The BREATHE Study: Bronchiolitis Recovery and the Use of High Efficiency Particulate Air (HEPA) Filters

Statistical Analysis Plan

Version [1.0]

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SAP Revision History

Protocol version	Updated SAP version number	Section number changed	Description of and reason for change	Date of change

Note: The “Last Revision Date” on the cover page must be updated accordingly.

Table of Contents

Abbreviations and Glossary

Abbreviation	Description
AE	Adverse Event
API	Application Programming Interface
CFR	Code of Federal Regulations
CMP	Clinical Monitoring Plan
COPD	Chronic Obstructive Pulmonary Disease
CPAP	Continuous Positive Airway Pressure
CRF	Case Report Form
DCOC	Data Coordinating and Operations Center
DSMB	Data Safety Monitoring Board
ECHO	Environmental Influences on Child Health Outcomes
ECMO	Extracorporeal membrane oxygenation
ED	Emergency Department
EDC	Electronic Data Capture
EPA	Environmental Protection Agency
GCP	Good Clinical Practice
HEPA	High Efficiency Particulate Air
HFNC	High Flow Nasal Cannula
HIPAA	Health Insurance Portability and Accountability Act
IAQ	Indoor Air Quality
ICF	Informed consent form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ISPCTN	IDeA States Pediatric Clinical Trials Network
kW	Kilowatt
kWh	Kilowatt Hour
LRTI	Lower respiratory tract infection
MOP	Manual of Procedures
NCT	National Clinical Trial
NIH	National Institutes of Health
NO_2	Nitrogen Dioxide
PI	Principal Investigator
PedsQL™	Pediatric Quality of Life Inventory
PM	Particulate Matter
PM2.5	Particulate matter <2.5 micrometers in diameter
QA	Quality Assurance
QC	Quality Control
QOL	Quality of Life
RCT	Randomized controlled trial

RSV	Respiratory syncytial virus
RUCA	Rural-Urban Commuting Area
RV	Rhinovirus
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Secure Digital
SFD	Symptom-free days
SMART IRB	A platform designed to ease common challenges associated with initiating multisite research and to provide a roadmap for institutions to implement the NIH Single IRB Review policy
SOA	Schedule of Activities
SOP	Standard Operating Procedure
UAMS	University of Arkansas for Medical Sciences
UC	Urgent Care
UP	Unanticipated Problem
UPIRTSO	Unanticipated Problem(s) Involving Risk(s) to Subjects of Others
US	United States

1. Introduction

The goal of the "Statistical Analysis Plan" (SAP) outlined here is to provide a comprehensive document that provides required details for the summary, visualization, and analysis of the data that is measured and/or observed during the course of the study "The BREATHE Study: Bronchiolitis Recovery and the Use of High Efficiency Particulate Air (HEPA) Filters". This document must be read together with the study protocol, data collection forms and any additional documents (e.g., survey tools) related to the study. This SAP is finalized based on the study protocol finalized on "November 29, 2023"; data collection form finalized on "November 4, 2022".

The protocol should be read with the understanding that the outlined methods related to summarizing, displaying, and analyzing the study data should be considered flexible and deviations from the pre-planned approach may be required. Many statistical analyses rely on satisfactorily meeting different assumptions that can be validated only during data analysis. Therefore, deviations from pre-planned analysis approach can be inevitable. A statistical and/or clinical description justifying the need for these deviations will be included.

In summary, air pollution is associated with respiratory symptoms and disease, particularly in sensitive populations, including infants. Air pollution is, therefore, a key intervention target. HEPA filters are efficacious in cleaning the air and improving multiple indicators of health. To date, however, no clinical trial has tested the efficacy of HEPA filtration units in increasing symptom-free days (SFDs) in infants hospitalized for bronchiolitis. The study aims to address this important gap and improve the health of infants who have experienced this severe and common respiratory event. Reduction in these symptoms may lead to decreased healthcare utilization and improve QOL for a large population. The current bronchiolitis care guidelines lack recommendations for post-hospitalization symptom reduction. If effective, HEPA filtration intervention can help fill this gap.

Research Question: For children <12 months of age hospitalized with bronchiolitis, will those who receive a HEPA filtration unit household intervention to reduce $PM_{2.5}$ have decreased respiratory symptom burden over 24 weeks compared to those who receive a control HEPA unit? Please refer to 'Section 2 Introduction' of the protocol for additional details on study overview.

2. Study Objectives

2.1. Primary Objective

To determine if use of a HEPA filtration unit home intervention reduces the respiratory symptom burden (symptom-free days; SFD) for 24 weeks compared to a use of a control unit.

2.2. Secondary Objectives

Secondary objective 1: To test the efficacy of a HEPA filtration home intervention, relative to the control arm, on difference in number of unscheduled healthcare visits for respiratory symptoms over 24 weeks.

Secondary objective 2: To test the efficacy of a HEPA filtration home intervention, relative to the control arm, on difference in quality of life (QOL).

Secondary objective 3: To test the efficacy of a HEPA filtration home intervention, relative to the control arm, on difference in particulate matter ≤2.5 micrometers in diameter ($PM_{2.5}$) levels in the home over 24 weeks.

Section 3. Study Methods

3.1. Study design

This is a multi-center, parallel, double-blind, randomized controlled clinical trial. Two hundred twenty eight children <12 months old with their first hospitalization for bronchiolitis will be randomized 1:1 (stratified by site) to receive 24 weeks of home intervention with active HEPA filtration units to improve IAQ or to a control group without a HEPA or carbon filter inside identical-appearing units. Children will be followed for respiratory symptoms during a pre-intervention period of up to two weeks following randomization and during an intervention period of 24 weeks. The scientific rationale for the study design is included in the protocol section 4.2. The study biostatisticians from the Data Coordinating and Operations Center (DCOC) will perform statistical analyses.

3.2. Randomization and Blinding

Participants in each of the 17 sites will be randomized 1:1 (stratified by site) to receive HEPA filtration (intervention group) or control filtration. Permuted block randomization with a block size of 4 or 2 participants (selected at random) will be employed. The block size and block permutation will be selected at random for each site. After selecting the block size and block permutation, a participant is assigned to the first control/intervention in the block, and the remaining slots are assigned as subjects continue to randomize within the site. As randomizations continue and no more slots are available in the previously assigned block, a new block is assigned and participants are randomized accordingly.

Families will be masked as to whether their Winix units are equipped with HEPA filters or control filters. Study coordinators, investigators, and other team members who interact with participants to obtain symptom diaries, troubleshoot equipment setup and operation, or have other interactions will remain masked through the duration of the study for individual participants. This includes masking as to which intervention the participants receive and household air quality measurements, including the baseline measurements (separate personnel will need to be on the receiving end for air quality measurement data). This will require more than one study coordinator or additional staff/technician on the study team.

Unmasked personnel (separate coordinator, technician, or other qualified personnel) will work on the HEPA units to ensure standardized appearance with tape and active or inactive filter

setup. They will perform any mailing of units or coordination of drop-offs etc. They will not assess outcomes.

3.3. Study Visits and Assessment

Evaluation/Procedures	Screen[1] (hospital)	Enroll / Randomize In hospital (+1 week)	Pre-intervention Weeks 1-2 after hospital discharge[2]	Intervention[2] Weeks 3-26	Week 26 (End of Participation)
Review inclusion/exclusion criteria	x	x			
Informed consent		x			
Document participant characteristics and risk factors for recurrent wheeze[3]		x			x
Pre-intervention period (in all study participant homes – both intervention and control): up to 2 weeks continuous home $PM_{2.5}$ monitoring via PurpleAir[4]			x		
Intervention period (in all study participant homes – both HEPA/control unit): Continuous home $PM_{2.5}$ monitoring via PurpleAir[4]				x	
Continuous HEPA/control unit use[5]				x	
Continuous use of kilowatt meter to measure HEPA/control unit adherence[5]				x	
Weekly submission: Symptom survey, number of medical visits, number of nights away from home, HEPA/control unit adherence[7]			x	x	
Check-in contact with study team[6]			x	x	
QOL Survey[7]					x

[1] Screening and enrollment ideally will occur during hospitalization. However, enrollment can occur after discharge to home *if the family can receive and set-up the air quality monitoring equipment ideally within7 days of discharge.* Other procedures can occur at home.

[2] **Day of hospital discharge is defined as day 1. Intervention ideally starts on day 14.**

[3] See Table 4 in the protocol.

[4] Families place PurpleAir monitors in the child's sleep space and in another common room. Baseline $PM_{2.5}$ measurements are collected for up to 14 days and then the family will begin using HEPA units in the same rooms (child's sleep space and another common room) that contain the PurpleAir monitors while $PM_{2.5}$ monitoring continues. HEPA units will have active filters in the intervention group and no HEPA or carbon filters in the control group.

[5] Kilowatt-hour meter is used to measure actual HEPA unit use. All devices are simple to plug in. The study team will work with the family remotely to confirm correct installation and placement of the devices at baseline and at the start of HEPA use and confirm data transmission from the PurpleAir monitor.

[6] Weeks 1-4, check-in with the enrolling site will occur weekly and as needed (minimum of weekly). Weeks 5-26, check-in with the enrolling site will occur weekly or monthly and as needed (minimum of monthly). During the check-in, the study team will assist or prompt EDC documentation as needed, assess equipment questions/concerns, and safety assessments will occur (AE, SAE, UPIRTSO).

[7] Family will receive an Electronic Data Capture (EDC) system survey link weekly by text (if allowed by the local site) or email. The family will submit the brief questionnaire (alternatively, the study staff can call the parent(s)/guardian(s) to read the questions and record the responses in the EDC system for the parent(s)/guardian(s)). QOL survey will also be administered electronically (with alternative of survey completion by phone with study staff).

3.4. Sample size Justification

We originally plan to enroll 218 participants, or 109 participants per arm. To account for an anticipated attrition rate of 10% per arm, the power analysis is based upon a sample size of 196 participants, or 98 participants per arm. From a similar previously published study, it was found the mean of days with symptoms was 70 days (equivalently the mean of SFDs was 98 days out of total 24 weeks or 168 days of observation), and the standard deviation was 43 days (Bisgaard et al., 2008). The proposed sample size will provide 90% power to detect an effect size of 0.465, or a difference of 20 symptom-free days with a standard deviation of 43 days, using a two-sample t-test.

Due to the increase in the dropout rate during follow-up, we modified the sample size to 228 participants, or 114 participants per arm, to account for an updated attrition rate of 14% per arm.

Using data from nine ISPCTN sites, we observed 3,209 admissions for bronchiolitis in infants less than 12 months of age during the 2019-2020 season. We estimate that we will have a minimum of 10-14 sites in this trial. Therefore, we will have a population of approximately 3,500-5,000 infants hospitalized from bronchiolitis to recruit from per year. Given that the recruitment period for this study is estimated at 2 years, we should have 7,000-10,000 eligible infants during the study period in our recruitment sites. Assuming a conservative recruitment rate of 20% would give us 700-1,000 infants, which is far greater than the recruitment targets for this trial. Additional sites could be added to the trial if recruitment falls short, as the ISPCTN has 18 awardees, some with multiple available recruitment sites.

3.5. Hypothesis and Study Framework

Primary Objective: To test the efficacy of a HEPA filtration unit home intervention, relative to the control arm, with respect to respiratory symptom burden (as measured by symptom-free days; SFD) over 24 weeks following activation of filtration.

> **Primary Endpoint:** Number of caregiver-reported SFDs over 24 weeks following activation of filtration (SFD defined as a 24-hour period without coughing, wheezing, or trouble breathing)

Statistical Hypothesis: Mean of SFDs in the HEPA filtration home intervention group is larger than mean of SFDs in the control group. (The hypothesis testing is the comparison of superiority.)

Secondary Objective 1: To test the efficacy of a HEPA filtration home intervention, relative to the control arm, on the number of unscheduled healthcare visits for respiratory symptoms over 24 weeks following activation of filtration.

Secondary Endpoint 1: Caregiver reported counts of unscheduled healthcare visits from each of the metrics, which include:

- Hospitalizations
- Emergency Department (ED) or Urgent Care (UC) visits
- Other unscheduled medical visits for respiratory complaints (cough, wheeze, or trouble breathing).
- A sum of counts (or total counts) of all metrics.

Statistical Hypothesis 1: Mean of counts of unscheduled healthcare visits for respiratory symptoms in the intervention group is smaller than mean of counts of unscheduled healthcare visits for respiratory symptoms in the control group.

Secondary Objective 2: To test the efficacy of HEPA filtration home intervention, relative to the control arm, on difference in QOL.

Secondary Endpoint 2: Total QOL score, as measured by the PedsQLTM Pediatric Quality of Life Inventory Infants Scales questionnaire at the end of the intervention period.

Hypothesis 2: Child QOL will be higher in families that receive the HEPA intervention compared to controls.

Secondary Objective 3: To test the efficacy of HEPA filtration home intervention, relative to the control arm, on $PM_{2.5}$ levels in the home over 24 weeks following activation of filtration.

Secondary Endpoint 3: Weekly average $PM_{2.5}$ levels as measured in each room separately and by the average of the two in-home PurpleAir monitors over 24 weeks and

scaled to the unit of $\mu g/m^3$ per week. Log transformations of the $PM_{2.5}$ data will be considered if data are skewed.

Hypothesis 3: Mean of $PM_{2.5}$ level in the intervention group is lower than the mean of $PM_{2.5}$ level in the control group.

3.6. Interim Analysis

An interim efficacy analysis has been planned for this study when 50% of study participants (49 participants in both groups) have completed the follow-up period. We will employ Lan & DeMets' alpha-spending function (GORDON LAN & DEMETS, 1983) together with O'Brien-Fleming boundaries (O'Brien & Fleming, 1979) to preserve the overall type I error rate at 0.05 and power at 90% in the final analysis. The boundaries and operating characteristics for the proposed analyses are provided in the table below. In the event that findings from interim analysis provide evidence in favor of futility, the study team may consider halting the study.

Analysis	Information fraction	Reject H_0 (Efficacy)	Overall α spent	Reject H_1 (Futility)	Overall β spent
Interim	0.50	$\|z\| > 2.963$	0.0003	$\|z\| < 0.200$	0.012
Final	1.00	$\|z\| > 1.969$	0.05	$\|z\| < 1.969$	0.102

3.7. Study Adherence

Intervention compliance will be assessed in two ways: caregiver-reported HEPA/control unit use and through kW meters. Participants who have used HEPA/control unit on both units >80% of the time (at least 19 weeks out of 24 weeks) with recommended setting (running continuously on the level 3/second from the highest) will be considered as good adherence. Since compliance data may be incomplete, additional analyses will define adherence as >80% use for weeks with available compliance information.

- Families will be instructed to place kW meters on the units at the onset of installation within the home to assess usage compliance with HEPA/control units. These meters enable assessment of power consumption and estimate corresponding costs for energy usage. Increasing kWh over the course of participation will indicate HEPA/control unit use. When available, we will also quantify actual kWh usage during the intervention period by comparing observed kWh

used to the usage predicted from laboratory tests. The observed kWh used will be divided by the predicted usage and this quantity multiplied by 100 to determine adherence.

- Weekly surveys will also include prompts for parent(s)/guardian(s) to report whether they used the HEPA/control unit that week and on what setting it was most commonly used. The survey questions include the usage of the air unit for each room during the last full week (Sunday through Saturday), whether there is a red glow, and what is the usual setting on the air unit.

Note that the kW meters require three-prong electrical outlets. If a home has only two-prong outlets, they will not use the kW meter. Since not all participants will have kW meters and since power interruptions will change kW meter settings, caregiver reported HEPA/control unit use on weekly surveys will be the primary measure of compliance.

3.8. Protocol Deviations

A deviation is any instance of failure to follow, intentionally or unintentionally, the requirements of the clinical trial protocol, ICH E6(R2) (i.e., "GCP"), the study-specific MOP, or other documents needed to complete study conduct. The instance of failure may be on the part of the participant, the investigator, or other study staff personnel. When deviations occur, the sponsor and/or site team(s) will ensure actions are taken to correct the problem and, as needed, prevent the deviation from recurring.

These practices are consistent with ICH E6(R2) (available at https://www.fda.gov/files/drugs/published/E6%28R2%29-Good-Clinical-Practice--Integrated-Addendum-to-ICH-E6%28R1%29.pdf). Specifically, sections:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

Sites must record all deviations in the trial source documents. Whenever a deviation occurs, the DCOC will ensure an appropriate assessment is conducted. The assessment should include documentation of the severity and risk of the deviation. Sites that have a system set up for assessing deviations and doing their own corrections via corrective and preventive action (CAPA) plans will do so according to their site SOPs/system. The site will send copies of their CAPA plan documentation to the DCOC. If a site does not have their own quality assurance

system to complete adequate deviation review and assessments, corrections, and CAPA plans, then the DCOC will provide that function for the sites. Details of these processes will be provided in the MOP and/or trial-specific SOPs. Essentially, the site and/or the DCOC will request/ensure that there is either a CAPA plan initiated or a simple one-time correction is performed, as appropriate.

3.9. Blinded Review of Data

Blinded review of the data is not planned for this study.

4. Analysis Populations

4.1. Intent-to-Treat Population

Intent-to-Treat (ITT) Population: The ITT population will include all participants who are randomized to either HEPA filtration (intervention group) or inactive filter unit (control group).

The primary set of analyses for this study will be based on ITT population. A separate analysis will be done with the Per-Protocol population.

4.2. Per-Protocol Population

Per-protocol (PP) Population: The PP population will include all participants who are randomized to either the HEPA filter (intervention group) or inactive filter (control group) and have used HEPA/control unit on both units >80% of the time with recommended setting (running continuously on the level 3/second from the highest). Intervention compliance will be assessed primarily by caregiver-reported HEPA/control unit use as well as kW meters as described in section 3.7.

4.3. Safety Population

The safety population includes all participants who were randomized to either the HEPA filter (intervention group) or the inactive filter (control group) and had received the HEPA filter or inactive filter.

5. Endpoints and Variables

Table 1. Endpoints and variables

Endpoint/Variable	Description
Primary Endpoint(s) ➢ Number of caregiver-reported SFDs over 24 weeks	➢ The primary endpoint is the number of caregiver-reported symptom free days (SFD) over 24 weeks following activation of filtration (SFD is defined as a 24-hour period without coughing, wheezing, or trouble breathing).
Secondary Endpoint 1 ➢ Counts of unscheduled healthcare visits (UHV)	➢ Caregiver reported counts of unscheduled healthcare visits (UHV) from each of the metrics including • Hospitalizations • Emergency Department (ED) visits or Urgent Care (UC) visits • Other unscheduled medical visits for respiratory complaints (cough, wheeze, or trouble breathing) • A sum of counts (or total counts) of all metrics
Secondary Endpoint 2 ➢ Total QOL score	➢ Total QOL score or Total Scale Score (TSS), is measured by the PedsQL™ Pediatric Quality of Life Inventory Infants Scales questionnaire at the end of the intervention period. Caregivers whose children are age 1-12 months will use a 36-item PedsQL; and those whose children are age 13-24 months will use a 45-item PedsQL. In each item, a score is assigned at 100 if the answer is "never a problem"; 75 if the answer is "almost never a problem"; 50 if the answer is "sometimes a problem", 25 if the answer is "often a problem"; and 0 if the answer is "almost always a problem" respectively (Varni et al., 2011).
Secondary Endpoint 3 ➢ Weekly average PM2.5 levels	➢ Weekly average $PM_{2.5}$ levels as measured by the average of the two in-home PurpleAir monitors over 24 weeks and scaled to the unit of $\mu g/m^3$ per week. ➢ Weekly average PM2.5 levels as measured in each room separately of the two in-home PurpleAir monitors over 24 weeks and scaled to the unit of $\mu g/m^3$ per week.

	Data related to PM2.5 will be generated from two PurpleAir monitors installed in each participating home in most cases. Some smaller homes will have only one PurpleAir monitor installed. The data will be collected by a research team (third party) at the University of Montana (UM) every day during the study or follow-up period for a total of 26 weeks (24 weeks after intervention). The UM team will then convert the original data into $PM_{2.5}$ levels using an EPA-defined formula, after data cleaning, quality assurance (QA) and quality control (QC). The UM team has developed a protocol of procedures for downloading, QA/QC checking, cleaning, computing, and saving PurpleAir data to ensure high quality $PM_{2.5}$ measurements. An R script has been developed, tested and saved in a server of the CPHR at UM.
Study Variables ➢ Demographic characteristics ➢ Environmental factors ➢ Medical history	Description ➢ Please see Table 2 in section 6.4 for variable information.

6. Analysis

6.1. General Considerations

Timeline for final statistical analysis*:*

The final analysis will be performed after data collection and entry is complete on 228 participants and bioinformatics team has completed the data transfer to the study biostatistician.

Statistical Software*:*

SAS software version 9.4 (SAS Institute, 2016) and R software version 4.4.1 (R Core Team 2021) will be used in the analysis.

Statistical Significance and Precision*:*

A p-value less than 0.05 is considered to be statistically significant.

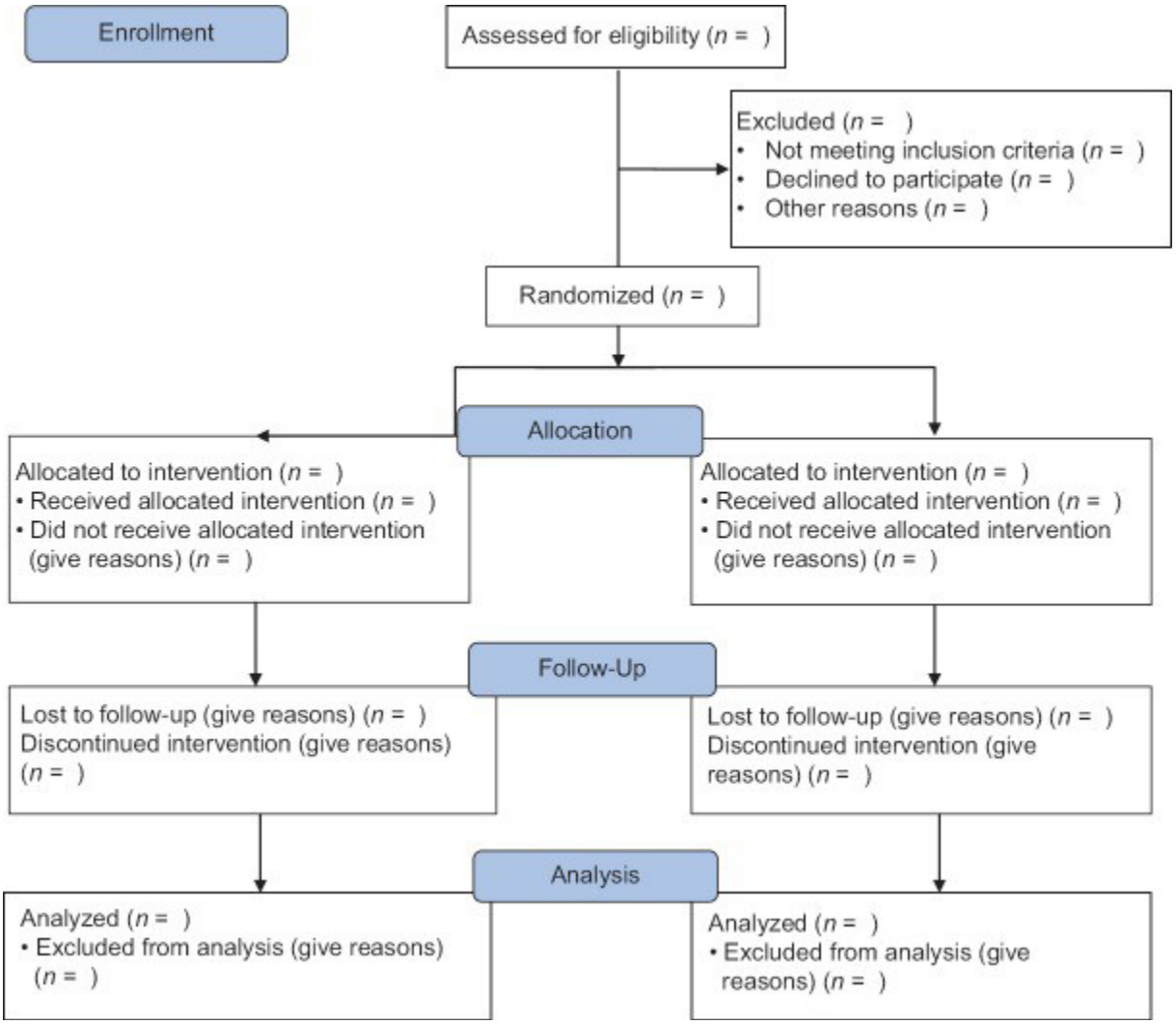
6.2. Data Screening

Summary/descriptive tables and/or graphical tools will be used to describe the distribution for continuous variables and proportions for categorical variables to ensure the levels are captured appropriately. The distribution of primary and secondary endpoints will be inspected for normal assumptions using the histogram plots. A transformation variable may be used in the parametric models to ensure the assumption of normality is met.

6.3. Subject Disposition

The flow of participants through the study will be displayed in a CONSORT flow diagram (see Figure 1 below for the template that will be used)

Figure 1. Consort flowchart template for subject disposition



6.4. Participant Demographics and Baseline Variables

The participant demographics and baseline variables are summarized in Table 2. The baseline variables were collected after randomization but prior to intervention initiation.

Table 2. Summary of Baseline Characteristics and Covariates

Sites	Variable	All participants N	Intervention Group	Control Group
	Infant demographics			
Site 1	Infant's Age at initial hospitalization for bronchiolitis (in months), mean (SD)			

	Gestational age at birth (in weeks), mean (SD)
	Infant's Sex, N (%)
	Infant's Race, N (%)
	Infant's Ethnicity, N (%)
	Rurality (RUCA code), N (%)
	Parent/caregiver/legal guardian education, N (%)
	Environmental factors
	Wood-burning stove ever used in the home (primary place of residence), N (%)
	Central air conditioning in the home (primary place of residence), N (%)
	Type of stove is used, N (%)
	A fume hood (or range hood) is above the primary cooking stove, N (%)
	Use fume hood while cooking, N (%)
	Has plumbed (running) water in the home, N (%)
	Furry pets in the home, N (%)
	Infant attends a child care center with at least 2 other infants / children who are not members of the infant's household, N (%)
	Number of people live in the home (primary place of residence), (IQR)
	Number of other children are living in the home (primary place of residence), (IQR)
	Number of the other children living in the home attend daycare or school, (IQR)
	Medical history
	Viral test results (first hospital admission for bronchiolitis), if available, per standard of care testing, N (%)

	Highest level of respiratory support during bronchiolitis admission, N (%)	
	Infant ever received systemic corticosteroids during hospitalization for bronchiolitis, N (%)	
	Previous history of wheezing with colds or other illnesses (besides this hospitalization), N (%)	
	History of asthma in the infant's parents or siblings, N (%)	
	Child has eczema or atopic dermatitis based on healthcare provider, N (%)	
	Child has been on chronic asthma medications at home any time before they were hospitalized, N (%)	
	Child ever used asthma medications with other breathing illnesses (such as colds, wheezing, or asthma) in the past (before this hospitalization), N (%)	
Site 2		
Etc…		

6.5. Data Summary and Visualization

All numerical variables will be summarized using mean ± standard deviation and median (minimum, maximum). All categorical variables will be summarized using frequency (%). The variables will be summarized using descriptive statistics overall and stratified by intervention groups (HEPA filtration versus control) as well as by sites. The variables will be compared between groups (HEPA filtration vs. control) using two sample t-tests; or Wilcoxon rank sum tests if they are numerical variables, and Chi-square tests or Fisher's exact tests if they are categorical variables. The choice of a t-test or a Wilcoxon rank sum test will depend on the empirical distribution of the tested numerical variable. The choice of a Chi-square test or a Fisher's exact test will depend on the sample size used in the test.

Under the study design of stratified randomization, it is unlikely to expect any significant association between baseline characteristics and the intervention effect. However, some of the

baseline characteristics could be associated with the endpoints. For a candidate covariate used in multivariate analyses, its correlation to the primary endpoint, or a secondary endpoint will be assessed using a Pearson's correlation coefficient or a Spearman's correlation coefficient when it is a numerical variable. When the covariate is categorical, its association to the primary endpoint, or each of the secondary endpoints will be assessed using an ANOVA model, or a Kruskal-Wallis test. A variable that is associated with the intervention and primary endpoint and secondary endpoints or the association is mentioned in the previous literature will be considered as the adjusting (controlling) covariate and will be added as adjusting independent variable in the statistical models proposed for primary and secondary analyses.

6.6. Efficacy Analysis

6.6.1. Primary Efficacy Analysis

For the primary efficacy measure, we will test the following null hypothesis:

Null hypothesis: Mean of SFDs in the HEPA filtration home intervention group will not differ from the mean of SFDs in the control group.

vs

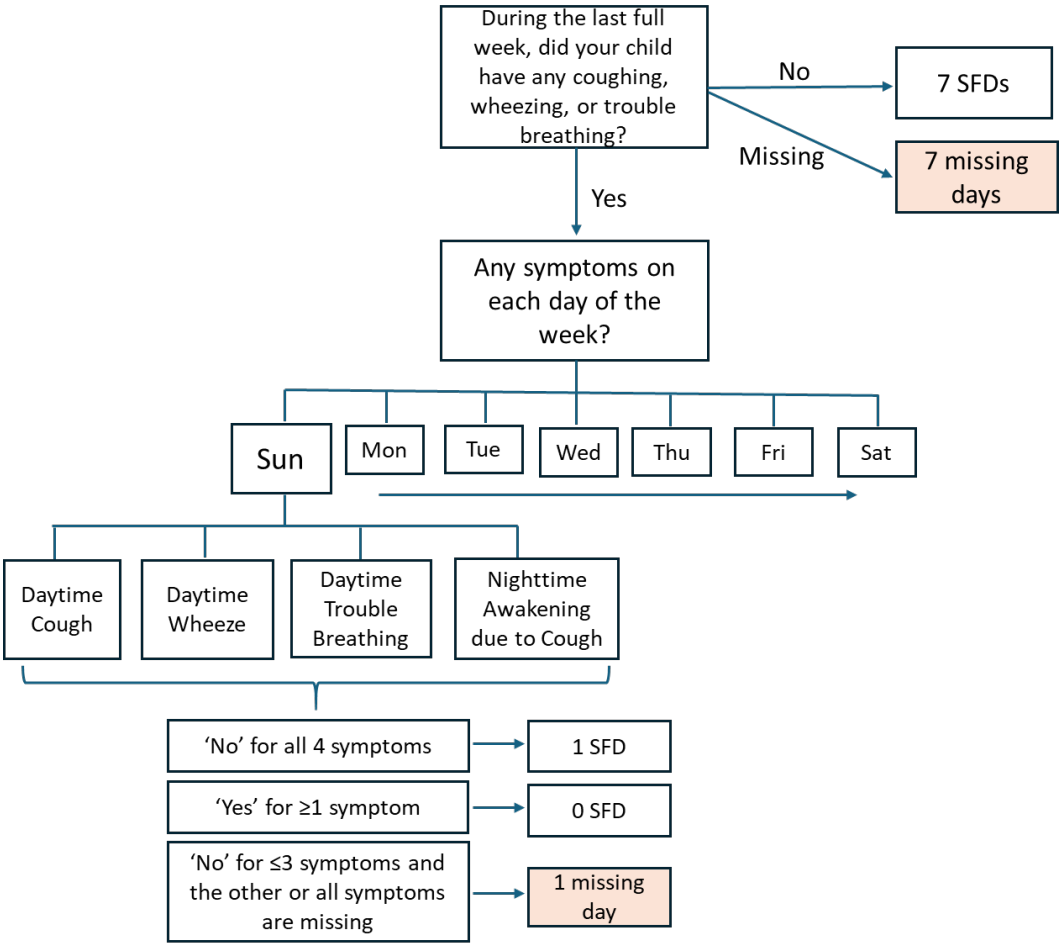
Alternative hypothesis: Mean of SFDs in the HEPA filtration home intervention group is larger than the mean of SFDs in the control group.

The primary endpoint is the number of caregiver-reported symptom-free days (SFD) over 24 weeks following activation of filtration (SFD is defined as a 24-hour period without coughing, wheezing, or trouble breathing). The SFD is observed through a survey each week. The survey is completed on each Sunday, and the symptom-free days are recorded from Sunday in the last week to Saturday, or the day before the survey date. The total SFDs range from 0 to 168 days (7*24 weeks) assuming no missing data. Complete case analysis is considered as the primary analysis. For situations of missing surveys, see Handling Missing Data section 6.8.

Figure 2 shows the flowchart of surveys of symptom-free days. If a caregiver replies a "No" to the question "During the last full week (Sunday through Saturday), did your child have any coughing, wheezing, or trouble breathing?", then the recorded SFDs in this week are seven. On the other hand, if the caregiver reports a "Yes" in the previous question, and answers a "Yes" in any of the following categories "Daytime Cough", "Daytime Wheeze", "Daytime Trouble

Breathing", and "Nighttime Awakening due to Cough" for all seven days in a week, then the SFD of the survey week will be zero.

Figure 2. Flowchart for weekly survey of symptom free days



Univariate Analysis

The primary endpoint will be measured as a count variable. The hypothesis will be tested using a generalized linear mixed model (GLMM) with a log link function. The Poisson or negative binomial distribution will be used based on statistical convergence and dispersion. The model uses the primary endpoint as the dependent variable, and the intervention effect (intervention vs. control) as the independent variable or the fixed effect with site as a random effect. We will calculate the point estimates (i.e., average SFDs) and their respective 95% CIs for each intervention group and for the difference in average SFDs between the intervention groups. Additionally, we will report the p-value of the difference in point estimates between intervention groups.

Multivariable analysis

Potential confounders will be adjusted for the impact of intervention effect on the SFDs using a multivariable GLMM. We will calculate adjusted point estimates (i.e., average SFDs) and respective adjusted 95% CIs for each intervention group and for the difference in average SFDs between the intervention groups. We will also report the adjusted p-value of the difference in point estimates between intervention groups.

6.6.2. Analysis of Secondary Endpoints

Secondary Endpoint 1: UHVS

For the secondary endpoint measure, we will test the following null hypothesis:

Null hypothesis: Mean of counts of unscheduled healthcare visits for respiratory symptoms in the intervention group will not differ from the mean of counts of unscheduled healthcare visits for respiratory symptoms in the control group.

vs

Alternative hypothesis: Mean of counts of unscheduled healthcare visits for respiratory symptoms in the intervention group is smaller than mean of counts of unscheduled healthcare visits for respiratory symptoms in the control group.

Caregiver reported counts of unscheduled healthcare visits (UHV) from each of the metrics including 1) hospitalizations; 2) Emergency Department (ED) or Urgent Care (UC) visits; 3) other unscheduled medical visits for respiratory complaints (cough, wheeze, or trouble breathing); and 4) A sum of counts (or total counts) of all metrics.

The counts of hospitalizations, ED or UC visits, and other unscheduled medical visits will be obtained from the caregiver's weekly survey or adverse event forms. The total count is the aggregation of counts of all unscheduled healthcare visits described above.

Univariate analysis

Similarly, the secondary endpoint will be measured as a count variable. The hypothesis will be tested using a generalized linear mixed model (GLMM) with a log link function and Poisson or negative binomial distribution. The model uses the secondary endpoint as the dependent variable, and the intervention effect (intervention vs. control) as the independent variable or the fixed

effect with site as a random effect. For all different types of unscheduled healthcare visits and the sum of all healthcare visits, we will calculate the point estimates (i.e., average hospitalizations, average ER visits, average unscheduled healthcare visits, and the average of all visits) and their respective 95% CIs for each intervention group and for the difference in average visits between the intervention groups. We will also report the p-value of the difference in point estimates between intervention groups.

Multivariable analysis

We will assess the impact of intervention effect on each type of unscheduled healthcare visit using a multivariable GLMM controlling for potential confounders. For all types of unscheduled healthcare visits and the sum of all visits, we will calculate adjusted point estimates (i.e., average hospitalizations, average ER visits, average unscheduled healthcare visits, or the average of total visits) and respective adjusted 95% CIs for each intervention group and for the difference in average unscheduled healthcare visits between the intervention groups. We will report the adjusted p-value of the difference in point estimates between intervention groups.

Secondary Endpoint 2: QOL score

We will test the following null hypothesis:

Null hypothesis: Child QOL will not differ in families that receive the HEPA intervention compared to controls.

vs

Alternative hypothesis: Child QOL will be higher in families that receive the HEPA intervention compared to controls.

Total QOL score or Total Scale Score (TSS), is measured by the PedsQL^{TM} Pediatric Quality of Life Inventory Infants Scales questionnaire at the end of the intervention period. Total QOL score will be calculated as the sum of all the items on the scales divided by the number of items answered. Caregivers with children at age 1-12 months will use a 36-item PedsQL, and those with children at age 13-24 months will use a 45-item PedsQL. In each item, a score is assigned at 100 if the answer is “never a problem”; 75 if the answer is “almost never a problem”; 50 if the answer is “sometimes a problem”, 25 if the answer is “often a problem”; and 0 if the answer is “almost always a problem” respectively (Varni et al., 2011).

Univariate analysis:

The total QOL score will be measured as a continuous variable. The hypothesis will be tested using a mixed effect model after accounting for within-cluster correlation. The model uses the QOL score as the dependent variable, and the intervention effect (intervention vs. control) as the independent variable or the fixed effect with site as a random effect. We will calculate the point estimates (i.e., average QOL score) and their respective 95% CIs for each intervention group and for the difference in average QOL score between the intervention groups. Additionally, we will present the p-value of the difference in point estimates between intervention groups.

Multivariable analysis:

Similarly, we will adjust for confounders for the impact of intervention effect on the QOL score using a multivariable mixed effect model. We will calculate adjusted point estimates (i.e., average QOL score) and respective adjusted 95% CIs for each intervention group and for the difference in average QOL score between the intervention groups. We will report the adjusted p-value of the difference in point estimates between intervention groups.

Secondary Endpoint 3: PM2.5

Data related to PM2.5 will be generated from two PurpleAir monitors installed in each participating home. Weekly average $PM_{2.5}$ levels as measured in each room separately and by the average of the two in-home PurpleAir monitors over 24 weeks and scaled to the unit of $\mu g/m^3$ per week. Log transformations of the $PM_{2.5}$ data will be considered if data are skewed. We will compare the weekly average $PM_{2.5}$ levels in each room separately and the average of the two monitors between intervention and control groups using the same unadjusted and adjusted mixed-effect models as described for the secondary endpoint QOL score.

6.6.3. Additional Analysis

Descriptive analysis will be conducted to compare baseline pre-intervention $PM_{2.5}$ levels by demographics, environmental factors, and medical history for the common room and sleep space, respectively. The average or median pre-intervention hourly $PM_{2.5}$ levels will be calculated for 1) all participants who have available $PM_{2.5}$ data; and 2) participants who have at least four days of pre-intervention $PM_{2.5}$ data. The demographics and baseline variables will include the infant's race, infant's ethnicity, rurality, parental education, the season of hospitalization, wood stove

use, use of central AC, type of cooking stove, presence of hood, use of hood while cooking, whether the participant infant attends daycare, number of other children in the home, number of other children in home attend daycare or school, any furry pets in the home, previous history of wheezing with colds or other illnesses, history of asthma, chronic asthma medication used before hospitalization, asthma medication with other breathing illnesses, and the highest level of respiratory support.

6.7. Safety Analysis

Any AE related to the study groups specific to child participants will be documented and summarized as overall and by study groups using descriptive statistics. Safety analysis and reports will be made as specified in the Data Safety and Monitoring Plan (DSMP).

6.7.1 Adverse Events

Adverse events constitute any untoward or unfavorable occurrence in a research participant associated with the involvement in the research that may or may not be related to their participation in research. The adverse events will be collected and coded in accordance with the guidelines specified in MedDRA/CTCAE.

Unexpected adverse events refer to adverse events occurring in participants when they deviate from the following guidelines:

- Risks outlined in study-related documents (e.g. approved research protocol, investigator brochure)
- Anticipated course of any pre-existing health conditions, diseases, or disorders in the affected individuals, considering their specific risk factors.

The analysis and listing of adverse events will be done for safety population. The summary statistics will be presented (if applicable) by organ class, study groups, study phase, severity, expectedness, and relatedness to the study intervention. The severity for adverse events will be presented as per the guidelines specified by “The Common Terminology Criteria for Adverse Events” (CTCAE) developed by the US National Cancer Institute (NCI).

Adverse events will be analyzed and listed as per the guidelines listed below:

1. If a participant experiences same AE multiple times during the study, the patient will be counted only once in the number of participants experiencing the event.

2. If a patient experiences same AE multiple times but with different severities during the study, the worst or most intense event will be counted.
3. If there are adverse events that are not coded, the summary table will use the exact description as reported in the database. The statistician may consult with the study investigator for further information to resolve this (i.e. whether it can be reassigned to one of the existing coding). Any decisions made in this regard will be documented (either by including as a footnote or as an appendix).

6.7.2 Deaths, Serious Adverse Events, and other Significant Adverse Events

Serious adverse events refer to the adverse events that meet at least one of the following criteria:

- Lead to death
- Considered life threatening (putting participants in immediate risk of death)
- Necessitate inpatient hospitalization or prolongation (if already hospitalized)
- Result in congenital anomaly or birth defect
- Result in persistent or significant disability/incapacity
- Require medical or surgical intervention to prevent outcomes listed in the above definition of SAE
- Cause significant psychological, social, economic, or legal harm to participants or others

6.7.3 Clinical Laboratory Data

No clinical laboratory data.

6.7.4 Pregnancy and pregnancy outcomes

No pregnancies and pregnancy outcomes.

6.7.5 Other DSMB reported findings

The number of weeks with high $PM_{2.5}$ concentrations (weekly average $PM_{2.5}$ level $\geq$100 $\mu g/m^3$) during the study will be summarized as overall and by study groups using descriptive statistics.

6.8 Missing Data

Missingness refers to the absence of anticipated data as well as the reasons associated with data being absent. The frequency (percentage) of missingness for each study variable will be

summarized and presented overall as well as by study group, site, and demographics (or any combination of these characteristics).

For the analysis of the primary endpoint, the findings from the complete case analysis will be presented together with the findings based on imputed data.

SOURCE OF MISSING DATA

Missing observations of primary endpoint of SFDs can occur if a caregiver fails to 1) complete and submit the weekly symptom survey in a week or in multiple weeks, or (2) complete all symptom questions in a weekly survey.

Missing observations of the secondary endpoint 1 of UHVs can occur if a caregiver fails to 1) complete and submit the weekly survey of healthcare visits in a week or in multiple weeks, or 2) report the counts of ED or UC visits and other medical visits for breathing problems.

Missing observations of the secondary endpoint 2 (total QOL score or TSS) can occur if a caregiver fails to complete and submit the PedsQL survey, or (2) complete all items in the PedsQL questionnaire.

Missing observations of the secondary endpoint 3 of $PM_{2.5}$ can occur if (1) the PurpleAir monitor fails to function and read the air quality properly, (2) the child's family disables the PurpleAir monitor(s) intentionally or unintentionally, or (3) the participant does not return the PurpleAir monitor(s) and no WiFi hotspot data is available.

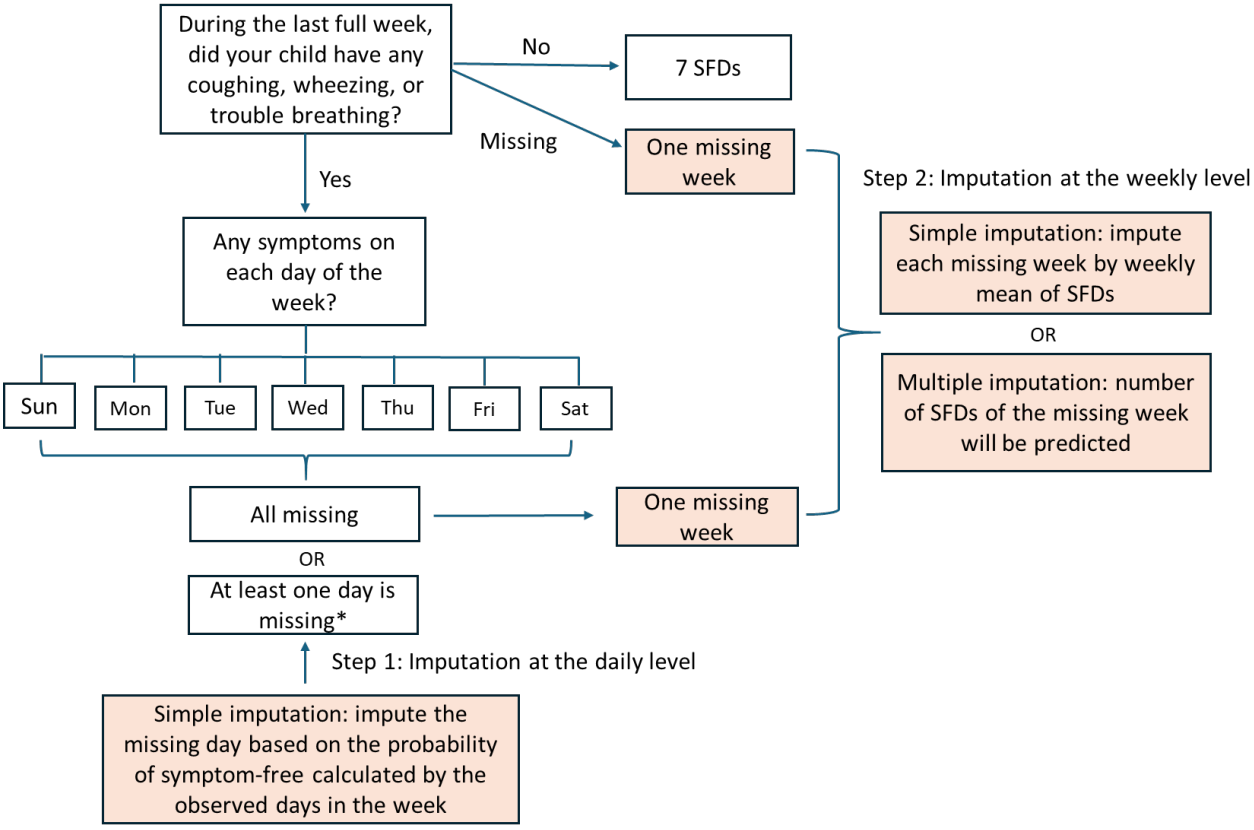
HANDLING MISSING DATA

The patterns of missing will be inspected to determine if the missing data are missing completely at random (MCAR), missing at random (MAR), or missing not at random (MNAR). Under the scenario of MCAR, the analysis will be performed using complete data or mean imputation. When MAR is assumed, a multiple imputation method will be used to impute missing data based on the relationships between the missing data and other observed variables. If the missing data are MNAR, then pattern mixture models will be used in imputation and inference (Little & Wang, 1996).

Primary endpoint of SFDs:

The complete case analysis may underestimate the total SFDs since all missing values will not be considered as SFDs. We will use both simple imputation and multiple imputation at the weekly level for missing values of SFDs and compare the results with a complete case analysis (Figure 3).

Figure 3. Flow chart of handling missing data for primary endpoint SFDs.



*The missing data at the daily level is defined as 1) all four symptoms are missing; or 2) answering 'No' for ≤3 symptoms and the other symptoms are missing.

Step 1: Simple imputation at the daily level

If the caregiver reports the child has symptoms in the week but fails to report symptoms (or no symptoms) for at least one day in seven days of the week, then we will compute the frequency of symptom-free days using the remaining observed days of the week and calculate the probability of symptom-free to impute the binary outcome of the symptom (or symptom-free) for the day with the missing observation.

Step 2 option 1: Simple imputation at the weekly level

If the caregiver fails to submit the weekly survey (i.e., During the last full week, did your child have any coughing, wheezing, or trouble breathing?) or fails to report any symptom information for seven days of the week, simple imputation will be conducted at the weekly level. The SFDs of the missing weeks will be imputed based on the average SFDs of the observed weeks.

Step 2 option 2: Multiple imputation at the weekly level

The simple imputation based on weekly mean SFDs may result in biased estimates if the data are missing for more than 10% of the participants or have a large proportion of missing weeks (Eekhout et al., 2014). After evaluation of the missing proportion and pattern, multiple imputations could be used. If the caregiver fails to submit the weekly survey or no information was provided for seven days of the week, the SFDs of the week will be coded as missing. The multiple imputation will be conducted at a weekly level. The number of missing observations ranges from 0 to 24 weeks. Multiple imputation will be used to predict how many SFDs per week by the selected observed weeks based on 1) the correlations between the missing weeks and other observed weeks; and 2) the duration of the bronchiolitis symptoms (number of weeks to be included). Multivariate Imputation by Chained Equations (MICE) R package (van Buuren & Groothuis-Oudshoorn, 2011) or MI procedure in SAS software (SAS Institute Inc., 2015) will be used. The imputation process will be repeated for at least 10 times at the weekly level (Stuart et al., 2009), after which the total SFDs across 24 weeks will be calculated by adding up the observed and imputed weekly SFDs for every imputed data set. Each of the imputed data sets will be analyzed using a mixed effect model, and the parameter estimates will be combined from all analyzed data sets at the pooling phase.

Secondary endpoint 1 unscheduled healthcare visits (UHV):

For the secondary endpoint 1 of UHV, if the missing data are caused by missed submission of the weekly survey or reporting the counts of the visits for a caregiver, the AE reports will be used as resources to calculate the number of visits during the missing weeks. The number of unscheduled healthcare visits of the missing week will not be imputed using a statistical algorithm since the imputation may overestimate the number of visits if there is no information in the AE reports about hospitalizations, ER, or other medical care visits.

Secondary endpoint 2 TSS:

For the secondary endpoint 2 of TSS, if more than 50% of the items are missing in the questionnaire, the total scale score will not be computed for the individual (Varni et al., 2011). If the missing data are caused by the missed submission of the PedsOL survey from caregivers, under the scenario of MCAR, the analysis will be performed using complete data. If MAR is assumed, a multiple imputation method will be used to impute missing data based on the relationships between the missing data and other observed variables. If the missing data are MNAR, then pattern mixture models will be used in imputation and inference.

Secondary endpoint 3 $PM_{2.5}$:

For the secondary endpoint 3 of $PM_{2.5}$, the UM team has developed a protocol to handle the missing data. Multiple steps have been used to identify, flag, and filter out missing data. Briefly, the analysis for secondary endpoint 3 will include participants who return the PurpleAir monitor(s) or have available WiFi hotspot data. If the monitoring time is less than 18 hours per day, the $PM_{2.5}$ data of the day will not be included in the average weekly $PM_{2.5}$ calculation.

7. Changes to SAP

Any changes to the statistical approaches described in protocol must be described here with justification.

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