.96).

As described in detail in Appendix B, each recording will be listened to by at least 2 blinded independent annotators and all explosive sounds will be annotated. Annotators will have been trained in best practices regarding data privacy (completed online: The Centers for Disease Control and Prevention's (CDC) NCHS Confidentiality Training) which is reinforced in the annotation SOPs developed by Hyfe (Appendix B). Because prolonged annotation is tedious and could cause errors, we anticipate having a group of at least 12 annotators.

Sounds for which the two labelers disagreed on the presence of a cough, the timing of a cough's start time by greater than 100 milliseconds, or the indication that the cough sound was distant or unclear, will be adjudicated by a third expert trained physician annotator who makes the final decision as to how that sound should be labeled. These expert annotators are members of the Hyfe team, with 3-4 years of experience in annotating short (0.5s) and long (60s) audio snippets for cough and cough-like sounds (e.g., throat clears, sneezes and other). The team of third listeners works at Hyfe and adjudicates annotation discrepancies blinded to the behavior of the model and in a manner that is not subject to any bias that would affect the outcomes of cough labeling. The analysis will be based on the final adjudicated annotations of "cough", "not sure" and "far".

These protocols have been designed and tested to ensure high inter- and intra-person consistency and to not be biased by individuals who may have been involved in the design and algorithms of the HCMS (Sanchez-Olivieri I, et al).

5. SELECTION OF STUDY SUBJECTS

5.1. Inclusion criteria

1. Be at least 21 years old
2. Have a problematic cough
3. Willing to have ambient sounds recorded continuously for 24 hours.
4. Residing in a domestic environment without unusually high and / or persistent background sound levels.
5. Willing to wear two watches during the day and keep them at their bedside (within 3 ft from the mouth) during the night.
6. Fluent in English.
7. Have access to a device which can be used for video calls (computer, tablet or mobile phone)
8. Have an iPhone to download the Hyfe Companion App and connect to the Hyfe Watch.
9. Be willing to accept the Terms of Use and Privacy Policy of the Hyfe Companion App.

5.2. Exclusion criteria

1. Have an ongoing business/consulting relationship with any company making a cough monitor.
2. Have participated in a cough monitor device study in the last 6 months.
3. Inability to avoid unusually prolonged loud environments for the duration of the 24-hour study period.
4. Individuals who have had significant change in antitussive therapy in the week preceding study.

The investigator shall try to ensure that the participant completes the required monitoring period. The data of withdrawn participants shall be collected up to the moment of withdrawal. Losses due to recording for fewer than 20 hours or participants who are lost to follow up will be replaced. A participant (or their family, or legal representative) may interrupt their participation in the study at any time and for whatever reason. The principal investigator may also withdraw a participant from the study if he considers that it is in the best interest of the participant.

5.3. Treatment modifications that are not permitted before and/or during the trial

No medications or specific treatments will be prescribed to participants as part of this study. Participants shall continue with any pre-established treatment but not have significantly changed their antitussive therapy, or new medications in the week preceding the study or during the study period.

6. DESCRIPTION OF THE TOOL STUDIED

6.1. Indication for use

The Hyfe Cough Monitor System is an over-the-counter device intended to be used to quantify coughing by passively detecting and timestamping coughs, which are reported as coughs per hour. The Hyfe Cough Monitor System is intended to be used in daily living environments (i.e, home, day to day living and work environments) by people who are 21 and older and considering seeking health care for their problematic cough.

A licensed health care professional's advice is required when making medical decisions based on the results from the Hyfe Cough Monitor System. The Hyfe Cough Monitor System should only be interpreted in the context of all available clinical information and should not be used as the sole basis for diagnosis, treatment or other patient management decisions.

6.2. Hyfe Watch

The HCMS uses locked algorithms to recognize and timestamp coughs. It is comprised of a two-step process: (1) a “peak detection” algorithm continually monitors sound levels (but is not “listening”) identifying short second sound snippets with loudness similar to that of a cough which is of sufficient length to be identified as a cough; (2) each snippet is run through a “cough recognition” algorithm to determine if the sound is or is not a cough. The Hyfe Watch runs the two step algorithm on the device to identify the snippets of sound that are coughs. Both of these steps are performed on the Hyfe Watch such that the only information that leaves the watch is the timestamp of coughs. The resulting timestamps of detected coughs and the times in which the watch was being worn, charged and the battery level will be held locally on the smartwatch until they can be transmitted via Bluetooth to the companion app.

6.3. Hyfe Companion App

Timestamps of cough are transmitted via bluetooth from the Hyfe Watch to the Hyfe Companion App. At the end of the study participants will be instructed to download the timestamp data file from the app and to electronically send this file to the Investigator. Clinical data itself will not be displayed to participants in the app during this study.

6.4. Hyfe Hardware

- H206, a 1.69" smartwatch sourced from Shenzhen Do Intelligent Technology Co., Ltd. with a rechargeable battery and Bluetooth interface that detects and timestamps coughs.

6.5. Hyfe Software

- Algorithm: 1.0.0
- H206 Firmware: 1.0.0
- iOS App: 1.0.0

6.6 Continuous Sound Recorder

Continuous sound will be recorded using a sound recording device worn on the participant’s wrist; this device will serve only as a recording device. The continuous sound data will be downloaded directly from the sound recording watch via a wired connection to the proprietary Hyfe sound annotation software by study personnel.

7. EVALUATION OF RESPONSE

7.1. Endpoints

The main objective of this study is to assess the overall performance of the Hyfe Cough Monitoring System when used by individuals with problematic coughs as they go about their usual activities under common living conditions. The primary objective will be based on hour to hour comparison of coughing rates using Bland Altman analysis. Secondary objective #1 will assess the same, but using linear analysis. Secondary objective #2 will assess the event to event concordance with human annotators reported as sensitivity, false positive rate, and positive predictive value. Similar analysis will be conducted for the exploratory endpoints. The statistical details of this are provided below in the Statistical Analysis Plan.

7.2. Schedule for evaluation, annotation and analysis of the accuracy parameter

Participant recruitment is set to start in Q4 2024. Annotation of continuous recordings and data analysis will be conducted on subject data as soon as possible after their monitoring period.

8. SAFETY APPRAISAL

This study uses smartwatches that are commercially available and used commonly in daily life. There are no anticipated clinical risks or clinical benefits from participants taking part in this study. All participants can withdraw from the study at any point without need to provide justifications.

Privacy concerns will be addressed by clarifying that no sound is ever stored by the HCMS. Access to the continuous audio recordings will be limited to selected researchers at Hyfe Inc, who will be trained for confidentiality and will follow the annotating procedures defined by the sponsor in its SOPs for cough annotation in continuous audio recordings. Measures taken to protect patient's privacy and confidentiality are detailed in the section 10.4 Confidentiality of patients.

9. STATISTICAL ANALYSIS PLAN

9.1. Ground truth data analysis

Ground truth coughs, established from human-annotated continuous sound recordings, will be compared to the results of the Hyfe Cough Monitoring System obtained during this same time period. A timestamped cough generated by the HCMS is considered to be a "match" (ie, a true positive) if its start time falls within 0.2 seconds of the start time of a ground truth cough, *and* that ground truth cough has not yet been matched to another HCMS timestamped cough (ie, one ground truth cough cannot be matched with multiple HCMS coughs; similarly, one HCMS cough cannot be matched to multiple ground truth coughs). A HCMS cough which has no match in ground truth is considered a "false positive" (ie, an incorrect detection); similarly, a ground truth cough which has no match in the HCMS timestamps is considered a "false negative" (ie, a "missed cough").

As described in detail in our publication (Sanchez-Olivieri et al. Performance evaluation of human cough annotators: optimal metrics and sex differences. BMJ Open Respir Res. 2023) most sounds are obvious and consistently annotated. However, in that study, 2.8 to 6.5 percent of explosive sounds were genuinely ambiguous and thus labeled as "not sure". Excluding these ambiguous sounds improves the intra and inter observer reproducibility of our standard operating procedure (Pearson correlation coefficients of 0.99 and 0.96 respectively). In that study, 2 to 4.1 percent of sounds are obviously coughs but are faint, likely due to being from another person who is distant from the watch. Excluding these far coughs decreases the likelihood of inappropriately attributing other people's cough to the HCMS user.

The primary analysis will only analyze "coughs" and exclude "not sure" and "far" sounds. A sensitivity analysis including "not sure" and "far" sounds as coughs will also be presented to determine the potential impact of these ambiguous sounds on the HCMS performance. Thus, the prevalence of such sounds will be calculated, reported and their potential impact on device accuracy assessed.

While there may be subtle distinctions between coughs caused by different diseases, the basic physiology and acoustic pattern (described in detail in the SOPs provided in Appendix B) are such that they need not be sub-recognized by cause for monitoring purposes. Thus, we will include (and report frequencies of) cough due to a variety of etiologies, but we will not pre-specify those conditions. Another common characteristic of all coughing is a diurnal pattern, in which frequencies are the lowest in the early morning hours when patients are in deep REM sleep. Thus, we will be conducting a full 24 hours of monitoring on all subjects and expect their cough rates to vary considerably through this period.

9.2 Missing data

We will exclude from analysis any subject with fewer than 20 hours of recording (with replacement). We will then analyze all remaining data without any corrections.

9.3. Methodology

The HCMS detects coughs on the device and timestamps them to the millisecond. Our trial's primary endpoint compares human annotated timestamps to those detected by the HCMS. The comparison is done in terms of cough-seconds defined as a second during which at least one individual cough occurs. The use of "cough seconds" rather than "coughs" has been discussed extensively in the literature and deemed suitable (Morice et al, 2007). Furthermore, in a prior study, the use of "cough seconds" as the unit of analysis reduced annotator discrepancies by 50% compared to counting individual coughs (Sanchez-Olivieri et al, 2023). For simplicity, we will henceforth refer to "cough seconds" as "coughs" in this document.

Primary Objective: Apply a Bland-Altman analysis to assess the overall performance of the Hyfe Cough Monitoring System on an hour by hour basis when used by individuals with problematic cough under common living conditions.

Secondary *objective #1:* Apply a linear analysis to assess the overall performance of the Hyfe Cough Monitoring System on an hour by hour basis when used by individuals with problematic cough under common living conditions.

Secondary *objective #2:* Calculate the sensitivity, false positive rate, and positive predictive value of the Hyfe Cough Monitoring System so as to assess its overall performance on an event by event basis when used by individuals with problematic cough under common living conditions. As explained in Section 9.2.3 below, specificity is not a relevant metric.

9.3.1. Bland-Altman analysis

Bland-Altman analysis (Bland et al., 1986 and 1999) assesses the agreement between the hourly cough counts obtained by the Hyfe Cough Monitoring System and those determined by the ground truth cough detections. Specifically, plotting the differences between these hourly cough counts against their averages provides a visual summary of agreement. The overall average of the differences in hourly cough counts estimates the systematic bias and accuracy of the Hyfe Cough Monitoring System, while the variability of these differences measures the Hyfe Cough Monitoring System's precision; both must be small for performance to be considered acceptable. The variability should also be uniform throughout the range of observed hourly cough counts. Validation based on a Bland-Altman analysis therefore consists of the following steps:

1. compute the bias (the mean difference of the hourly cough counts) and the corresponding 95% cluster bootstrap confidence interval,
2. compute the 95% Limits of Agreement (LOA) and their 95% confidence intervals using the cluster bootstrap,
3. inspect plots of the hourly differences against their averages, and
4. analyze the regression lines that fit these plots to quantify any proportional bias or other structural defects.

The bootstrap is needed to avoid inappropriate distributional assumptions about hourly cough counts; moreover, the cluster bootstrap is needed to account for correlations within subjects.

9.3.2 Linear analysis

A linear validation analysis proceeds from the assumption that the paired person-hour counts enjoy *a* linear relationship; having checked the plausibility of this assumption visually with a scatterplot, the strength of this linear relationship can be quantified with the Pearson correlation coefficient. A correlation coefficient close to +1 is a necessary criterion for satisfactory performance of the Hyfe Cough Monitoring System, but this only implies that the paired counts cluster tightly about *some* line; we must then analyze the coefficients of the regression line to verify that it is close (in an appropriate metric) to the ideal line y=x. These observations lead to a validation process with four components: using the paired hourly cough-second counts, we will

1. compute the sample Pearson correlation coefficient and the corresponding 95% bootstrap confidence interval,
2. compute the slope of the line of best fit through these points and the corresponding 95% bootstrap confidence interval,
3. compute the intercept of the line of best fit through these points and the corresponding 95% bootstrap confidence interval, and
4. inspect a plot of the residuals for evidence of proportional bias and heteroscedasticity.

For validation purposes, bootstrap confidence intervals are superior to traditional null hypothesis-based significance tests because hourly cough-second counts, which are necessarily non-negative integers, tend to be overdispersed and follow negative binomial distributions, thereby violating traditional assumptions of normality (as needed for power calculations, t-tests for regression coefficients, etc.).

9.3.3 Sensitivity, false positive rate and positive predictive value

To evaluate the Hyfe Cough Monitoring System's success at detecting individual cough events, its sensitivity, false positive rate, and positive predictive value will be calculated.

By comparison with ground truth annotations, each Hyfe Cough Monitoring System timestamp is either a true positive or a false positive. According to the usual formulas,

$$\text{Sensitivity} = \text{Number of true positives} / \text{Total number of coughs},$$

where the denominator is determined by ground truth,

$$\text{False positive rate} = \text{Number of false positives} / \text{Total number of hours of monitoring},$$

and

$$\text{Positive predictive value} = \text{Number of true positives} / \text{Total number of true and false positives}.$$

While specificity is often reported alongside these three metrics, it is not meaningful for cough detection. A “true negative” event in this context would be an instant or an interval of time that is correctly identified as not containing a cough; since coughs can occur at any time and times between coughs can be of arbitrary nonnegative duration, defining such an instant or interval of time is not practical. False positives, on the other hand, are easy to define and identify relative to ground truth annotations, so the false positive rate replaces specificity and is the relevant performance metric.

As detailed above, in prior studies sounds annotated as “far” and “not sure” are uncommon. A sensitivity analysis will also be presented including these sounds as coughs so as to determine the variance of the results based on these events.

9.3.4 Sample size

The number of subjects needed for this validation study is based on the primary endpoint, Bland-Altman analysis. Assuming independent pairs of observations in the following calculations provides conservative sample sizes, as repeated measures designs control for variability between subjects and thereby provide higher power with fewer subjects.

Bland-Altman analysis: To achieve satisfactory agreement, the confidence intervals surrounding the Bland-Altman Limits of Agreement (LOA) should not exceed the allowed difference between hourly cough counts. This is the basis of the sample size procedure outlined in Lu 2016 (Lu et al, 2016), which can be applied once we have reasonable values of the allowed difference , the bias , and the standard deviation σ of the hourly differences. In our pilot validation study:

- 95% of the hourly differences were between -4 and 5. We therefore take δ=5.
- The standard deviation of all hourly differences was 2.15; excluding the 2 extreme outliers, the standard deviation was 1.88. To be conservative, we take σ= 2.1.
- The mean of the hourly differences (whether including or excluding the 2 extreme outliers) was 0.23.

Using these values, δ/σ =2.4 and μ/σ = 0.1. Referring to Table 1 below from Lu 2016 (Lu et al, 2016), we see that the necessary sample size with a power of 80% (β=0.2) is 538 pairs of observations, which translates into 27 subjects who monitor for at least 20 hours per day.

Table 1: Sample size (n) for Bland–Altman method with non-central t-distribution for different standardized differenc limits (μ/σ), different standardized agreement limits (δ/σ), and different type II error (β). ($\alpha = 0.05$).

δ/σ	μ/σ β	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
2.0	0.2	19,152									
2.1	0.2	1,570	14,307								
2.2	0.2	538	1,174	14,307							
2.3	0.2	271	403	1,174	14,307						
2.4	0.2	164	206	402	1,174	14,307					
2.5	0.2	110	128	203	402	1,174	14,307				
2.6	0.2	80	89	123	203	402	1,174	14,307			
2.7	0.2	61	66	84	123	203	402	1,174	14,307		
2.8	0.2	49	51	61	83	123	203	402	1,174	14,307	
2.9	0.2	40	42	48	61	83	123	203	402	1,174	14,307
3.0	0.2	33	35	39	47	60	83	123	203	402	1,174
2.0	0.1	23,685									
2.1	0.1	1,941	19,152								
2.2	0.1	665	1,570	19,152							
2.3	0.1	334	538	1,570	19,152						
2.4	0.1	202	271	538	1,570	19,152					
2.5	0.1	136	166	271	538	1,570	19,152				
2.6	0.1	99	113	164	271	538	1,570	19,152			
2.7	0.1	75	83	110	164	271	538	1,570	19,152		
2.8	0.1	60	64	80	110	164	271	538	1,570	19,152	
2.9	0.1	49	52	62	80	110	164	271	538	1,570	19,152
3.0	0.1	41	43	49	61	80	110	164	271	538	1,570

Sample size: We propose enrolling until 30 subjects have completed at least 20 hours of monitoring which is consistent with the estimate based on the primary endpoint.

9.4 Criteria for success

The goal of this study is to demonstrate the performance of the Hyfe Cough Monitoring System as a device that continually and unobtrusively monitors cough. As has been agreed upon with the FDA, we are not pre-specifying a performance goal for the HCMS, but will report this information in the labeling material. Therefore, the success of this study will be judged based on demonstrating adequate precision of our endpoints as quantified by their margin of error. Thus, the study will be considered satisfactory if the following criteria are satisfied:

9.4.1 Bland-Altman analysis:

1. The bootstrap confidence interval for the bias has endpoints of magnitude at most 0.5.
2. The endpoints of the bootstrap confidence intervals surrounding the 95% Limits of Agreement have magnitude at most 5.
3. The regression line fitting the Bland-Altman plot has a slope that does not differ significantly from 0. This will confirm that the hourly differences do not suffer from proportional bias.

These 95% Bland-Altman Limits of Agreement (LOA) criteria correspond to the extreme acceptable differences between Hyfe's cough-second counts and those of human annotators; by requiring these limits to have magnitude at most 5, we expect most of the differences to have magnitude less than 5. Note that the accompanying criterion for success for the bias is that its confidence interval have endpoints of magnitude at most 0.5, so we expect the average difference to be close to 0. Also, as pointed out in the sample size calculation above, these criteria are consistent with and inspired by the results of our pilot validation study.

9.4.2 Linear analysis

1. The bootstrap confidence interval for the Pearson correlation coefficient has a lower endpoint greater than or equal to 0.90.
2. The bootstrap confidence interval for the slope of the line of best fit contains 1, with a lower endpoint greater than or equal to 0.8 and an upper endpoint less than or equal to 1.2.
3. The bootstrap confidence interval for the intercept of the line of best fit has endpoints of opposite sign, each less than 20% of the average of the ground truth counts in magnitude.
4. The OLS residuals are randomly scattered, exhibiting no patterns of any kind.

9.4.3 Sensitivity, false positive rate and positive predictive value

1. The margin of error of the confidence interval estimating the HCMS sensitivity for cough-second detection is at most 5%.
2. The margin of error of the confidence interval estimating the false positive rate is at most 0.5 false cough-seconds per hour.
3. The margin of error of the confidence interval estimating the HCMS positive predictive value is at least 10%.

9.5 Exploratory Analyses

9.5.1 Daytime versus nighttime performance

Given that there may be value in monitoring cough only during the day or night, a secondary analysis will compare HCMS performance during these periods; "nighttime" is defined as time spent in bed and "daytime" is defined as time not spent in bed. When going to bed, subjects will note their bedtimes and will place the HCMS and the continuous sound recorder in chargers by the bed; reported bedtimes will be validated against the charging times reported by the HCMS. The performance metrics described in Section 9.3 will then be calculated separately for subjects' daytime and nighttime monitoring periods.

9.5.2 Individual performance

HCMS performance will vary from subject to subject; in particular, the HCMS may fail to recognize extremely unusual coughs or perform differently in challenging acoustic backgrounds. Trained human annotators face similar issues, leading to disputed annotations that will be resolved by a third expert annotator.

To understand the impact of atypical coughers on HCMS performance, the performance metrics defined in Section 9.3 will be calculated separately for each individual participant. The distributions of these accuracy metrics will be investigated and summarized, and a post hoc analysis of audio from any subject who has exceptionally poor performance will be done to determine the causes of degraded performance.

9.5.3 Dependence of performance on cough rate

It is possible that HCMS performance could depend on the frequency with which different subjects cough. To assess the impact of this variable, subjects will be stratified into tertiles according to their average cough rates; a subject's average cough rate is that individual's total number of coughs divided by the number of hours of monitoring. The performance metrics defined in Section 9.3 will be calculated separately for these tertiles and compared.

9.6. Criteria for premature termination of the study

N/A

9.7. Procedure for reporting all the deviations from the original statistical plan

Any deviations from the original statistical analysis plan shall be included in the final report of the clinical trial.

10. ETHICAL ASPECTS

10.1. Good Clinical Practice

The study shall be carried out in accordance with the International Conference on Harmonization (ICH) regarding good clinical practice and the corresponding regulatory requirements. The investigator shall be completely familiar with the correct use of the investigational tool as described in the protocol. The essential clinical documents shall be kept to demonstrate the validity of the study and the integrity of the collected data. The main files should be established when the study starts, shall be kept over the course of the study and stored in accordance with the relevant legislation.

10.2. Ethical considerations

The study shall be carried out in accordance with the ethical principles of the latest revision of the Helsinki Declaration and with legislation currently in force. Each component of this study may only start after a favorable decision in writing is received from the ethical committees of WCG IRB for the different recruitment sites/strategies.

Recording of sounds poses specific ethical concerns regarding privacy of data. We propose those are addressable at several independent levels. Device level – While prolonged sound recordings can be used to identify participants (e.g., Amazon's Alexa device), short snippets (< 0.5 seconds) cannot. Conversations or acoustic environments are not recorded by the HCMS, though they are by the voice recorders in this study. Study level – at all points, participants can opt out of the study and apps can be turned off or removed from watches. Our consent process will explicitly describe exactly what is and is not recorded. The use of unique PIN IDs on watches will ensure that only the investigators can link cough data to personal identifiers. Data sharing data transfer agreements will be designed and implemented to ensure codified digital cough data and medical metadata are shared by the investigator in non-identifiable ways.

Datasets shared with other research partners apart from the sponsor will only include deidentified data. Datasets including this kind of information will be kept under lock, and in password-protected PCs exclusively by the investigator. The HCMS solely collects non-identifiable data from users. These data will be stored in servers in the US.

10.3. Information for the participant and informed consent

Once the entire study is explained via a phone or video call, a digitally signed informed consent shall be obtained from the participant before their participation in the study may be made effective. The method used to obtain and document the informed consent and its contents will comply with the International Conference on Harmonization (ICH) regarding good clinical practice and with all the relevant regulatory requirements.

The investigator (or delegated person) shall digitally sign and date the Consent Forms. The investigator shall file the forms in the Investigator's File with Hyfe.

The participant shall receive the Informed Consent Form and shall be informed that participation in the study is voluntary and may be withdrawn at any time without prejudice to the prior medical care. Neither the Study Diary, nor the Informed Consent can be modified without agreement from the corresponding ethical committee and the sponsor.

A copy of the Informed Consent Form will be provided to the participant by email.

10.4. Confidentiality of participants

With a view to respecting patients' privacy, the participants shall be identified with an assigned participant number in all the case report forms, accountability records, reports and communiqués of the study. The investigator shall provide inspectors and any possible monitors or collaborators appointed by the sponsor and the regulatory authorities with access to original records of the participants so that they can verify the data in the case report forms and audit the data collection process. Confidentiality shall be maintained and the participant’s identity shall not be made public, to the extent permitted by relevant legislation and regulations.

10.5. Compensation for the participant

The investigational procedures deriving from the study shall be financed by the sponsor. The sponsor shall not bear the costs of the habitual care of the participant, these are any procedures that would be practiced or treatments that would be received independently of their participation in the study.

Every participant in the study will receive a $100 debit card as compensation for the participation.

10.6. Compliance with protocol

The investigator shall carry out the study in accordance with the protocol provided by the sponsor and once approval or a favorable decision is obtained from the corresponding ethics committee and the relevant regulatory authorities. The protocol should not be changed without the consent of the investigator and the sponsor. Any relevant changes to the protocol require approval or a favorable decision in writing from the corresponding ethics committee prior to its implementation unless the modification is necessary to prevent immediate risks to participants. The sponsor shall present all the changes made in the protocol to the regulatory authorities in accordance with legislation currently in force.

When an immediate deviation of the protocol is required to prevent immediate risks to participants, the investigator shall contact the sponsor, if the circumstances permit, to consider the measures to be adopted. Any deviation from the protocol should be documented in detail in the study file. The conduct of the study will be monitored by the Hyfe team and a compliance file maintained.

11. PRACTICAL CONSIDERATIONS

11.1. Direct access to source data/documents

The sponsor shall guarantee in the protocol or other written agreement that the investigator or the institution shall permit direct access to the source data or documents for monitoring, auditing, revision by the corresponding ethics Committee, and for inspection of the trial by the medical authorities.

11.2. Responsibilities of all the participants in the trial

Investigator

The investigator should agree with this protocol and have an in-depth knowledge of the properties of the products used in the clinical trial. He/she shall sign the applications for authorization sent to the corresponding ethics Committee and/or the institutional review board and is responsible for the same, including its execution, commencement and completion.

The investigator should review the information sheet with the participant and collaborate with him/her to help them understand the explanation provided in the document. It is important for the investigator to inform participants that their participation in the study is totally voluntary and that it does not affect the doctor/patient relationship, and to assure them that all the persons involved in the study shall respect the confidential nature of any information relating to the participant.

The Principal Investigator or one of his/her collaborators shall be responsible for correctly collecting, interpreting, and reporting the data (including precise start/stop times) and shall ensure that any serious or unexpected adverse events shall be reported within 24 hours.

It is the duty of the investigator to regularly inform the corresponding ethics committee of the progress of the study and he/she shall be jointly responsible with the sponsor in preparing the final report.

Sponsor

The sponsor of the study (Hyfe, Inc) is the natural person or legal entity that has an interest in completing the study. The sponsor shall also be responsible for ensuring compliance with the relevant legal standards.

The sponsor takes on the obligations of a sponsor contained in legislation currently in force, providing all the resources and collaborators required to fulfill said responsibility with full guarantees.

The sponsor shall provide the investigator with an Investigator's File. This file shall be used for all the relevant documents related to the study. The investigator shall be responsible for updating the Investigator's File, checking that all the required documents are included during and after the study. The file shall be inspected during the monitoring visits and shall be kept by the investigator after the study.

11.3. Audit/monitoring

The regulatory authorities, the corresponding ethics committee and the sponsor or an appointed representative may ask for access to all the original documents, case report forms of the participants and other documentation of the study in order to carry out an audit or inspection at the center. The investigator should guarantee direct access to these documents and collaborate at all times in said activities.

12. DATA MANAGEMENT

12.1. Case Report Forms

The Case Report Forms (CRFs - see Appendix G) shall be completed by the investigation team, transcribing the data from the original documents that form the participant's clinical history. The CRF may be completed by any authorized person, whose signature is recognized. Any changes shall be made in a clearly visible manner and shall have the initials of the person who made the correction and the date when they were made. The completed CRF shall be checked for completeness upon completion of the participant's study activities by study personnel.

Any outcome outside the range of normality shall be duly commented.

12.2. Documents to be kept by the investigator

The investigator shall keep all the original documents of the study diary, CRFs and the identification list of the subjects for a period complying with the local clinical trial regulations or seven years (whichever is longer). This documentation shall not be destroyed without written consent from the trial sponsor.

The investigator shall keep all the records of the study in accordance with the good clinical practices of the International Conference on Harmonization (ICH) and the corresponding regulatory requirements.

12.3. Study archive

The sponsor shall maintain the Principal Archive of the Study for a period of at least 2 years following the date on which an application for a research or marketing permit, in support of which the results of the nonclinical laboratory study were submitted, is approved by the Food and Drug Administration.

12.4. Final report

The investigation team shall draw up a report suitable for presentation to the relevant authorities.

13. PUBLICATION OF RESULTS OF TRIAL AND USE OF INFORMATION

The results of this clinical trial shall be published in scientific journals and shall mention the corresponding Ethics Committee, which approved the study.

Basic regulations of the trial

The results of this study will be used to support market approval applications, including a de novo application of the Hyfe Cough Monitoring System to the FDA.

In order to guarantee data accuracy, the Principal Investigator may not transmit to third parties, divulge and/or publish the results obtained in this trial with prior written consent from the sponsor.

They should in any case respect the following conditions:

The results of this study may not be published until completion of the primary objective, or before, if all parties agree to do so.

1. The sponsor shall not cite the names of the investigators without their authorization, except when referring to work that is already published.
2. The sponsor shall permit publication of the data obtained in this trial in journals of recognized scientific prestige and the divulgation of its contents in seminars and conferences in the professional medical sphere, as long as the conditions in paragraphs a) and b) of this section are respected and if the final draft of the article may be reviewed within a maximum of thirty days. In any case, the legitimate interests of the trial sponsor shall be protected, such as in obtaining optimal protection of patients, coordination in the presentation of documents to medical authorities or other studies in progress in the same field, protection of confidential data and information, etc.
3. These regulations are understood to be applicable to information obtained in uncompleted trials or studies that were suspended before termination.

Future use of data

De-identified data collected in this study may be used by the Sponsor for future research and development, such as the development of additional algorithms and medical devices. The Sponsor will ensure that the data is used in accordance with local laws and regulations.

14. REFERENCES FROM LITERATURE

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15. SIGNATURES

This Test Plan was prepared by:

Signature Date

Printed Name

This Test Plan is Approved for Use.

Approval Granted by:

Signature Date

Printed Name

Title	Title
Telephone	Telephone

16. REVISION HISTORY prior to Confluence (2nd Dec 2024)

Version	Description	Effective Date
V 1.0	Initial release	8-20-24
V 1.1	Update to objectives, endpoints and statistical analysis following FDA Q sub meeting	11-11-24
V 1.2	Update to include use of Hyfe Companion App to pair with Hyfe Watch and download data file for analysis	12-02-24

Appendix A - Manuscript: Validation of the Hyfe cough monitoring system: a multicenter clinical study

Chaccour C, Sánchez-Olivieri I, Siegel S, Megson G, Winthrop KL, Berto-Botella J, de-Torres JP, Jover L, Brew J, Galvosas M, Rudd M, Small P Validation and accuracy of the Hyfe cough monitoring system: a multicenter clinical study. Scientific Reports. (in peer-review). Manuscript available HERE

Appendix B - SOP for Cough Annotation

linked here

Appendix C - Recruitment Material

Validation User Recruitment Material

Appendix D - Phone Screener

Validation Screener

Appendix E - Consent Form

Validation Consent Form

Appendix F - Study Diary

Validation Diary

Appendix G - Case Report Form

Validation CRF

Document Activity

User	Action	State	Comment	Date	Version
Document Management	Changed state to **In Approval**	o In Approval		December 4th 2024 at 5:26:49 PM (UTC)	v. 3
Peter Small	Signed and approved **Approval**	o In Approval		December 4th 2024 at 5:26:49 PM (UTC)	v. 3
Stephanie Swift	Signed and approved **Approval**	o Draft		December 4th 2024 at 3:36:19 PM (UTC)	v. 3
Parmjeet Gill (Unlicensed)	Signed and approved **Approval**	o Draft	*"Validation Protocol, H206 reviewed and approved"*	December 4th 2024 at 6:45:51 AM (UTC)	v. 3
Parmjeet Gill (Unlicensed)	Assigned **Stephanie Swift** to **Approval**	o Draft		December 4th 2024 at 6:44:36 AM (UTC)	v. 3
Parmjeet Gill (Unlicensed)	Assigned **Parmjeet Gill (Unlicensed)** to **Approval**	o Draft		December 4th 2024 at 6:44:36 AM (UTC)	v. 3
Parmjeet Gill (Unlicensed)	Assigned **Peter Small** to **Approval**	o Draft		December 4th 2024 at 6:44:36 AM (UTC)	v. 3
Parmjeet Gill (Unlicensed)	Changed state to **Draft**	o Draft		December 4th 2024 at 4:07:03 AM (UTC)	v. 3
Parmjeet Gill (Unlicensed)	Changed state to **Draft**	o Draft		December 4th 2024 at 3:04:56 AM (UTC)	v. 2
Tamsin Chislett	Assigned **Stephanie Swift** to **Approval**	o Draft		December 3rd 2024 at 10:10:36 PM (UTC)	v. 1
Tamsin Chislett	Assigned **Peter Small** to **Approval**	o Draft		December 3rd 2024 at 10:10:36 PM (UTC)	v. 1
Tamsin Chislett	Assigned **Parmjeet Gill (Unlicensed)** to **Approval**	o Draft		December 3rd 2024 at 10:10:36 PM (UTC)	v. 1
Document Management	Changed state to **Draft**	o Draft		December 3rd 2024 at 9:36:59 PM (UTC)	v. 1