CLINICAL STUDY PROTOCOL

FOR THE

MAKANI SCIENCE RESPIRATION MONITORING SYSTEM

Pivotal Clinical Study Protocol

Protocol CP-0001

07/29/2022

Makani Science, Inc.
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U.S.A.

Contents

Investigator Approval

Study Title:

Pivotal Clinical Study for the Makani Science Respiration Monitoring System

Study Device:

Makani Science Respiration Monitoring System (RMS)

Study Sponsor:

Makani Science, Inc.

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U.S.A.

Principal Investigator Acknowledgement Signature:

I have received and reviewed this version of the above noted study protocol and will conduct the study in accordance with the outlined protocol requirements, all attachments, and applicable local and Food and Drug Administration (FDA) regulations.

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Principal Investigator's Name (print)

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Principal Investigator's Signature **Date**

Version History

Version	Date	Author	Description of Change	Justification
A	22-Apr-2022	Michael Chu	Initial Version	
B	07/29/2022	Michael Chu	Changed protocol and reference measurement.	Based on feedback on data from first study.

Study Summary

Study Title	Pivotal Clinical Study for the Makani Science Respiration Monitoring System
Study Device	Makani Science Respiration Monitoring System
Study Objective	The study objective is to compare the accuracy of the Makani Science Respiration Monitoring System (RMS) to manual respiration rate counting by a trained individual (reference) for measuring respiratory rate in adult subjects, supporting its indications for use as a non-invasive system intended to monitor a patient's breathing in both supine and reclined positions using two Makani Science sensors. It graphically displays respiration versus time and reports an approximate respiratory rate value. Its measurements are used as an adjunct to other clinical information sources. The Makani Science RMS is intended to be used on adults by professionals in healthcare facilities and dental offices.
Indications for Use	The Makani Science Respiration Monitoring System (RMS) is a non-invasive system intended to monitor a patient's breathing. It graphically displays respiration versus time and reports an approximate value of respiratory rate. Its measurements are used as an adjunct to other clinical information sources. The Makani Science RMS is intended to be used by healthcare professionals in healthcare facilities and dental offices on adult patients.
Study Design	Prospective, non-blinded, open-label, single-arm study
Enrollment/Number of Sites	Single-center/two sites
Subject Population	30 healthy adult subjects: 15 subjects with normal BMI; 15 subjects with overweight BMI
Primary Endpoint	To evaluate the respiratory rate (RR) accuracy of the Makani Science RMS compared to manual respiratory rate counting by a trained individual in with subject in a supine and reclined positions.
Secondary Endpoints	1. RR bias of the Makani Science RMS compared to manual respiratory rate counting by a trained individual. 2. RR precision of the Makani Science RMS compared to manual respiratory rate counting by a trained individual.
Subgroup Analysis	Subgroup analyses of primary and secondary endpoints will be presented by subject BMI (normal: BMI < 25 and overweight: BMI ≥ 25).
Safety Analysis	Any and all adverse events will be recorded for the duration of the study period. The severity of all adverse events will be assessed based on risk management procedures and will be recorded. Serious adverse events will be recorded.
Statistical Considerations	The null and alternative hypotheses for the primary endpoints are as follows: $H_{01}: \sigma_1 \geq PG$ v. $H_{a1}: \sigma_1 < PG$, $H_{02}: \sigma_2 \geq PG$ v. $H_{a2}: \sigma_2 < PG$

	where σ_1 is the RR accuracy represented by the root-mean-squared-percentage-error (RMSPE) for data collected in the supine position, σ_2 is the RMSPE for data collected in the reclined position, and PG is the performance goal. The PG for the primary endpoint is set at 20% defined based as in Atkinson et al. using the ExSpiron respiratory volume monitor by Respiratory Motion Incorporated. The primary hypotheses will be sequentially tested using a gate-keeping approach, each with a one-sided significance level of 0.025. If $H_{01}$is rejected, H_{02} may be tested. If $H_{01}$is not rejected, H_{02} will not be tested. Once 50% of the planned subjects have completed the study, an interim analysis of RR accuracy will be performed and sample size re-estimated. The new sample size will be calculated as the sample size necessary to yield an 80% conditional probability of trial success (i.e., meeting both primary endpoints), given the interim results. If the conditional probability of demonstrating accuracy in the supine position is less than 50%, the study will be stopped for reassessment of the protocol. If the conditional probability is greater than or equal to 50%, the study will proceed to completion with the new sample size, capped at a maximum of 50 participants. If the study continues following the interim analysis, the sponsor will remain blinded to the primary endpoint results and thus, no overall alpha adjustment is required.
Inclusion Criteria	• Healthy adult subjects between ages of 22 and 99 years old. • Body mass index between 15 and 35 kg/m^2.
Exclusion Criteria	• Medical history of serious cardiac (e.g., heart failure, coronary artery disease) or pulmonary diseases/conditions (e.g., poorly controlled asthma, chronic obstructive pulmonary disease, chronic cough). • History of serious skin irritation (severe rash or blisters) caused by medical adhesives (tape). • Subject cannot lie still on their back for one (1) hour. • Subject is pregnant. • Subject is unable to give written, informed consent.
Principal Investigator	Dr. Vinit Joshi
Study Sponsor	Makani Science, Inc. 5270 California Ave, Ste 300 Irvine, CA 92617 U.S.A.
Study Management Contact	Michael Chu, Ph.D. michael@makaniscience.com

Makani Science Pivotal Clinical Study Protocol

1 Introduction

1.1 Clinical Background

Continuous respiratory monitoring is critical for situations where the patient's breathing is at risk [1, 2, 3]. For example, sedated patients undergoing, or recovering from, medical procedures are at a higher risk of developing respiratory failure and require continuous monitoring [4, 5]. Failure to detect such events in a timely manner can lead to preventable complications and even death, commonly described as "found dead in bed". [6] Respiratory failure events that occur after medical procedures involving sedatives or opioids are significant contributors to complications, death, and prolonged hospital length of stay [7, 8].

Respiratory rate can be measured by a trained individual counting the number of breaths a patient breathes per minute. This is the standard practice for measuring rate during patient spot checks, but it is has neglected by health care professionals for a number of different reasons [9]. Breathing can also be measured continuously using certain monitoring systems. However, the current standard of care monitors for respiration, pulse oximetry and capnography, are ill-suited for detecting incipient respiratory failure. A recent study across 16 sites in 1,335 patients receiving parenteral opioid showed that capnography and pulse oximeters were least accurate measure for respiratory depression in these patients [10]. Additionally, both systems have known shortcomings described below.

Pulse oximetry is a measure of a patient's blood oxygenation through an optical sensor. While this type of monitor is ubiquitous and low cost, it is known to be a late indicator of respiratory depression. The Joint Commission Sentinel Event Alert, Issue #49, on the Safe Use of Opioids in Hospitals recommends that health care workers should be educated not to rely on pulse oximetry alone because it can indicate adequate oxygen saturation even in patients who are actively experiencing respiratory depression, especially when they are receiving supplemental oxygen [11]. Other important limitations of pulse oximetry include: (1) frequent false alarms; (2) its susceptibility to motion artifact; (3) its poor ability to predict respiratory decline; and (4) insensitivity to early signs of hypoventilation. Moreover, on February 19th, 2021, the US Food & Drug Administration issued a Safety Communication stating that pulse oximetry frequently gives inaccurate readings based on patient's skin color, skin thickness, and temperature [12].

Capnography measures the partial pressure of exhaled carbon dioxide ($EtCO_2$) and is widely used for patients under general anesthesia [13]. However, use of the system outside the operating room is inconsistent and facilities have been slow to adopt this technology [14, 15]. While capnography better detects respiratory failure than pulse oximetry, it also has limitations, including: (1) delayed response time; (2) frequent false alarms; (3) readings that often are inaccurate and unclear; (4) complicated to use and interpret; (5) accuracy highly dependent on sensor position, which often can be dislodged from the patient's nose; and (6) uncomfortable to wear over time.

While pulse oximetry and capnography can monitor patient respiration continuously, respiratory depression and failure can be best detected by measuring the patient's ventilation (breathing rate and depth) [15]. This is currently done via spot checks by a trained individual in hospital settings [9]. Changes in ventilation can indicate respiratory failure much earlier than the current standard of care [2, 16]. Respiratory rate in particular, is an underutilized ventilatory parameter that can be important in assessing a patient's health [17, 18]. Additionally, a patient's respiration is a dynamic process that changes over time [2, 19]. The ability to monitor changes in breathing patterns can provide better information about the patient and may even permit inference about future adverse respiratory events. Therefore, a need exists for an accurate monitor to provide continuous information on the patient's ventilatory status.

Patient ventilation can be continuously monitored through different methods including direct measurement of flow in line with the airway, through bioimpedance measurements of the chest wall, acoustically through the airways, or by measuring torso movements.

Direct Airway Measurement

The current gold standard for continuous ventilatory monitoring is through capnography measuring respiratory rate using a mask or nasal cannula. Use of capnography is included in many standards and is well accepted by the field. Direct measurement of ventilation from the airway can also be made through other flow-meter type devices, such as the Linshom monitor [20], which hooks onto a capnography mask. Apart from the general problems associated with capnography, direct measurement of ventilation in line with the airway requires access to the patient's mouth or airway, which is not always possible.

Bioimpedance Measurement

The flow of air into and out of the lungs will change the overall impedance of the chest cavity. The change in impedance can be measured by injecting an electrical signal into the body and measuring it's change with breathing. This data can then be used to calculate respiratory rate and volume. Systems like Respiratory Motion's ExSpiron 1Xi monitor uses this method to great effect [21]. However, while this method can provide both respiratory rate and volume, it adds another wire, which tethers the patient to a large, bulky monitor.

Acoustic Measurement

Breathing will generate turbulence in the airway, which can be measured acoustically [22]. Monitoring for breathing sound not only provides respiratory rate information, but can also indicate other patient states, such as wheezing, etc. Equipment like a precordial stethoscope makes it possible to listen to the patient's respiration; however, acoustic systems are very susceptible to motion artifacts and noise, limiting its use to certain environments.

Chest Wall Movement

Patient chest wall and abdomen will naturally move from breathing. Medical professionals are trained to look at the chest wall movement to assess the patient's respiratory status. Quantitatively measuring the

chest wall movement can also provide ventilatory information; for example, respiratory inductive plethysmography (RIP) uses two large bands placed around the patient's torso to measure the change in circumference. While simple to use, this type of system is prone to motion artifacts and the bands slipping away from their required placement locations. Chest rise and fall can also be monitored using a contactless method via radar (Circadia Health) [23]. This method requires direct line of site to the patient's chest wall and offers the advantage of not adding an additional wire to the patient. However, direct line of sight limits the use case of such a system to environment where the patient's chest is exposed.

While all the methods described above monitor ventilation continuously, they are either cumbersome to use or are subject to certain common environmental factors. The Makani Science Respiration Monitoring System provides a ventilatory monitoring solution that removes the cumbersomeness of wires and a bulky monitor while providing a simple way to monitor ventilation that medical professionals are already familiar with (chest wall movement).

1.2 Study Rationale

The purpose of this study is to demonstrate that the Makani Science Respiration Monitoring System (RMS) can accurately measure respiratory rate, compared to a trained individual manually counting breathing for subjects in both supine and reclined positions.

The Makani Science RMS is a non-invasive system intended to monitor a patient's breathing. It graphically displays respiration versus time and reports an approximate value of respiratory rate. Its measurements are used as an adjunct to other clinical information sources. The Makani Science RMS is intended to be used on adult patients by trained professionals in healthcare facilities, and dental offices.

2 Study Device

Figure 2-1. Representative diagram of the Makani Science RM starting from the sensor placed on the torso (left), Bluetooth communication to the iPad (middle) and image of the Makani Science app displaying the chest wall waveform and respiratory rate.

2.1 Device Description

The Makani Science RMS (subject device) uses a wireless, wearable sensor that can measure respiratory rate based on the expansion and contraction of the patient's chest (Figure 2-1a). The device uses highly stretchable soft strain sensors to measure torso expansion and contraction from respiration. The change in sensor length is then used to calculate respiratory rate.

The concept of using thoracic deflection to calculate the breathing rate is well documented and has been applied with respiratory inductive plethysmography (RIP), commonly used in sleep labs. RIP measures the change in transverse circumference of the chest using large bands; the changes in chest wall circumference has been shown to have a linear correlation with tidal volume waveform [24]. In a previous publication, it was demonstrated that the concept behind RIP can be preserved even when the measurement length of the chest wall is reduced to a 1 cm length of skin on the torso by using a highly sensitive strain sensor (Figure 2-1b). [25] This significantly reduces the footprint needed for the measurement, making a wearable system more practical.

The Makani Science RMS consists of two disposable "Band-Aid®" size sensors and an iPad, which serves as the monitor. The sensors consist of a Bluetooth-enabled circuit connected to the soft strain sensor; the iPad has a custom app that connects with each sensor via Bluetooth and displays the respiratory rate and waveform. The sensors are attached to the skin with medical grade adhesives, to measure the torso expansion and contraction. A single sensor can be used to capture movement from the abdomen; two sensors can be combined to capture the movement of both the chest wall and abdomen. As the app receives the raw waveforms from the sensors, it will calculate the respiratory rate and display a filtered waveform representing the chest wall rising and falling (Figure 2-2).

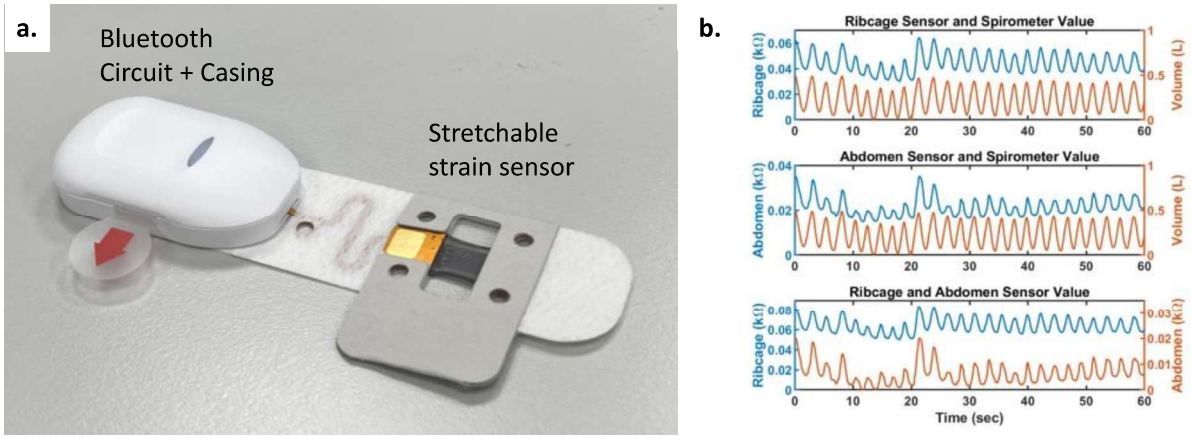

Figure 2-2 (a) Image of the Makani Science RM sensor. The Bluetooth circuit and the stretchable strain sensors are labeled within the image. (b) Waveforms showing the ribcage and abdomen rise and fall compared against the tidal volume waveform from a flow meter. [25]

The Makani Science RMS is an adjunct device that is to be used simultaneously with another standard respiratory monitoring device (e.g., capnography). The Makani Science RMS will be an FDA Class II device.

2.2 Indications for Use

The Makani Science RMS is a non-invasive system intended to monitor a patient's breathing. It graphically displays respiration versus time and reports an approximate value of respiratory rate. Its measurement is used as an adjunct to other clinical information sources.

The Makani Science RVM is intended to be used on adults by professionals in healthcare facilities and dental offices.

3 Study Overview

This is a prospective, non-blinded, open-label, single-arm study. Participants will follow the protocol-specified breathing procedures and respiratory data will be simultaneously recorded for each participant using : (1) the Makani Science RMS and (2) manual respiratory rate counting by a trained individual. This study uses the same reference for measuring respiratory rate as Respiratory Motion for their ExSpiron device [26]. Enrollment will be targeted to balance normal (BMI < 25) and overweight (BMI ≥ 25) subjects.

3.1 Study Objective

The study objective is to compare the accuracy of the Makani Science Respiration Monitoring System (RMS) to manual counting by a trained individual for measuring respiratory rate in adult subjects, supporting its indications for use as a non-invasive system intended to monitor a patient's breathing in the supine and reclined positions using two Makani Science sensors. It graphically displays respiration versus time and reports an approximate respiratory rate value. Its measurements are used as an adjunct to other clinical information sources.

The Makani Science RMS is intended to be used on adults by professionals in healthcare facilities and dental offices.

3.2 Study Endpoints

3.2.1 Primary Endpoint

- Respiratory rate (RR) accuracy of the Makani Science RMS compared to manual counting by a trained individual for both supine and reclined positions. Accuracy is estimated using the root-mean-squared-percentage-error (RMSPE) comparing the Makani Science RMS and the reference device.

3.2.2 Secondary Endpoints

- RR bias of the Makani Science RMS compared to manual counting by a trained individual for both supine and reclined positions. Bias is estimated using the mean paired difference comparing the Makani Science RMS and the reference.
  RR precision of the Makani Science RMS compared to manual counting by a trained individual for both supine and reclined positions. Precision is estimated using the standard deviation of the paired differences of the Makani Science and the reference.

3.2.3 Subgroup Analysis

Subgroup analyses of primary and secondary endpoints will be presented by subject BMI (normal: BMI < 25 and overweight: BMI ≥ 25).

3.3 Study Population

3.3.1 Inclusion Criteria

Subjects must meet the following inclusion criteria to participate in the study:

- Healthy adult subjects between ages of 22 and 99 years old.
- Body mass index (BMI) between 15 and 35 kg/m^2.

3.3.2 Exclusion Criteria

Subjects who meet any of the following exclusion criteria will not be eligible to participate in the study:

- Medical history of serious cardiac (e.g., heart failure, coronary artery disease) or pulmonary diseases/conditions (e.g., poorly controlled asthma, chronic obstructive pulmonary disease, chronic cough).
- History of serious skin irritation (severe rash or blisters) caused by medical adhesives (tape).
- Subject cannot lay still on their back for one (1) hour.
- Subject is pregnant.
- Subject is unable to give written, informed consent.

3.3.3 Patient Enrollment Plan

- A representative sample of the target patient population will be enrolled for this study.

- Specific enrollment numbers will be targeted for participants with normal and overweight BMI (15 normal subjects, defined as BMI between 15 and 25, and 15 overweight subjects, defined as BMI between 25 and 35).

3.4 Study Data to be Recorded

3.4.1 Patient Baseline Data

The following baseline data will be recorded for each participant.

- Birthdate (age)
- Sex
- Weight
- Height
- Abdomen circumference
- Torso circumference

3.4.2 Data for Primary and Secondary Endpoints

The following data will be collected and analyzed specifically for the primary and secondary endpoints. The data will be recorded by the investigator as well as captured via video recording of the screen.

- Subject respiratory rate via manual counting by a trained individual for both supine and reclined positions.
- Concurrent respiratory rate over time from the Makani Science RMS system for both supine and reclined positions.
- Baseline patient information recorded by the clinical researcher

3.4.3 Data for Subgroup Analysis

The following data will be collected for the subgroup analyses of the primary and secondary endpoints presenting the data by subject BMI:

- Normal BMI <25
- Overweight BMI ≥ 25

3.4.4 Safety Analysis and Adverse Event Recording

The safety analysis shall be performed by recording adverse events (AE) for the study period. The study period is defined as the period from the start of any study procedures to the end of the study follow-up. For this study, the subject's study period is defined as the start of placing the non-invasive Makani Science device on the subject until removal of the device, which defines the subject's study exit.

Information on any and all adverse events shall be recorded immediately in the source document and in the appropriate adverse event module of the Case Report Form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedure results will be recorded in the source documentation.

Any and all adverse events occurring during the study period will be recorded and reported in the associated Clinical Study Report. The clinical course of each event will be followed until resolution,

stabilization, or until it has been determined that the study participation or device is not the cause. The severity of all adverse events will also be assessed based on current risk management procedures and will also be reported in the Clinical Study Report.

Serious adverse events (SAE) that are still ongoing at the end of the study period must be followed-up to determine the outcome. Any serious adverse event that occurs after the study period and is possibly related to study participation will be recorded and reported immediately to Makani Science. Adverse events definitions are detailed in Appendix 2 – Adverse Events Definition.

3.4.5 Protocol Deviations

Any deviations from this protocol will be recorded in the associated Clinical Study Report. Potential major deviations will be reported using the following categories:

- **Makani Science RMS Failure**
  If the Makani Science RMS fails to operate at any point of the study (e.g., the subject device fails to turn on, turns off, disconnects, shows non-continuous waveforms, or any other unforeseen condition that causes the investigator to pause the study for a prolonged period of time), all study results will be discarded, a new Makani Science RMS sensor will be applied to the subject, and the study will be restarted. This will be considered a device failure, and not a failure of the study. The Makani Science RMS failure will be recorded and a root cause analysis for the failure will be performed.
- **Deviation from Guided Breathing**
  If the subject noticeably deviates from the guided breathing protocol (e.g., falls asleep or is showing difficulty following the guided breathing), the study results from that specific segment will be discarded, and the subject will be asked to repeat the breathing protocol for that segment. If the subject deviates excessively from the protocol (i.e., over twice per respiratory maneuver), their data will be removed from the overall analysis. All cases described here will be considered as the subject's failure to follow protocol and the deviation will be recorded.
- **Failure to record baseline information**
  If the investigator fails to record the subject baseline information or has reason to believe the patient baseline information is not accurate, the investigator will follow up with the subject via phone. The deviation will be recorded.

3.5 Risk Analysis

3.5.1 Risks

The subject device does not have known direct major risks to the health and safety of the patient. There is no SAE anticipated for this study.

Anticipated minor risks includes:

- Minor skin irritation in certain participants from the adhesive used in the Makani Science RMS sensor.

3.5.2 Minimization of Risks

Precautions that will be taken to avoid risks related to the study include:

- Well-defined clinical study protocol, including specific inclusion and exclusion criteria to enroll appropriate subjects in the trial.
    - Patients with significant and extreme skin reaction to adhesives will be excluded from the study.
- Use of approved medical grade adhesives.
- Explanation of potential study risks and adverse events to each subject, and
- Ensuring that each subject fully understands the Informed Consent process and has provided their written consent prior to proceeding with any study procedures.

4 Study Design

This is a prospective, non-blinded, open-label, single-arm study. Participants will follow the protocol-specified breathing procedures and respiratory data will be simultaneously recorded for each participant using: (1) the Makani Science RMS and (2) a trained individual manually counting respiration for two positions (supine and reclined). Enrollment will be targeted to balance normal (BMI < 25) and overweight (BMI ≥ 25) subjects. To satisfy select design requirements (detailed in section 4.8), all testing will include an operational and active multi-vital monitor (Phillips MP50), be done with the Makani Science RMS sensors pre-stretched, and be done with the application site of the Makani RMS sensors cleaned with alcohol wipes.

4.1 Performance Goal

The study objective is to compare the accuracy of the Makani Science RMS to manual respiratory rate counting by a trained individual (reference) for measuring respiratory rate in adult subjects to support its indications for use as a non-invasive system intended to monitor a patient's breathing in the supine and reclined positions. The Makani Science RMS graphically displays respiration versus time and reports an approximate value of respiratory rate. Its measurements are used as an adjunct to other clinical information sources. The Makani Science RMS is intended to be used on adult patients by professionals in healthcare facilities and dental offices.

The primary study objective is to demonstrate that the RR accuracy of the Makani Science RMS compared to manual counting by a trained professional is within a performance goal (PG) of 20%. The PG for the Makani Science RMS was determined to be 20% based on the PG used by Respiratory Motion ExSpiron; this study also uses the same reference as the Respiratory Motion ExSpiron device.

4.2 Study Procedure

Health adult subjects will be recruited to participate in this study. The subject's respiration will be monitored simultaneously by the Makani Science RMS and a trained individual. Each subject will be asked to breath according to a number of pre-defined maneuvers. The order of the pre-defined breathing maneuvers and the position of the subjects will be randomized [27].

Details for the steps of the study procedure are provided in the **Appendix 1 – Study Procedure**.

4.3 System Verification and Calibration

The algorithm used by the Makani Science RMS will be the final version to be implemented in the Makani Science RMS product. Any Makani Science sensors that has been used and reprocessed for the clinical study will undergo the same inspection and testing done during factory assembly to ensure that the performance will be equivalent. The sensors will also be documented and tracked through the clinical study.

The Phillips MP50 vital monitor will be calibrated for all vital signs.

4.4 Data Acquisition

Respiratory data will be simultaneously recorded for each participant from: (1) the Makani Science RMS and (2) by a trained individual counting respiration manually (reference). The clinical investigator will be recording the respiratory rate every 60 seconds, and will be blinded from any respiratory rate information from the monitor and guided breathing. A video camera will also be recording the values from the monitors present as well as the movement from the subject's chest.

Data from both the Makani Science system and the Phillips MP50 vital monitor will also be recorded electronically. Data from the Phillips MP50 vital monitor will be recorded continuously over time via an ethernet cable to an open-source software (VitalSignsCapture by Xeonfusion) as a comma separated value format [28]. Data from the Makani Science RMS will be downloaded afterwards. The electronically recorded data will not be used in the analysis of this clinical study, but will as exploratory for future studies.

All subject baseline data will be recorded prior to the start of the study by the investigator.

4.5 Data Handling

4.5.1 Initial Data Storage

All data recorded by the clinical investigator will be assigned a de-identified file name and inputted digitally as an excel file format. Notes taken during the study, including patient baseline information and deviation from the protocol, will be scanned, and assigned a de-identified file name. Video recording of the monitor screen will also be assigned a de-identified file name. All the files will then be uploaded onto an external memory drive and kept in a secure location.

4.5.2 Data Processing and Quality Control

The clinical investigator will be a qualified individual who can manually count the patient respiration rate. During the study, they will be asked to assess for and record any abnormal breathing events, which will be reported [29, 30]. The trained individual manually counting respiratory rate will be blinded to all monitor information as well as any guided breathing rates so that they remain unbiased while making the measurement.

The respiratory rate data will be saved as an excel file with the columns as time, manually counted respiratory rate, and respiratory rate from the Makani Science RMS. The processed data will be provided

for the statistical analysis. Data from the supine and reclined position will be stored as separate tabs. Table 1 shows an example of the data format.

Table 1. Example of the table provided for final analysis

Time (HH:MM:SS)	Manually Counted RR (BPM)	Makani Science RR (BPM)
4:01:51	5	4
4:02:56	5	5
4:03:55	6	5
4:04:58	6	6
4:05:50	6	6

4.6 Data Analysis and Statistical Considerations

Detailed statistical analysis and considerations are provided in the Statistical Analysis Plan (SAP) for this pivotal study. The primary analysis will be conducted twice. An interim analysis will be generated as described in Section 4.6.6, once 50% of subjects have been enrolled. The final analysis will take place once all subjects have completed the study and all data have been downloaded, monitored and processed. All analyses will be performed using all subjects who meet the inclusion and exclusion criteria and have simultaneous monitoring using the two study devices for any amount of time.

4.6.1 Primary Study Hypothesis and Analysis

The null and alternative hypotheses for the primary endpoint are as follows:

$$H_{01}: \sigma_1 \geq PG \quad \text{v.} \quad H_{a1}: \sigma_1 < PG,$$

$$H_{02}: \sigma_2 \geq PG \quad \text{v.} \quad H_{a2}: \sigma_2 < PG$$

where σ_1 is the accuracy represented by the RMSPE for data collected in the supine position, σ_2 is the RMSPE for data collected in the reclined position, and PG is the performance goal. The PG for the primary endpoint is set at 20% defined based as in Atkinson et al. [31] using the ExSpiron respiratory volume monitor by Respiratory Motion Incorporated. The primary hypotheses will be sequentially tested using a gate-keeping approach, each with a one-sided significance level of 0.025. If $H_{01}$is rejected, H_{02} may be tested. If $H_{01}$is not rejected, H_{02} will not be tested.

For each primary hypothesis, the percentage RMSPE for RR will be calculated using the paired difference between log-transformed Makani Science and reference RR measurements analyzed in a mixed-effects linear model with random subject effects to account for repeated measurements. The RMSPE will be estimated as the sum of the squared bias, the within-subjects variance and the between-subjects variance, back-transformed with the usual equation for coefficients of variation: $\sqrt{e^{\sigma^2} - 1}$ and expressed as a percentage. Bootstrapped confidence intervals for RMPSE will be calculated by randomly sampling subjects with replacement and including all of the selected subjects' replicates in the analysis. Following the sequential gate-keeping approach, if the upper one-sided 97.5% confidence interval for the RMSPE is less than the corresponding performance goal, then accuracy can be claimed.

4.6.2 Secondary Study Endpoint Analyses

The RR percentage bias and precision for both supine and reclined positions will be calculated using a similar mixed-effects linear model as described for the primary endpoint analysis. The bias is the estimated geometric mean paired difference from the model, back-transformed and expressed as a percentage. The precision is the standard deviation of the square root of the sum of the within-subjects variance and the between-subjects variance, back-transformed and expressed as a percentage. Bootstrapped 95% confidence intervals will be generated for percentage bias and precision.

4.6.3 Demographic and Baseline Characteristics

Demographic and baseline characteristics will be summarized descriptively. Demographic characteristics include age, sex, weight, height, BMI, abdomen circumference, and torso circumference.

4.6.4 Subgroup Analysis

For each monitoring position (supine and reclined), a subgroup analysis for the primary endpoint will be generated for BMI comparing subjects in the Normal (BMI<25) and Overweight (BMI≥25) categories. The RMSPE will be estimated for each BMI category using the same methods as described in Section 4.6.1 and will be compared using a randomization test. Secondary endpoints will also be summarized for each monitoring position by BMI category with no formal statistical comparisons.

4.6.5 Sample Size Estimation

The sample size to achieve an 80% probability of rejecting the null hypothesis at a significance level of 0.025, assuming the standard deviation of the RMSPE is 4.51% as observed in a pilot study for data collected in supine position and the observed RMSPE in the Pivotal study is 17.5%, is 28 subjects. This was calculated using the One Sample T-tests Procedure of NCSS Power Analysis Statistical Software, Version 20 (PASS 20, [32]). Assuming a potential 5% dropout rate, 30 subjects (28/0.95) will be enrolled. An interim analysis is planned once 50% of the subjects have completed the study to verify these assumptions and re-estimate the sample size if needed.

4.6.6 Interim Analysis

Once 50% of the planned subjects have completed the study, an interim analysis of RR accuracy will be performed and sample size re-estimated. The conditional probability of demonstrating accuracy in the supine position at the end of the trial, given the interim results, will be calculated. If this conditional probability is less than 50%, the study will be stopped for reassessment of the protocol; if the conditional probability is greater than or equal to 50%, the study will proceed to completion with the new sample size, capped at a maximum of 50 participants. The new sample size will be calculated as the sample size necessary to yield an 80% conditional probability of trial success (i.e., meeting both primary endpoints), given the interim results. If the study continues following the interim analysis, the sponsor will remain blinded to the primary endpoint results and no overall alpha adjustment is required. This procedure has been shown by Gao et al. [33] to control the overall significance level at 5%.

4.7 Ethical and Regulatory Considerations

4.7.1 Role of Makani Science as Study Sponsor

As the study sponsor, Makani Science, Inc. has the overall responsibility for conducting the study, including any applicable national requirements. In this study, Makani Science will have certain direct responsibilities and may delegate others to qualified consultants. This protocol and any amendments will be documented and maintained at Makani Science.

4.7.2 IRB Approval

Prior to conduction of this study, investigator review board (IRB) approval will be received by Advarra.

4.7.3 Informed Consent

Informed consent forms will be obtained from all participants of the study prior to start of study conduction.

4.7.4 Subject Confidentiality

Subject confidentiality will be maintained throughout the clinical study. A unique subject de-identification code will be assigned and used to allow identification of all data reported for each subject.

Study data may be made available to third parties, e.g., in the case of an audit, provided the data is treated confidentially and that the subject's privacy is guaranteed. The identity of a subject will never be disclosed in the event that the study data is published.

5 Appendices

5.1 Appendix 1. Study Procedure

Respiration rate will be measured concurrently on healthy adult participants using capnography and the Makani Science RMSS. 15 patients will initially be enrolled for the study.

5.1.1 Study Material

- Makani Science RMS
- Phillips MP40 Vital Monitor + Sensors
- Computer with VitalSignsCaptureMP Software available
- NIST calibrated stop watch or timer
- Ethernet Crossover Cable
- Scale
- Tape measure
- iPad with paced breathing video
- Camera
- USB drive and USB to iPad adapter
- Chairs
- Resting/massage tables or bed

5.1.2 Primary and Secondary Endpoint Study Procedure

1. The procedure will be explained to the subject and consent will be acquired.
2. The subject baseline information will be collected.
3. The subject will be asked to lay in the supine or recline position (order will be randomized per subject).
4. The subject's skin will be cleaned with alcohol wipes.
5. The Phillips MP50 vital monitoring sensors will be placed on the subject. This includes:
   5.1. ECG
   5.2. Capnography
   5.3. Pulse oximeter
   5.4. Blood pressure cuff
6. The subject will be asked to perform the following breathing exercises in a random order [29] [30]. They will be given a break between each exercise or as needed.
   6.1. The subject will be asked to breath quietly for 90 seconds.
   6.2. The subject will be asked to breath at a slow, but consistent rate for 90 seconds.
   6.3. The subject will be asked to breath at a medium, but consistent rate for 90 seconds.
   6.4. The subject will be asked to breath at a fast, but consistent rate for 90 seconds.
   6.5. 15 breaths-min^{-1} for 90 seconds.
   6.6. 12 breaths-min^{-1} for 90 seconds.
   6.7. 9 breaths-min^{-1} for 90 seconds.
   6.8. 6 breaths-min^{-1} for 90 seconds.

6.9. 18 breaths-min^{-1} for 90 seconds.
6.10. 21 breaths-min^{-1} for 90 seconds
6.11. 24 breaths-min^{-1} for 90 seconds.

7. The subject will be moved into the supine or recline position (whatever position they did not lay in step 3).
8. A new set of Makani Science sensors will be placed on the subject.
9. The subject will repeat the breathing exercises in step 6.

5.1.3 Follow up Assessment

- No follow up assessment is required.

5.2 Appendix 2. Adverse Events Definitions

Adverse Event (AE):

Any untoward medical occurrence, unintended disease or injury, or untoward clinical signs (including abnormal laboratory findings) in subjects, users, or other persons, whether related to the study medical device.

Serious Adverse Event (SAE):

An adverse event when the patient outcome is:

a) Death

b) Life-threatening

c) Hospitalization (initial or prolonged); including emergency room visits that do not result in admission to the hospital but were evaluated for one of these other serious outcomes.

d) Disability or permanent damage

e) Required intervention to prevent permanent impairment or damage

f) Other serious important medical events (if serious event does not fit the other outcomes, but event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes (i.e., seizures/convulsions that do not result in hospitalization)

Note that planned hospitalization for a pre-existing condition, or a procedure required by the study protocol without serious deterioration in health, is not considered a serious adverse event.

Device-Related Adverse Event (DRAE):

An adverse event or effect related to the use of the investigational or study medical device.

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