Makani Science:

Pivotal Clinical Study for the Makani Science Respiration Monitoring System

Statistical Analysis Plan

Version 2.0 – 19 Jul 2024
Based on Protocol CP-0004

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1. SYNOPSIS

The purpose of this study is to demonstrate that the Makani Science Respiration Monitoring System (RMS) can accurately measure respiratory rate in adult subjects compared to manually scored etCO2 recordings from an FDA-cleared capnography device. 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 adults by professionals in healthcare facilities and dental offices.

This is a prospective, open-label, single-arm study. Participants will follow the protocol-specified breathing procedures and respiratory data will be simultaneously recorded for each participant from: (1) Makani Science RMS, and (2) manually scored etCO2 recordings from an FDA-cleared capnography device. The planned enrollment is 30 subjects, and eligible participants will be adults 22-99 years of age with body mass index (BMI) between 15 and 40 kg/m^2. Enrollment will be targeted to balance normal (BMI < 25) and overweight (BMI ≥ 25) subjects, biological sex, and provide a representative range of age.

2. ABBREVIATIONS

Abbreviation	Description
AE	Adverse Event
BMI	Body Mass Index
BPM	Beats Per Minute
ITM	Intent to Monitor
MAE	Mean Absolute Error
PG	Performance Goal
RMS	Respiration Monitoring System
RR	Respiratory Rate
SD	Standard Deviation

3. STUDY OBJECTIVES

The primary objective of the study is to demonstrate the respiratory rate (RR) accuracy of the Makani Science RMS compared to manual, clinician-scored etCO2 waveform (capnography) within 3 bpm.

Additional objectives include:

- Demonstrate the Makani Science RMS system's ability to recover after motion artifacts.
- Demonstrate the Makani Science RMS system's performance in side-lying and reclined subjects.
- Demonstrate the Makani Science RMS system's performance for BMI and biological sex subgroups.

4. DEFINITIONS AND CALCULATIONS

4.1 Endpoint Definitions

Endpoints for this study include RR accuracy and bias.

RR accuracy will be estimated using mean absolute error (MAE). MAE will be calculated as a weighted average. For each subject, the average of the absolute value of the paired difference between Makani Science RMS and capnography RR measurements will be calculated:

$$MAE_{subject\ i} = \frac{\sum\left|\left(RR_{Makani} - RR_{capnography}\right)\right|}{number\ of\ measurements}$$

The subject-level MAE values will then be averaged across subjects:

$$MAE_{overall} = \frac{\sum_i MAE_i}{number\ of\ subjects}$$

Similarly, RR bias will be calculated as a weighted average. For each subject, the average of the actual difference between Makani Science RMS and capnography RR paired measurements will be calculated:

$$bias_{subject\ i} = \frac{\sum\left(RR_{Makani} - RR_{capnography}\right)}{number\ of\ measurements}$$

The subject-level bias values will then be averaged across subjects:

$$bias_{overall} = \frac{\sum_i bias_i}{number\ of\ subjects}$$

4.2 Performance Goal (PG) for Accuracy

The performance goal (PG) for the primary endpoint is set at 3 bpm, to be consistent with the predicate device (PMD Solution, RespiraSense) [1].

4.3 Bootstrapping Methods

For the endpoints of accuracy (MAE) and bias, 95% bootstrap confidence intervals will be calculated using a cluster bootstrapping approach with sample selection at the subject level to account for the correlation within subjects.

- Bootstrap samples will be generated using the original analysis data. Sampling will be performed with replacement and each bootstrap sample will have the same number of subjects as the original data. For each subject selected to be in the bootstrap sample, all available data from that subject will be included. If a subject is sampled more than once, that subject's data will be included in the bootstrap sample more than once.
- 5000 bootstrap samples will be generated. For each bootstrap sample, MAE and bias will be calculated, resulting in 5000 estimates of MAE and 5000 estimates of bias.
- Percentiles will be used to determine confidence limits, using the empirical distribution of the bootstrap samples as an approximation of the population distribution. For each endpoint, the 95% CI will be calculated as the 2.5^{th} and 97.5^{th} percentiles of the distribution of estimates calculated from all the bootstrap samples.

5. SAMPLE SIZE

Capnography data were collected during Clinical Validation Study for the Makani Science Respiration Monitoring System (CP-0003) and simultaneous results were matched with the Makani RMS device and summarized over 60 second breathing intervals. Matched results were available for 16 subjects in the supine position. Accuracy between the Makani device and capnography was summarized as mean absolute error (MAE). MAE was 1.694 bpm with a standard deviation (SD) of 1.662 bpm, estimated using the bootstrapping methods defined in Section 4.3.

The t-distribution was used as a conservative approach for sample size estimation. The required number of subjects to achieve at least 90% probability of rejecting the null hypothesis comparing to a performance goal (PG) of 3 bpm given the true MAE as calculated from CP-0003 data was estimated based on a one-sided test with an alpha of 0.025 in the One-Sample T-Tests Procedure in PASS [2].

A sample size of 20 subjects yields 90% power to reject the null hypothesis when the PG is 3 bpm, and the assumed MAE is 1.694 with a SD of 1.662.

A minimum of 30 subjects will be enrolled to facilitate the balance between normal (BMI < 25) and overweight (BMI ≥ 25) subjects, biological sex, and provide a representative range of age.

6. ANALYSIS POPULATIONS

All subjects who meet the inclusion and exclusion criteria and are enrolled in the study will be considered Enrolled Subjects.

Since it is required that both Makani Science RMS and capnography RR measurements are available to calculate the study endpoints, only time frames in which both were properly measured will be utilized. All Enrolled Subjects with simultaneous measurements for any amount of time will be included in the Intent to Monitor (ITM) population.

All summaries and analyses described in this Statistical Analysis Plan will be performed using the ITM population.

7. STATISTICAL METHODS

The number of observations, mean, standard deviation (SD), median, minimum and maximum will be calculated for continuous variables, unless otherwise stated. The number of significant digits reported will be as follows: minimum and maximum will be reported with the same number of significant digits as the raw data; the mean, median, and SD will be reported with one more significant digit than the raw data. Frequencies and percentages will be calculated for categorical data using one significant digit for percentages.

As a routine function in applying statistical procedures, the assumptions underlying those procedures will be evaluated. Tests for normality will not be done for continuous variables, but data will be inspected for symmetry through histogram plots. Parametric test procedures will be supported by non-parametric procedures as appropriate.

Statistical procedures specified in this analysis plan include bootstrapping methods to calculate confidence intervals and a Bland-Altman analysis to compare the two methods of measurement.

7.1 Data Pooling

This study will be conducted at one study site, thus data pooling is not a consideration.

7.2 Missing Data

Per study procedures, RR will be recorded simultaneously from the Makani Science RMS and the capnograph monitor approximately every 2 minutes rather than at a specific number of timepoints. Therefore, subjects may have differing numbers of measurements. These differences will not be considered "missing" and all available paired measurements from all subjects will be included in the primary analysis.

7.3 Multiplicity

There are no multiplicity considerations for the primary analysis of this study.

For additional endpoint and subgroup analyses, 95% confidence intervals may be presented as descriptive statistics. No multiplicity adjustment will be made for these analyses and the confidence intervals are not intended as statistical inference.

8. SUBJECT ENROLLMENT AND ACCOUNTABILITY

The number of subjects Enrolled and in the ITM population will be presented. Any protocol deviations will be listed.

9. DEMOGRAPHIC AND BASELINE CHARACTERISTICS

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

Relevant medical conditions will be summarized descriptively with full details provided in a listing. Current medications will be listed.

10. PRIMARY ENDPOINT

10.1 Primary Endpoint

The primary endpoint is RR accuracy of the Makani Science RMS as compared to capnography, which will be estimated using mean absolute error (MAE).

10.2 Primary Endpoint Analysis

The null and alternative hypotheses for the primary analysis are:

$$H_0: \mu \geq PG \quad \text{v.} \quad H_a: \mu < PG$$

where μ is the RR accuracy estimated by MAE and PG is the performance goal of 3 bpm, as defined in Section 4.2.

A bootstrapped confidence interval for MAE will be calculated as detailed in Section 4.3. If the upper one-sided 97.5% confidence limit for the MAE is less than the performance goal of 3 bpm, then RR

accuracy as compared to capnography can be claimed.

11. ADDITIONAL ENDPOINTS AND ANALYSES

Bias in RR measurements will be calculated as defined in Section 4.1 and bootstrapped 95% confidence intervals will be calculated.

A Bland-Altman analysis will be performed to further evaluate agreement between Makani Science RMS and capnography. Limits of agreement for RR bias will be calculated as described in Bland and Altman (2007) [3] using the bootstrap samples to estimate variance. Results will be presented graphically.

Additional paired RR data will be collected following breathing artifacts as described in the protocol. Accuracy (MAE) and bias will be summarized descriptively for all combined post-artifact data.

Paired RR data will also be collected with subjects lying in different positions: on the left side, the right side, and in the reclined position. For these measurements, accuracy (MAE) and bias will be summarized descriptively by position.

12. SUBGROUP ANALYSES

A subgroup analysis of accuracy (MAE) will be presented separately by subject BMI (normal: BMI < 25 and overweight: BMI ≥ 25). MAE will be calculated for each BMI category and a randomization test will be employed to test whether subjects in the two BMI categories differ with respect to MAE. The difference in MAE between the two groups will be calculated and compared to the distribution of possible differences assuming a null distribution of homogeneity of groups. Specifically, subjects' BMI category will be randomly permuted to generate the null distribution of the differences. The p-value for BMI effect is defined as the proportion of replicates in the null distribution where the difference between BMI groups is greater than or equal to the observed difference.

Bias will also be summarized by BMI category using descriptive statistics with no formal statistical comparisons.

Additional summaries of accuracy (MAE) and bias will be presented by biological sex (female, male) using the methods described for BMI.

13. SAFETY ANALYSES

All adverse events (AE) reported during the study will be listed, including the event description, time of occurrence, severity, outcome, action taken, expectedness and relationship to the study procedure and/or device. Serious and study- or device-related AEs will be presented separately. AEs will be tabulated by event category if needed.

14. DATA HANDLING

The following participant data will be collected:

- Respiratory rate over time from the Makani Science RMS
- Manually scored respiratory rate from the capnography monitor
- Baseline subject information

- Adverse events
- Protocol deviations

Respiratory rate data will be acquired from the Makani Science RMS and capnography and stored as described in the study protocol. Results will be provided in Microsoft Excel files for analysis.

Baseline subject data, AEs, and protocol deviations will be recorded by the clinical researcher and stored in Microsoft Excel files.

All study data will be imported into SAS datasets (or other format as needed) to perform the summaries and analyses detailed in this document.

15. STATISTICAL SOFTWARE AND QUALITY CONTROL

All statistical analyses will be generated using SAS® software, version 9.4 or later, or other appropriate statistical package. Statistical analyses will be independently quality checked by a second statistician.

16. REFERENCES

[1] FDA, "510(k) Premarket Notification," 19 August 2022. [Online]. Available: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm?ID=K220111. [Accessed 22 February 2024].

[2] PASS 2023 Power Analysis and Sample Size Software (2023). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass.

[3] Bland, J. Martin and Altman, Douglas G. Agreement Between Methods of Measurement with Multiple Observations Per Individual, Journal of Biopharmaceutical Statistics 2007, 17:4, 571 – 582. DOI: 10.1080/10543400701329422

17. TABLES AND FIGURES

The following tables, figures, and listings are planned:

Table	Title
1	Subject Enrollment and Study Populations
2	Demographic and Baseline Characteristics
3	Medical History
4	Primary Analysis of Respiratory Rate (RR)
5	Analysis of Respiratory Rate (RR) After Breathing Artifacts
6	Analysis of Respiratory Rate (RR) by Body Position
7	Subgroup Analyses of Respiratory Rate (RR) by BMI Category
8	Subgroup Analyses of Respiratory Rate (RR) by Biological Sex

Figure	Title
1	Bland-Altman Limits of Agreement Comparing Makani Science RMS and Capnography Respiratory Rate (RR)

Data Listing	Title
1	Protocol Deviations
2	Medical History and Medications
3	Adverse Events

18. DOCUMENT VERSION HISTORY

Version	Issue Date	Author	Significant changes from previous version
1.0	15 May 2024	Meredith Decker	Initial version
2.0	19 Jul 2024	Meredith Decker/ Leslee Willes	Update to MAE and bias calculations. Rationale: Study subjects had differing numbers of measurements available for analysis. The calculations specified in the prior version of the SAP gave equal weight to all observations, and results would be biased toward subjects with more observations. The endpoint definitions were updated to give equal weight to each subject by performing calculations within each subject and then averaging across subjects.