2.1 Adherence Issues

Participants enrolled in the Microbiome protocol are involved in study activities throughout the trial. A great deal is asked of participants, and the quality of the study results is a function of the participants' level of protocol adherence. Each participant must be given every opportunity to be compliant and successful.

Factors That Affect Adherence
It is important to be aware of factors that may affect a participant's adherence level.

Participant Characteristics

- ability to comprehend and recall instructions
- support of family members for study participation
- satisfaction with care and caregivers
- degree of concern about health
- perception of disease severity
- perceived costs and benefits of treatment

Performance Site Personnel Characteristics

- consistency of AsthmaNet personnel with whom participants have contact during the study
- demonstration of interest and genuine concern for the participant's health
- warm and caring demeanor; approachable
- engagement in social conversation and active interchange
- presentation of clear instructions
- proficiency in clinical activities
- accessibility when the participant has questions, concerns or emergency needs

Clinic Characteristics

- positive and warm environment (unhurried and comfortable)
- timely appointments
- organized and efficient

Characteristics of Regimen (determined by the protocol)

- most important determinant of adherence
- should not be too complex
- side effects of study drug should not be a big problem/concern
- regimen should be adaptable to participant's life and work, not the other way around

Improving Adherence
A number of approaches can be used to improve adherence in the Microbiome trial:

- Associate the regimen with daily activities

  Encourage the participant to associate the required study activities with his/her daily routine to help make these steps automatic. This point can be reinforced while reviewing the Daily Activities handouts at each visit (P3_DAILYACT1, P3_DAILYACT2).

- Educate the participant

  - Make sure the study activities are understood
  - Demonstrate the activities and have the participant do the same
  - Present instructions as clearly as possible
  - Have the participant repeat instructions
  - 'Quiz' the participant on the instructions
  - Teach the regimen in a stepwise fashion (i.e., step 1, step 2, step 3 for AM and PM activities)
  - Review 1 or 2 of the participant handouts at each visit
  - Use phone contacts to reinforce instructions and to ensure that the participant is performing activities correctly

- Provide positive reinforcement for excellent participant adherence

- Encourage support of family and friends during study participation

- Prepare participants for what will happen at upcoming visits

- Run the clinic on schedule and make good use of the participant's time

- Make sure the clinic is accessible with flexible hours and ample, convenient parking

- Avoid no-shows with a reminder phone call in advance of the visit date. Call the participant’s residence and cell phone immediately if there is a no-show

- Ensure that clinic personnel are easily accessible by phone, pager, and e-mail

- Develop a friendly and caring relationship with the participant

  An integral part of the visit is interacting with the study personnel. A feeling of attachment or obligation to an individual improves adherence and reduces withdrawals.

Tools for Monitoring and Improving Adherence during the Microbiome Trial
The following tools are in place to improve and/or monitor adherence (form name is given in parentheses, where applicable):

Visit Scheduler Reports
Missed visits and poorly timed visits are forms of non-adherence. In order to allow the participant and the performance site to plan for upcoming visits, visit scheduler reports have been programmed that list the ideal dates and lower and upper regular and extended windows for upcoming visits per the protocol.

For Microbiome there are no defined time intervals for the screening phase, other than the constraint that Visit 1 must occur no later than 4 weeks following Visit 0. Therefore, no visit scheduler reports are in place for this portion of the study. Starting with Visit 1, a visit scheduler report will generate the ideal date and lower and upper regular and extended windows for future visits as follows:

- At Visit 1, a scheduler will provide the acceptable dates for scheduling Visit 2.
- At Visit 2, a scheduler will provide the acceptable dates for scheduling Visits 3-5 (asthmatics only).
- At Visit 5, a scheduler will provide the acceptable dates for scheduling Visit 6 (asthmatics only).

A complete listing of visit dates should be generated with the participant's daily schedule in mind. At Visit 1, multiple potential schedules may be generated for each participant by entering several start dates into the schedulers for Visit 1, Visit 2 and Visit 5. Start dates entered into the Visit 2 and 5 schedulers must be consistent with those allowed by the previous schedulers so that a given set of reports covers the entire study and keeps the participant's visits within the visit windows. Generating several complete lists of visit dates allows the participant to choose the most convenient schedule for him/her. See the Visit Schedule discussion in this section and Section 3 of this manual for further details on the creation of visit scheduler reports.

Visit Handouts and Study Folder
A series of handouts is presented and reviewed with the participant at Visit 1 and at various subsequent visits as new procedures and concepts are introduced. Because it may be difficult to comprehend and execute all instructions initially, and because activities may change during the study depending on the study phase, participants are asked to bring this folder to each visit for review and replacement of certain materials. A brief description of each of the Microbiome handouts follows:

Daily Activities Handouts – Asthmatics only
Microbiome Daily Activities: Visit 2 (P3_DAILYACT1)
Microbiome Daily Activities: Visit 5 (P3_DAILYACT2)

These handouts contain simple summaries of the study activities that must be carried out each day, including dosing with the study Diskus. These handouts also provide the participant a quick reference for his/her rescue use values for determination of significant exacerbation conditions. See the Daily Activities Handout discussion in this section for further details.

How to Use Your Metered Dose Inhaler (HTMDI) – Asthmatics only
This handout provides general instructions for proper inhalation technique for home use of the study inhalers.

Participants must demonstrate proper metered-dose inhaler (MDI) inhalation technique as assessed through the MDI Inhalation Technique Checklist (No Spacer) (TECH_MDI_NOSP) before leaving Visit 1. Completed TECH_MDI_NOSP form(s) should be filed in the participant's study folder at the performance site. See the Inhalation Technique Assessment discussion in this section for further details.

Microbiome Participant Identification Card (P3_ID) – Asthmatics only
The Microbiome Participant Identification Card (P3_ID) facilitates the identification, treatment, and handling of worsening asthma symptoms by the participant and by healthcare providers. Baseline peak flow and high rescue use values are completed on the ID card at Visit 2. See the Participant Identification Card discussion in this section for further details.

If Your Asthma Gets Worse (P3_ASWORSE) – Asthmatics only
This handout contains instructions for recognizing and treating asthma attacks. It outlines proper use of the Ventolin® RESCUE inhaler in detail. It is important for the integrity of the study for the participant to understand how to use this inhaler as outlined in the protocol. This handout should be thoroughly covered at Visit 1 and reviewed at subsequent visits. For further information regarding treatment of asthma exacerbations, see the Significant Asthma Exacerbation and Study Medications discussions in this section.

Microbiome Visit Preparation Checklist (P3_VISPRP_A or P3_VISPRP_C)
This handout is a tool for improving adherence with respect to the participant's preparation for each visit. The P3_VISPRP handout contains a checklist to help asthmatic participants to remember to bring all necessary medications, and materials to each visit. It also includes reminders to ensure that the participant refrains from using certain medications, foods, and beverages within protocol-specified periods prior to each visit. Clinic personnel should review this handout with the participant before he/she leaves each visit to be sure the information in the checklist is understood.

<u>Inhalation Technique Assessment – Asthmatics only</u>
The MDI Inhalation Technique Checklist (Without Spacer) (TECH_MDI_NOSP)
Proper inhalation technique using a metered-dose inhaler (MDI) (e.g., rescue Ventolin®) is important to the study. Improper technique is a form of non-adherence with study procedures. Instruction in proper technique and continual coaching serve to improve

adherence. The MDI Inhalation Technique Checklist (Without Spacer) (TECH_MDI_NOSP) is used to document that each participant has achieved proper MDI inhalation technique at Visit 1. See the Inhalation Technique Assessment discussion in this section for further details.

Diskus Inhalation Technique Checklist (TECH_DISKUS)
Proper inhalation technique using a Diskus (e.g., study Flovent® or placebo Diskus) is important to the study. Improper technique is a form of non-adherence with study procedures. Instruction in proper technique and continual coaching serve to improve adherence. The Diskus Inhalation Technique Checklist (TECH_DISKUS) is used to document that each participant has achieved proper Diskus inhalation technique at Visit 2. Proper inhalation technique is an eligibility criterion assessed at Visit 2.

Counseling for Non-Adherence
At each visit the participant's level of adherence with study procedures must be assessed. Individuals who have maintained high levels of adherence should be applauded. If adherence levels are low, this should be addressed with the participant.

During each visit, review the necessity of correct study medication use and the importance of avoiding medications that are not allowed during the study. Discuss the importance of rescue use information that is collected at home. Remind the participant that correctly following study procedures is crucial to the study; it is a part of the commitment he/she made when agreeing to participate.

When addressing problems, try to be constructive and helpful:

Acceptable: "I noticed that you have not been taking your study Diskus twice daily regularly. Is there anything we can do to help you?

Unacceptable: "You are not doing what you are supposed to do. What is your problem?"

When dealing with problems it is best to re-explain procedures slowly and thoroughly and to rationalize and persuade logically. Attribute lack of adherence to a misunderstanding between clinic personnel and the participant. Ensure that the participant is aware of the resources available to help him/her understand the study procedures, such as study handouts and the availability and willingness of clinic personnel to answer questions whenever they arise.

2.2 Adverse Events

Definition and Reporting
Adverse events include the following:

- Clinical Adverse Events:

  Any unintended worsening in structure or function of the body; any illness that occurs during the trial. These events are documented on the Clinical Adverse Events (AECLIN) form.

- Laboratory Adverse Events:

  Occurrences of abnormal laboratory tests or other test (e.g., ECG) results. These events are documented on the Clinical Adverse Events (AECLIN) form.

- Significant Asthma Exacerbation:

  See the discussion of Significant Asthma Exacerbations in this section for the protocol criteria. These events are documented on the Clinical Adverse Events (AECLIN) form as well as on protocol-specific forms.

- Serious Adverse Events:

  Any experience that poses a significant hazard to a participant is considered a serious adverse event. With respect to human clinical experience, a serious adverse event includes any experience that meets at least one of the following criteria:

  1. Results in death
  2. Is life threatening (places the participant at immediate risk of death from the event as it occurred)
  3. Results in a significant or persistent disability/incapacity
  4. Requires inpatient hospitalization or prolongation of an existing hospitalization
  5. Results in a congenital anomaly/birth defect
  6. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the participant's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition. Examples include allergic bronchospasm requiring intensive treatment in an emergency department or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or abuse.

  Serious adverse events are reported on the Serious Adverse Events Reporting Form (SERIOUS) as well as on the Clinical Adverse Events (AECLIN) form.

For detailed information on adverse events, see Section 4 in the AsthmaNet General Manual of Operations.

In general, ICD-9 codes describing an adverse event of any type should be obtained by searching the AsthmaNet ICD-9 Codes Excel spreadsheet. This spreadsheet can be accessed on the secure website in the Applications folder or through a link provided in concurrent forms entry. The spreadsheet includes the ICD-9 code for a particular diagnosis, along with long and short text descriptions of the related diagnosis. Clinical personnel can search the spreadsheet for a specific condition to find an appropriate code. Codes and their associated descriptions were downloaded from the Department of Health & Human Services, Centers for Medicare & Medicaid Services (CMS) website. They are from version 27 of the full and abbreviated code titles of the ICD-9-CM codes effective October 1, 2009. This code library will be used for the duration of AsthmaNet to ensure standardization across trials. Note that no other ICD-9 code references are acceptable.

For AsthmaNet, reported ICD-9 codes should describe the underlying condition or disease that resulted in a particular adverse event. For example, if a participant is hospitalized for a hysterectomy that was necessitated by uterine fibroids, the ICD-9 code for uterine fibroids should be recorded on the Clinical Adverse Events (AECLIN) form. The procedure code for hysterectomy is unavailable in the master spreadsheet and should not be recorded. In general, procedure codes will not be reported.

Specific ICD-9 codes of interest for the Microbiome study include:

- 493.92: Significant asthma exacerbation

Visit 0
Record any adverse events that have occurred since the participant signed the informed consent on the Clinical Adverse Events (AECLIN) form

If the participant experienced any adverse events between the date he/she signed the informed consent form (original signature date) and the date of Visit 0, record the events on the Visit 0 AECLIN form. If no adverse events are recorded for the participant at Visit 0, check the ‘None’ box.

A brief medical history is taken during Visit 0 to establish the participant’s study eligibility. Pre-existing conditions should be documented in the participant’s clinic notes and stored in his/her study folder for future reference; they should not be recorded on the AECLIN form.

Visit 1
Follow up clinical and laboratory adverse events from previous visit and record any new events (AECLIN)

A comprehensive medical history is taken during Visit 1. As part of this history it is important to probe for pre-existing conditions, both those related to asthma and those unrelated to asthma. This baseline knowledge is necessary to determine if conditions experienced during the Microbiome study should be considered adverse events (i.e., worsening of a chronic condition or a condition that appears for the first time during the study). Pre-existing conditions should not be recorded on the Clinical Adverse Events (AECLIN) form, but they should be noted in the clinic notes that are stored in the participant's study folder.

Visits 2-5 (Visits 3-5 for asthmatics only)
Follow up clinical and laboratory adverse events from previous visit and record any new events (AECLIN)

The Clinical Adverse Events (AECLIN) form should be updated each time the clinic has contact with a participant, whether for a scheduled visit or phone contact, impromptu visit, or unexpected phone call.

In preparation for each contact, review the participant's file to determine if there were any ongoing adverse events at the last visit/contact. If an ending date for an ongoing adverse event becomes available, update the AECLIN form with this new information. Probe the participant for the occurrence of any new adverse events and record these on AECLIN.

An AECLIN form should be completed for each participant at each visit, even if the participant has not experienced any new adverse events since the previous visit. If no new adverse events are being recorded for the participant at a visit, check the 'None' box. If new information is available, record it and have the participant review it for accuracy.

Visit 6 (asthmatics only) or other early termination visit
Events that are ongoing at the time a participant leaves the study should be left open for stop dates (i.e., coded as 'ongoing at final visit'). The participant should be probed for any stop dates that are now known to close out previously-recorded events. All AECLIN forms for a given individual should be forwarded to the DCC following his/her study termination.

General Information
If an adverse event is deemed serious by the above definition, a Serious Adverse Event Reporting Form (SERIOUS) should be completed and faxed to the DCC as soon as possible, preferably within 72 hours of clinic notification. Promptly faxing this form to the DCC expedites communicating the details of the adverse event to the Steering Committee, Data and Safety Monitoring Board (DSMB), and Institutional Review Boards (IRBs) if the event was deemed unexpected and possibly related to the study.

The term ‘study drug’ on the AECLIN form should be interpreted to mean any drug dispensed as part of the study, including open-label Flovent® Diskus and blinded Flovent®/placebo Diskus. If an adverse event is thought to be related to at least one of these medications, this fact should be documented in Q1080 on the AECLIN form. In addition, if the dose of the medication was altered as a result of the adverse event, this should be noted in Q1090.

Significant asthma exacerbations that occur during the Microbiome trial should be recorded on the Significant Asthma Exacerbation (P3_SIGEX) form at the visit when they are reported. In addition, significant asthma exacerbations should be recorded on the AECLIN form using ICD-9 code 493.92. If a participant experiences a significant asthma exacerbation during the run-in period, he/she is ineligible for randomization and should be terminated. See the discussions of Significant Asthma Exacerbations in this section for further details.

See Section 10 of the AsthmaNet General Manual of Operations for further details on AECLIN form completion and submission.

2.3 Appointments: Confirming and Scheduling

Visit 0
No formal visit windows have been established for scheduling Visit 1; however, the window between Visit 0 and Visit 1 should be no longer than 4 weeks. Also, before Visit 1 can occur, Phadiatop results must be received.

Visit 1
Confirm/Schedule upcoming appointment(s) and review Visit Preparation handout (P3_VISPRP)

Review the Microbiome Visit Scheduler Report and confirm the date of Visit 2. Write the scheduled date on the participant's copy of the Visit Scheduler Report for his/her reference, and enter the date into the clinic's appointment book or scheduling calendar. If convenient, set up future appointments at the same time.

Visits for a given participant should be scheduled for the same time of day (+/-3 hours) to avoid the introduction of circadian variability into the assessment of lung function. If a participant needs to be scheduled outside the 3-hour window, the Microbiome scientific coordinator at the DCC should be contacted to obtain an exception.

Review the Microbiome Visit Preparation (P3_VISPRP) handout with the participant. Remind him/her of the substances that must be avoided prior to each scheduled visit. For asthmatics, also remind the participant to bring his/her study medications, Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG), and handout folder to each visit. Review the checklist on side 2 of the handout.

Visits 2-4 (asthmatics only)
Confirm/Schedule upcoming appointment(s) and review Visit Preparation handout (P3_VISPRP)

At each visit, review the Microbiome Visit Scheduler Report and confirm the date of the next regular appointment. Write the scheduled date on the participant's copy of the Visit Scheduler Report for his/her reference, and enter the date into the clinic's appointment book or scheduling calendar. If convenient, set up future appointments at the same time.

Review the Microbiome Visit Preparation (P3_VISPRP) handout with the participant. Remind him/her of the substances that must be avoided prior to each scheduled visit. Also remind the participant to bring his/her study medications, Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG), and handout folder to each visit. Review the checklist on side 2 of the handout.

Visit 5 (asthmatics only)
Confirm/Schedule upcoming appointment and review Visit Preparation handout (P3_VISPRP)

Review the Microbiome Visit Scheduler Report and confirm the date of Visit 6. Write the scheduled date on the participant's copy of the Visit Scheduler Report for his/her reference, and enter the date into the clinic's appointment book or scheduling calendar.

Review the Microbiome Visit Preparation (P3_VISPRP) handout with the participant. Remind him/her of the substances that must be avoided prior to each scheduled visit. Also remind the participant to bring his/her study medications, Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG), and handout folder to each visit. Review the checklist on side 2 of the handout.

Split Visit 1
Visit 1 can be split following Methacholine Challenge if desired due to visit length. The first and second half of a split visit should occur within 24-48 hours. For the second half of Visit 1, spirometry testing must be re-done to assess eligibility for sputum induction. New Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK) and Spirometry Testing (SPIRO) forms must be completed with the current date, and should be entered into the Microbiome database as single forms (in addition to the regular packet forms). A single SPIRO_RPT should be printed containing the data recorded on the single SPIRO form. All data collected on the P3_PULMONARYCHK and SPIRO forms for both parts of the visit should be entered into the study database.

Note: When the participant returns for the second half of Visit 1, review Eligibility Checklist 1 (P3_ELIG1A, P3_ELIG1C) to make sure nothing has changed since first completed. If something has changed, the form should be updated accordingly, and the visit date changed to the current date. If the participant is eligible to continue, proceed with completion of Pulmonary Procedure Checklist (P3_PULMONARYCHK) and Spirometry Testing (SPIRO), before returning to Microbiome Visit Procedure Checklist 1 (P3_VISIT1) for completion of the remainder of Visit 1. Do not have the participant redo previously completed questionnaires at the second half of the visit; the questionnaires completed on the original visit date will be submitted with the visit packet.

See the Visit Schedule and Visit Windows discussions in this section for further details.

2.4 Asthma Control Questionnaire (asthmatics only)

Visits 0-6
Administer Asthma Control Questionnaire (ACQ)

Visits 0, 2, 5
Calculate Asthma Control Questionnaire Score (ACQ_SCORE*)

The Asthma Control Questionnaire (ACQ) was developed by Elizabeth Juniper from the Department of Clinical Epidemiology and Biostatistics at McMaster University Medical Centre.

The goal of this questionnaire is to measure asthma control using recognized psychometric methods. The ACQ has strong measurement properties and can be used with confidence to measure the adequacy of clinical asthma control. It has been fully validated for use in both clinical practice and clinical trials.

The administration of the ACQ is one of the first procedures performed at a visit. This timing in the visit structure was intentional so that a participant's responses are not affected by other study procedures, such as spirometry and diary review. Study Coordinators should observe the order of procedures as they are laid out on the visit procedure checklists to ensure that ACQ results are not biased by other study activities.

If a given visit has been partially completed and then rescheduled for a later date, a new ACQ must be completed at the beginning of the rescheduled visit. Do **not** allow the participant to refer to or update his or her previously completed questionnaire. Old copies of the questionnaires should be filed in the participant's study folder; they should not be entered into the study database or forwarded to the DCC.

In administering the questionnaire, request that the participant complete the entire 6-question form and provide answers as completely and as accurately as possible. The form is self-administered and participant completed. No stated or implied time limit should be set. If the participant requests help with or clarification of any question, the Study Coordinator should instruct the participant to reread the instructions and to give the best answer possible to each question. The Study Coordinator should not provide an answer to any question. Providing guidance may bias the participant's responses.

Elizabeth Juniper gives the following guidelines for ACQ administration to ensure the best quality data:

- Provide the participant a quiet place to complete the questionnaire.
- Participants should be alone when they complete the questionnaire. Friends and relatives should wait in a separate room.
- Before the participant completes the ACQ, the Study Coordinator should do the following:
    - Tell the participant that <u>all</u> questions should be answered.

- Tell the participant that only one response may be given for each question.
- Remind the participant that he or she is scoring problems experienced due to asthma and not because of any other problems.
- Remind the participant that the ACQ is collecting data about their asthma over the past week (7 days).

Participants should use a black pen to complete the questionnaire. If the participant wishes to change a response, the original response should be crossed out with a single line and then dated and initialed by the participant. The final response should be circled for clarification. Once the participant returns the questionnaire to study personnel, no changes are allowed.

When the participant is finished with the questionnaire, collect it and review it for completeness before proceeding with the visit. If a question has been left blank, ask the participant to do his or her best to answer it. The answers to all of the questions are very important. Check that the participant's responses are clearly marked.

No source documentation will be provided on this questionnaire due to copyright constraints.

At Visits 0, 2 and 5, when the ACQ score factors into eligibility for the study or bronchoscopy, the Asthma Control Questionnaire Score (ACQ_SCORE) administrative form should be completed to aid in calculation of ACQ score.

2.5 Asthma Monitoring Log (asthmatics only)

The Asthma Monitoring Log (P3_ASTHMA_LOG) was created to document participant's rescue use (in puffs) each day. With this, the participant can assess how his/her rescue Ventolin® use may have changed over recent days, possibly signaling the onset of a significant exacerbation. The log also includes space to record any non-study medications that are taken between visits, and any medical problems the participant experiences. This information is useful in recording concomitant medications and adverse events at the participant's next study visit. The participant should be instructed to complete this form and to return it at his/her next visit.

The Asthma Monitoring Log will be used throughout the Microbiome study, dispensed at each visit starting at Visit 1. The logs provided to the clinic sites by the DCC should be given to participants, since they are printed on heavier weight paper. Asthma logs serve as a daily log and should be completed by the participant once daily before the participant goes to bed. Total daily rescue inhaler puffs for that day (in past 24 hours) should be recorded at that time.

Dispensing the P3_ASTHMA_LOGs
Before dispensing the logs, complete the upper right-hand corner and the dates (month and day), starting with the current date and ending with the participant's next visit date.

The P3_ASTHMA_LOG form is set up as a fillable PDF file with an auto-populating date field. When preparing a log for a participant, the coordinator should complete the current date (date of the visit) in the first date field at the top of the form. All dates will be completed automatically throughout the rest of the form. The participant should begin completing the log the evening of the visit.

Visits 1-5
Complete and distribute Asthma Monitoring Log (P3_ASTHMA_LOG)

At each of Visits 1 through 5, a new P3_ASTHMA_LOG form should be completed with participant information in the key fields area and dates, starting with the date of the current visit. The participant's 'High Rescue Inhaler Use' value from P3_ID should be recorded in the blank field in the text for Visits 2 – 5. The form should be given to the participant to complete until the next regularly scheduled visit.

Emphasize that the number of *puffs* of RESCUE inhaler should be recorded, not the number of *uses.*

Explain that non-study medications and medical conditions should be documented on the back of asthma log. The participant should be instructed that preventive bronchodilator puffs (taken routinely prior to exercise and other strenuous activities) should not be recorded in daily rescue use counts.

Encourage the participant to record the information each and every day. It is helpful if the recording of the data can be associated with specific daily activities (e.g., brushing teeth). Emphasize that data should not be *made up* or *recalled* more than one day back if days are missed.

Visits 2-6

Collect Asthma Monitoring Log (P3_ASTHMA_LOG)

Near the beginning of each visit, the participant's completed P3_ASTHMA_LOG form should be collected and reviewed with him/her for any recorded comments, concomitant medications, or adverse events experienced since the last visit.

At Visit 2, the participant's baseline rescue use should be completed in Q1000. Baseline rescue use equals the total number of rescue puffs divided by the total number of days for the period between Visits 1 and 2.

Review the asthma logs with the participant for completeness and legibility. Make sure the entries are completed in black ink. If they are not, remind the participant to use black ink. Also make sure the entries are easy to read. If numbers seem unclear, request clarification from the participant and have him or her update the values on the asthma log. If a page of the asthma log is difficult to read, ask the participant to copy the information onto a new asthma log before leaving the clinic. Note that clinical personnel should not make changes to the logs; only the participant may alter this information.

Examine the back of the asthma logs. This information is important for the documentation of adverse events and concomitant medications. If appropriate, complete the corresponding form(s) (AECLIN, CMED, and CMED_NON).

2.6 Baseline Peak Flow and Rescue Use Values (asthmatics only)

Baseline peak flow (PEF) and rescue medication use values for asthmatics are determined at Visit 1 and Visit 2, respectively. The baseline PEF is recorded on the Participant Identification Card (P3_ID), while the rescue use value is recorded on the Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG) and entered into the study database. The rescue use value is used by the participant and clinical personnel to identify when the participant meets certain significant asthma exacerbation criteria; the baseline PEF is used in the treatment of a significant asthma exacerbation.

Visit 2
Complete Asthma Monitoring Log (P3_ASTHMA_LOG)

Baseline Rescue Use Value
At Visit 2, the baseline rescue use value is the participant's average daily use (in puffs) of albuterol (RESCUE medication) during the week prior to the visit as completed on the Asthma Monitoring Log (P3_ASTHMA_LOG). Average daily use is calculated by taking total number of puffs divided by total number of days. Round to the nearest puff when calculating the value. Preventive puffs (e.g., pre-exercise puffs or puffs taken in advance of allergen exposure) should not be included in this calculation. Note that one use of nebulized albuterol should be counted as two puffs.

The baseline rescue use value is recorded in Q1000 on the P3_ASTHMA_LOG form. The participant's High Rescue Inhaler Use value is calculated by adding 8 to the baseline rescue use value. The High Rescue Inhaler Use value is used to determine when a participant meets certain asthma exacerbation criteria. If the participant uses at least the number of puffs per day denoted by the High Rescue Inhaler Use value, for a two day period, then he/she meets significant asthma exacerbation conditions. The High Rescue Inhaler Use value is recorded on the Participant Identification Card (P3_ID), as well as on several of the participant handouts.

Once established at Visit 2, the participant's baseline rescue use value will not change for the remainder of the trial.

Baseline Peak Flow (PEF)
Baseline PEF is the spirometry peak flow value corresponding to the best effort during baseline spirometry at Visit 1 (converted to liters/minute). This value is obtained by multiplying the value from Q1050 (FEF Max) on the Spirometry Testing (SPIRO) form by 60 and rounding to the nearest whole liter/minute.

The baseline PEF value is recorded on the Participant Identification Card (P3_ID) and is used as a reference in treatment of significant asthma exacerbations.

2.7 Blood draw (Biomarker analysis and allergen-specific IgE)

Visit 1

Obtain blood for biomarker analysis and allergen-specific IgE determination (one red-top tube)

Log biomarker and allergen-specific IgE serum samples

Enter biomarker and allergen-specific IgE sample data into Biological Sample Tracking module

Supplies

The following supplies are required to collect the Biomarker analysis and allergen-specific IgE samples for Microbiome:

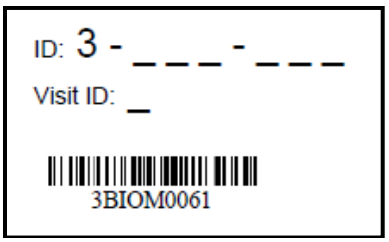
Item	Vendor	Catalog #	# Per Collection
10 ml red-top vacutainer (BD #367820)	Fisher Sci.	02-685-112	1
Red-top vacutainer label (Avery #5160)	Staples	209882	1
2.0 ml self-standing Saf-T-Seal screw cap tube, natural (USA Scientific, no substitutions)	Fisher Sci.	1420-9700	9
White Laser Cryo-Tags barcode label (Cryo-Tags 1.5”x0.75”)	Diversified Biotech	LCRY-1200	9
Sterile pipette			1

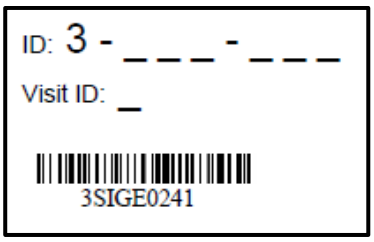
Fill one 10 mL red-top vacutainer with the participant’s blood. The vacutainer must be labeled with participant ID, initials and visit number. A template for labels for the red-top tubes (Avery #5160) can be found on the AsthmaNet secure website in the Protocols: Microbiome: Labels folder. For all participants, complete an entry for the blood draw on the Microbiome Biomarker Serum Sample Log (P3_BIOM_SAMP_LOG). For those participants with a positive Phadiatop, complete an entry on the Microbiome Allergen-specific IgE Serum Sample Log (P3_SIGE_SAMP_LOG) too. Complete the participant’s Microbiome ID number, visit number, and collection date/time.

Allow the blood sample to clot at room temperature between 20 minutes to 1 hour. At the end of the clotting period, complete the time spinning is initiated on P3_BIOM_SAMP_LOG and P3_SIGE_SAMP_LOG. Centrifuge the clotted blood at a minimum of 2800 RPMs for 15 minutes to separate the serum from the red blood cells.

Using a sterile pipette, carefully remove the serum from above the clot. For those participants with a positive Phadiatop, place 1 mL into one 2.0 ml screw cap tube for allergen-specific IgE testing. For all participants, place 0.5 mL into eight screw cap tubes for biomarker analysis. If less than 5 mL serum is collected, first place 1 mL serum into tube for allergen-specific IgE testing. Remaining serum should be aliquoted by 0.5 mL into however many screw cap tubes serum volume will allow for biomarker analysis.

Label the serum tubes with barcode labels (Cryo-Tags) generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system. Labels should be placed vertically on tube so that the barcode can be scanned. The length of the label is 1.5" so the label should be placed as high as possible (just under the screw top). For the allergen-specific IgE sample, place a barcode label that starts with "3SIGE" on it. The sample type associated with this tube in the BST module is "MCBM Allergen-specific IgE". For the biomarker analysis samples, place a barcode label that starts with "3BIOM" on it. The sample type associated with this tube in the BST module is "MCBM Biomarker Analysis". The participant's Microbiome ID and visit number should be written in the space provided on the label with a Sharpie marker. Sample Microbiome Biomarker and Specific IgE serum barcode labels follow:





Complete the tube barcode numbers and sample volumes on P3_BIOM_SAMP_LOG and P3_SIGE_SAMP_LOG.

Immediately after labeling the serum tubes, access the BST module and scan the barcodes to insert records for the samples. Input the participant ID information to link the barcodes to the correct Microbiome participant. It is imperative that all samples be scanned the day of collection so that the associated dates and times of the serum samples are available in the BST database. For details on accessing and interacting with the BST Module in the AsthmaNet Database Application, see the AsthmaNet Computing and Networking Environment details in Section 7 of the AsthmaNet General Manual of Operations.

Store the serum samples at -80° C until the shipment day. Record the date/time the sample is placed in the freezer and the current freezer temperature on P3_BIOM_SAMP_LOG and P3_SIGE_SAMP_LOG.

Sample Shipping

Samples for Biomarker analysis and Allergen-specific IgE testing will be stored at the site until the last participant has been enrolled at the site. The samples will then be

shipped in a batch, biomarker samples sent to the Boushey Lab at UCSF and allergen-specific IgE samples sent to Advanced Diagnostic Laboratories in Denver. Shipments must be sent Monday – Thursday only.

Note: Allergen-specific IgE samples can only be frozen for 1 year. If approaching one year since serum for allergen-specific IgE was collected for first participant, allergen-specific IgE samples collected to date should be mailed to Denver for analysis rather than waiting for last participant to be enrolled. When last participant has been enrolled, remaining allergen-specific IgE samples should be mailed to Denver.

Preparing Biomarker Samples for Shipment to Boushey Lab

A few days prior to shipment, e-mail the Boushey Lab to notify them of the shipment:

Snehal Nariya (snehal.nariya@ucsf.edu)
Homer Boushey (homer.boushey@ucsf.edu)
Yvonne Huang (yvonne.huang@ucsf.edu)
Kelsey Wollen (kelsey.wollen@ucsf.edu)

To create a shipment, scan the barcodes for all samples available to ship into the AsthmaNet BST system. Include a shipment comment detailing the contents of the shipment (i.e., human serum). Each shipment (from each site) will receive a unique shipment ID number. A shipment inventory will be generated that contains: date of shipment, tracking number, site of origination, shipment ID, and an inventory detailing all the tubes in the shipment with their barcode numbers and participant information (study ID number, initials, visit number and blood draw date). Print the shipment inventory for inclusion in the shipment.

Once the shipment is confirmed in the BST module, an e-mail will automatically be sent to the Boushey Lab. The e-mail will include an export file from the database that shows the information from the shipment inventory. A summary of the shipment will be included in the body of the e-mail message.

Packaging Biomarker Samples for Shipment to Boushey Lab

Before packaging available samples for shipment, they must be scanned into the BST system and an inventory of the shipment generated as described above. After the samples have been scanned and the shipment has been confirmed by the performance site, the samples should be packaged for shipment. The following materials are required:

Item	Vendor	Catalog #	# Per Shipment
Insulated Shipper Container (e.g. ThermoSafe)	Fisher Sci.		1
Dry Ice			
Cardboard freezer storage box (Deep-lid, premium cardboard box without grid/divider)	USA Scientific	9023-8100	1
100-well cardboard dividers	Fisher Sci	TF4000013	1
Ziploc bag			2
Packaging tape	Staples	380107	
Exempt Human Specimen labels Therapak 2.5”x2”	Fisher Sci.	22-130-070	1
Biohazard labels (large) 3”x3”	Fisher Sci.	18-999-936	1
Dry Ice labels (DRY ICE – UN 1845)	Air Sea Containers http://www.airseacontainers.com 1-866-272-9880	Dry Ice UN 1845 Label, Roll of 500 (No product number)	1
Shipment inventory from BST			1

Assembly instructions:

1. Place about 10 lbs. dry ice in the Styrofoam container.
2. Place the cardboard freezer storage box with divider holding the biomarker tubes in a Ziploc bag.
3. Further seal the Ziploc bag by securely taping it closed with packaging tape.
4. Pack the storage box in the Styrofoam container.
5. Place the shipment inventory in a Ziploc bag and insert into container.
6. Seal the shipping container.
7. Attach one large “Biohazard” label and one “Exempt Human Specimen” sticker to the shipping container.
8. Attach dry ice label “DRY ICE – UN 1845” to the container. Mark the approximate weight of dry ice in kg for each shipment.

9. Address the shipment to:

   Attention: Snehal Nariya
   UCSF
   Health Sciences East, Room 1355B
   513 Parnassus Avenue
   San Francisco, CA 94143
   Phone: (415) 476-5985

10. Specify priority overnight shipment for AM receipt.

Preparing Allergen-specific IgE Samples for Shipment to Denver Lab

A few days prior to shipment, e-mail the Denver Lab to notify them of the shipment:
Brock Harper (HarperB@njhealth.org)
Samantha LeRoy (LeroyS@njhealth.org)
Michael Leyden (leydenm@njhealth.org)

To create a shipment, scan the barcodes for all samples available to ship into the AsthmaNet BST system. Include a shipment comment detailing the contents of the shipment (i.e., human serum). Each shipment (from each site) will receive a unique shipment ID number. A shipment inventory will be generated that contains: date of shipment, tracking number, site of origination, shipment ID, and an inventory detailing all the tubes in the shipment with their barcode numbers and participant information (study ID number, initials, visit number and blood draw date). Print the shipment inventory for inclusion in the shipment.

Once the shipment is confirmed in the BST module, an e-mail will automatically be sent to the Denver Lab. The e-mail will include an export file from the database that shows the information from the shipment inventory. A summary of the shipment will be included in the body of the e-mail message.

Packaging Allergen-specific IgE for Shipment to Denver Lab

Before packaging available samples for shipment, they must be scanned into the BST system and an inventory of the shipment generated as described above. After the samples have been scanned and the shipment has been confirmed by the performance site, the samples should be packaged for shipment. The following materials are required:

Item	Vendor	Catalog #	# Per Shipment
Insulated Shipper Container (e.g. ThermoSafe)	Fisher Sci.		1
Dry Ice			
Cardboard freezer storage box	USA Scientific	9023-8100	1

(Deep-lid, premium cardboard box without grid/divider)			
100-well cardboard dividers	Fisher Sci	TF4000013	1
Ziploc bag			1
Packaging tape	Staples	380107	
Exempt Human Specimen labels Therapak 2.5”x2”	Fisher Sci.	22-130-070	1
Biohazard labels (large) 3”x3”	Fisher Sci.	18-999-936	1
Dry Ice labels (DRY ICE – UN 1845)	Air Sea Containers http://www.airseacontainers.com 1-866-272-9880	Dry Ice UN 1845 Label, Roll of 500 (No product number)	1
Shipment inventory from BST			1

Assembly instructions:

1. Place about 10 lbs. dry ice in the Styrofoam container.
2. Place the cardboard freezer storage box with divider holding the allergen-specific IgE tubes in a Ziploc bag.
3. Further seal the Ziploc bag by securely taping it closed with packaging tape.
4. Pack the storage box in the Styrofoam container.
5. Place the shipment inventory in a Ziploc bag and insert into container.
6. Seal the shipping container.
7. Attach one large “Biohazard” label and one “Exempt Human Specimen” sticker to the shipping container.
8. Attach dry ice label “DRY ICE – UN 1845” to the container. Mark the approximate weight of dry ice in kg for each shipment.
9. Address the shipment to:

   Advanced Diagnostic Laboratories at National Jewish Health
   ATTN: Client Services
   1400 Jackson Street
   Room M013
   Denver, CO 80206
   Phone: (303) 270-2579

10. Specify priority overnight shipment for AM receipt.

Denver Lab Contact
Name: Brock Harper
Phone: 303-270-2579

2.8 Blood draw (CBC and BUN/Creatinine)

Visit 1
Obtain blood for CBC/differential (one purple-top tube)
Obtain blood for BUN/Creatinine (one tiger-top tube)

CBC/Differential Procedures
For eligible participants only, fill one 5 ml purple-top tube with blood for CBC/differential determination. These samples will be analyzed in the clinical center's local lab. Samples should be transported to the lab within **two hours** of the blood draw. Record the participant's CBC/differential values on the P3_LAB form. Refer to Section 4 for more details on how to complete the P3_LAB form.

BUN / Creatinine Procedures
For eligible participants only, fill one 5 ml tiger-top tube with blood BUN / Creatinine determination. These samples will be analyzed in the clinical center's local lab. Samples should be transported to the lab within **two hours** of the blood draw. Record the participant's values on the P3_LAB form. Refer to Section 4 for more details on how to complete the P3_LAB form.

Visit 2
Obtain blood for CBC/differential (one purple-top tube)

CBC/Differential Procedures
Prior to bronchoscopy, as part of immunophenotyping blood draw, fill one 4 ml purple-top tube with blood for CBC/differential determination. The preferred protocol will be to draw the blood from the IV itself at the time of placement (after first using a separate syringe to pull back through the IV to clear blood diluted with saline introduced at the time the IV catheter was inserted). If that is not possible, a separate phlebotomy will be acceptable. These samples will be analyzed in the clinical center's local lab. Samples should be transported to the lab within **two hours** of the blood draw. Record the participant's CBC/differential values on the P3_LAB form. Refer to Section 4 for more details on how to complete the P3_LAB form.

2.9 Blood draw (Immunophenotyping)

Visit 1
E-mail Visit 2 date to Ansel Lab (laura.christian@ucsf.edu) and Microbiome Scientific Coordinator (adyer@psu.edu) as soon as possible

The machine used to analyze the immunophenotyping blood and BAL samples needs to be reserved a week prior to the arrival of samples. Near the end of Visit 1, the Visit 2 date should be confirmed and communicated to the Ansel Lab and Scientific Coordinator. It is important that you e-mail these individuals so they can schedule appropriately and keep track of bronchoscopy visits.

If a scheduled visit date changes or the visit is cancelled, notify the Ansel Lab as soon as possible. Unforeseen circumstances (i.e. rescheduled visit) will be accommodated.

Visit 2, 5
Obtain blood for immunophenotyping (two 5 mL Cyto-Chex® BCT tubes)
Enter immunophenotyping sample data into Biological Sample Tracking module

Visit 4
E-mail Visit 5 date to Ansel Lab (laura.christian@ucsf.edu) and Microbiome Scientific Coordinator (adyer@psu.edu) as soon as possible

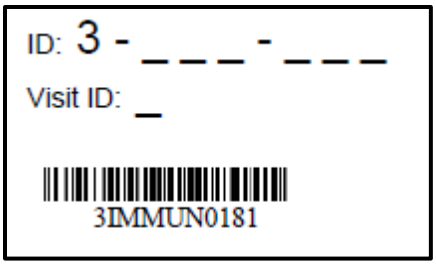
Supplies

Item	Vendor	Catalog #	# Per Collection
Supplied by UCSF 5 mL Cyto-Chex BCT blood collection tubes	Fisher Sci.	11-716-358	2
White Laser Cryo-Tags *Or* Tough-Tags barcode label (1.50” x 0.75”)	Diversified Biotech	LCRY-1200 *or* TTGP-1050	2

Prior to bronchoscopy, 10 mL of blood should be drawn into two 5 mL Cyto-Chex BCT blood collection tubes (2 x 5 mL). Invert gently 8 times and store at room temperature. The preferred protocol will be to draw the blood from the IV itself at the time of placement (after first using a separate syringe to pull back through the IV to clear blood diluted with saline introduced at the time the IV catheter was inserted). If that is not possible, a separate phlebotomy will be acceptable.

Label the serum vial with a barcode label (Cryo-Tags *or* Tough-Tags) generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system. The barcode label for the immunophenotyping blood starts with “3IMMUN”, which represents the sample type (3 for Microbiome protocol and ‘IMMUN’

for immunophenotyping blood), and is followed by a 4 digit number. The sample type associated with the serum tubes in the BST module is “MCBM Immunophenotyping Blood.” The participant’s Microbiome ID should be written in the space provided on the label. A sample Microbiome immunophenotyping blood barcode label follows:



Immediately after labeling the serum vials, access the BST module and scan the barcodes to insert records for the samples. Input the participant ID information to link the barcodes to the correct Microbiome participant. It is imperative that all samples be scanned the day of collection so that the associated dates and times of the serum samples are available in the BST database. For details on accessing and interacting with the BST Module in the AsthmaNet Database Application, see the AsthmaNet Computing and Networking Environment details in Section 7 of the AsthmaNet General Manual of Operations.

Store the blood sample at room temperature. Samples must be shipped on the same day as bronchoscopy by overnight mail to the Ansel Lab at UCSF. Samples cannot be shipped on Fridays. BAL cell pellet in Streck Cell Preservative will be shipped with immunophenotyping blood.

Preparing Samples for Shipment to Ansel Lab
Immunophenotyping samples must be shipped the day they are collected!

E-mail the UCSF Immunophenotyping Core (Ansel Lab) at least 48 hours in advance of bronchoscopy to insure they are ready for your shipment:

Laura Christian (laura.christian@ucsf.edu)
Sana Patel (sana.patel@ucsf.edu))
Mark Ansel (mark.ansel@ucsf.edu)

To create a shipment, scan the barcodes for samples ready to ship into the AsthmaNet BST system. Include a shipment comment detailing the contents of the shipment (i.e., human blood and BAL cell pellet). Each shipment (from each site) will receive a unique shipment ID number. A shipment inventory will be generated that contains: date of shipment, tracking number, site of origination, shipment ID, and an inventory detailing all the tubes in the shipment with their barcode numbers and participant information (study ID number, initials, visit number and blood draw date). Print the shipment inventory for inclusion in the shipment.

Once the shipment is confirmed in the BST module, an e-mail will automatically be sent to the Ansel lab. The e-mail will include an export file from the database that shows the information from the shipment inventory. A summary of the shipment will be included in the body of the e-mail message.

Packaging Samples for Shipment to Ansel Lab
Before packaging samples for shipment, they must be scanned into the BST system and an inventory of the shipment generated as described above. After the samples have been scanned and the shipment has been confirmed by the performance site, the samples should be packaged for shipment.

Item	Vendor	Catalog #	# Per Shipment
Cardboard Shipper Box			1
Parafilm	Fisher Sci.	13-374-10	
Absorbant pad	Fisher Sci.	19-075-383C	
Ziploc bag			2
Bubble wrap			
Packaging tape	Staples	380107	
Exempt Human Specimen labels Therapak 2.5”x2”	Fisher Sci.	22-130-070	1
Biohazard labels (large) 3”x3”	Fisher Sci.	18-999-936	1
Shipment inventory from BST			1

Assembly instructions:

1. Wrap the cap of the BAL immunophenotyping vial with parafilm to insure against leakage.
2. Place BAL immunophenotyping vial, blood collection tubes, and absorbant pads into Ziploc bag. Seal bag.
3. Wrap the sealed bag in bubble wrap. Use tape to secure the bag inside the bubble wrap.
4. Place in box, using additional packing material generously to prevent breakage.
5. Place the shipping inventory in waterproof Ziploc bag at the top of the shipping box. Seal box.
6. Attach one large “Biohazard” label and one “Exempt Human Specimen” sticker to the shipping box.

7. Ship by Fedex to Ansel Lab (Immunophenotyping Core).

   Ansel Lab (c/o Laura Christian)
   University of California San Francisco
   513 Parnassus Avenue, HSE-201
   San Francisco, CA 94143-0414
   (Phone: 1-415-476-5373)

8. Use Fedex website or manually send an e-mail message containing the tracking number and date of shipment to all of the addresses below:

   Laura Christian (laura.christian@ucsf.edu)
   Sana Patel (sana.patel@ucsf.edu)
   Mark Ansel (mark.ansel@ucsf.edu)

9. Specify priority overnight shipment for AM receipt.

Supplies Provided to Sites

Cyto-Chex BCT blood collection tubes will be shipped to each site from San Francisco site (Immunophenotyping Core). Inform Laura Christian (laura.christian@ucsf.edu) if your supplies are getting low and allow at least 2 weeks for delivery.

2.10 Blood draw (Phadiatop and IgE)

Visit 0
Obtain blood for Phadiatop and total IgE determination (one 5 ml red-top tube)
Log Phadiatop sample
Enter Phadiatop sample data into Biological Sample Tracking module

Supplies
The following supplies are required to collect the Phadiatop and IgE samples for Microbiome:

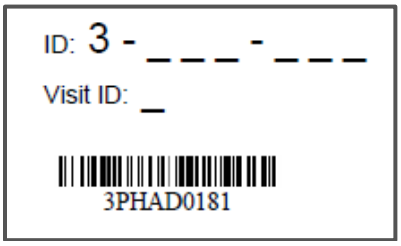
Item	Vendor	Catalog #	# Per Collection
5 ml red-top vacutainer (BD #367814)	Fisher Sci.	02-687-94	1
Red-top vacutainer label (Avery #5160)	Staples	209882	1
2.0 ml self-standing Saf-T-Seal screw cap tube, natural (USA Scientific, no substitutions)	Fisher Sci.	1420-9700	1
White Laser Cryo-Tags *Or* Tough-Tags barcode label (1.50" x 0.75")	Diversified Biotech	LCRY-1200 *Or* TTGP-1050	1
Sterile pipette			1

Fill one 5 ml red-top vacutainer with the participant's blood. The vacutainer must be labeled with participant ID and initials. A template for labels for the red-top tubes (Avery #5160) can be found on the AsthmaNet secure website in the Protocols: Microbiome: Labels folder. Complete an entry for the blood draw on the Microbiome Phadiatop and IgE Serum Sample Log (P3_PHAD_SAMP_LOG). Complete the participant's Microbiome ID number, visit number, and collection date/time.

Allow the blood sample to clot at room temperature between 20 minutes to 1 hour. At the end of the clotting period, complete the time spinning is initiated on P3_PHAD_SAMP_LOG. Centrifuge the clotted blood at a minimum of 2800 RPMs for 15 minutes to separate the serum from the red blood cells. Using a sterile pipette, carefully remove the serum from above the clot and place 0.5 ml into one 2.0 ml screw cap tube for Phadiatop and total IgE.

Label the serum tube with a barcode label (Cryo-Tags) generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system. Label should be placed vertically on tube so that the barcode can be scanned. The length of the label is 1.5" so the label should be placed as high as possible (just under the screw

top). The barcode label for the Phadiatop blood draw with “3PHAD”, which represents the sample type (3 for Microbiome protocol and ‘PHAD’ for Phadiatop), and is followed by a 4 digit number. Total IgE will be run on the same sample. The sample type associated with the serum tube in the BST module is “MCBM Phadiatop.” The participant’s Microbiome ID should be written in the space on the side of the tube with a Sharpie marker. A sample Microbiome Phadiatop serum barcode label follows:



Complete the tube barcode number and sample volume on P3_PHAD_SAMP_LOG.

Immediately after labeling the serum tube, access the BST module and scan the barcodes to insert records for the samples. Input the participant ID information to link the barcodes to the correct Microbiome participant. It is imperative that all samples be scanned the day of collection so that the associated dates and times of the serum samples are available in the BST database. For details on accessing and interacting with the BST Module in the AsthmaNet Database Application, see the AsthmaNet Computing and Networking Environment details in Section 7 of the AsthmaNet General Manual of Operations.

Store the serum sample at 4 degrees Celsius (refrigerated) until the shipment day. Record the date/time the sample is placed in the refrigerator and the current refrigerator temperature on P3_PHAD_SAMP_LOG. Serum may be refrigerated no longer than 2 weeks prior to analysis at Denver Lab.

A minimum of 0.3 ml of serum is required for the Phadiatop and total IgE assay. Note: Blood draw for allergen-specific IgE will be performed at Visit 1 on those participants who have a positive Phadiatop result.

Preparing Samples for Shipment to Denver (every Monday and Thursday)
Sites should ship all available samples to Denver Lab each Monday and Thursday, priority overnight for AM receipt in Denver.

To create a shipment, scan the barcodes for all samples available to ship into the AsthmaNet BST system. Include a shipment comment detailing the contents of the shipment (i.e., human serum). Each shipment (from each site) will receive a unique shipment ID number. A shipment inventory will be generated that contains: date of shipment, tracking number, site of origination, shipment ID, and an inventory detailing all the tubes in the shipment with their barcode numbers and participant information (study ID number, initials, visit number and blood draw date). Print the shipment inventory for inclusion in the shipment.

Once the shipment is confirmed in the BST module, an e-mail will automatically be sent to the Denver lab. The e-mail will include an export file from the database that shows the information from the shipment inventory. A summary of the shipment will be included in the body of the e-mail message.

Packaging Samples for Shipment to Denver Lab (every Monday and Thursday)
Before packaging available samples for shipment, they must be scanned into the BST system and an inventory of the shipment generated as described above. After the samples have been scanned and the shipment has been confirmed by the performance site, the samples should be packaged for shipment. The following materials are required:

Item	Vendor	Catalog #	# Per Shipment
Styrofoam mailer (ThermoSafe 440)	Fisher Sci.	03-528-10	1
Corrugated cardboard mailing sleeve (Thermosafe 440KD)	Fisher Sci.	03-520-7	1
Convoluted foam padding (Thermosafe 441)	Fisher Sci.	03-5206	2
Biohazard specimen bags 6”x10” (Fisherbrand)	Fisher Sci.	19-075-388D	2
Desiccant (Multisorb Drimop 0.5g liquid absorber 0201083CG08)	Fisher Sci.	NC9905534	1 for every 10 ml of sample (round up)
Gel packs (Thermosafe 8 oz 429)	Fisher Sci.	03-528-6	1
Packaging tape	Staples	380107	
Exempt Human Specimen labels Therapak 2.5”x2”	Fisher Sci.	22-130-070	1
Biohazard labels (large) 3”x3”	Fisher Sci.	18-999-936	1
Shipment inventory from BST			1

Assembly instructions:

1. Place tubes in a biohazard specimen bag. Add 1 desiccant pack for every 10 ml of sample and seal the bag tightly.
2. Further seal the specimen bag by securely taping it closed with packaging tape.
3. Place the sealed specimen bag inside a second biohazard specimen bag and seal the bag tightly.
4. Place 1 frozen gel pack in the bottom of the Styrofoam mailer. Cover with one sheet of the convoluted foam padding (egg carton side up).
5. Place the specimen bag on top of the foam padding and cover it with a second sheet of foam padding, egg carton side down. The specimen bag should be sandwiched between the sheets of foam.
6. Place a copy of the shipment inventory on top of the foam and close the Styrofoam mailer tightly. Use several loops of packaging tape to ensure that the mailer is secured and that it cannot leak if a sample tube opens during shipment.
7. Place the Styrofoam mailer inside a mailing sleeve. Attach one large "Biohazard" label and one "Exempt Human Specimen" sticker to the mailing sleeve. Address the shipment to:

   Advanced Diagnostic Laboratories at National Jewish Health
   ATTN: Client Services
   1400 Jackson Street
   Room M013
   Denver, CO 80206
   Phone: (303) 270-2579

8. Specify priority overnight shipment for <u>AM receipt</u>.

Receipt of Samples at Denver Laboratory (Tuesday and Friday mornings)
Denver Lab will receive shipments on Tuesday and Friday mornings. Lab staff will scan the barcodes of all received samples into the AsthmaNet BST system to reconcile the shipments and identify/resolve any problems. Lab staff can generate an export file (.csv format) through the BST system that contains the following variables: barcode number, participant ID number, participant initials, visit number, collection date and shipment ID. Export files can be generated separately for each shipment ID, or a combined export file that includes all shipments received on a given day (or days) can be created.

Analysis for Phadiatop and IgE will be performed on Tuesday and Friday afternoons, following receipt of shipments from all AsthmaNet sites that confirmed a shipment on a given Monday or Thursday.

Table 3 outlines shipment, analysis and result availability days. Please notice that Phadiatop results for visits occurring on Tuesday or Wednesday will not be ready until the following Wednesday. Phadiatop results for visits occurring on all other days will be available within one week of the visit.

Table 3.

Collection Day	Shipment Day	Lab Receipt/ Analysis Day	Results Ready	Report Run and Received at DCC
Monday	Monday	Tuesday	Thursday	Friday
Tuesday	Thursday	Friday	Tuesday	Wednesday
Wednesday	Thursday	Friday	Tuesday	Wednesday
Thursday	Thursday	Friday	Tuesday	Wednesday
Friday	Monday	Tuesday	Thursday	Friday
Saturday	Monday	Tuesday	Thursday	Friday
Sunday	Monday	Tuesday	Thursday	Friday

Phadiatop Result Availability
Phadiatop results will be received at the DCC every Wednesday and Friday afternoon, as indicated above, assuming all shipments are received in Denver on time and no problems are encountered with reporting.

Results of Phadiatop testing will be accessible via the Microbiome Participant Report on the AsthmaNet website. The report can be found under Reports > Participant Status Reports > Microbiome.

Denver Lab Contact
Name: Brock Harper
Phone: 303-270-2579

2.11 Bronchoscopy

Visits 1, (4)
E-mail Visit 2 (Visit 5) date to Ansel Lab (laura.christian@ucsf.edu) and Microbiome Scientific Coordinator (adyer@psu.edu) as soon as possible

The machine used to analyze the immunophenotyping blood and BAL samples needs to be reserved a week prior to the arrival of samples. Near the end of Visit 1, the Visit 2 date should be confirmed and communicated to the Ansel Lab and Scientific Coordinator. Near the end of Visit 4, the Visit 5 date should be confirmed and communicated to the Ansel Lab and Scientific Coordinator. It is important that you e-mail the above individuals so they can schedule appropriately and keep track of bronchoscopy visits.

If a scheduled visit date changes or the visit is cancelled, notify the Ansel Lab as soon as possible. Unforeseen circumstances (i.e. rescheduled visit) will be accommodated.

Visits 2, 5
Complete Bronchoscopy Checklist (P3_BRONCHCHK_A or P3_BRONCHCHK_C)
Perform bronchoscopy (P3_BPD, P3_LAB_BAL)
Enter bronchoscopy sample information into Biological Sample Tracking module

General Information
Bronchoscopy should ***not*** be scheduled for Fridays, due to same day shipping requirements for some samples. Due to Thursday shipping problems, which have resulted in Saturday and Monday deliveries, it is preferred for bronchoscopies to be scheduled for Monday – Wednesday. Bronchoscopies can be scheduled for Thursdays; however, BAL cell pellet and immunophenotyping samples should be shipped to UCSF First Overnight rather than Priority Overnight. Also, should there be concerns about a shipment (due to existing or impending extreme weather, holiday, etc.), ship First Overnight.

The bronchoscopy procedure is required for Microbiome because the samples are central to the study objectives. The bronchoscopy procedure will be performed at Visit 2 for those participants who successfully complete Visit 1, remain eligible at Visit 2 up to the bronchoscopy procedure, and are eligible for bronchoscopy according to the Bronchoscopy Checklist (P3_BRONCHCHK_A or P3_BRONCHCHK_C). If the participant is ineligible to proceed with bronchoscopy at Visit 2, the participant is ineligible for Microbiome and a Termination of Study participation form (P3_TERM_A or P3_TERM_C) should be completed. The bronchoscopy procedure will be performed at Visit 5 for those asthmatic participants who are eligible to proceed according to the Bronchoscopy Checklist (P3_BRONCHCHK) form.

Prior to bronchoscopy at Visits 2 and 5, the participant may NOT eat or drink 8 hours prior to the procedure. Follow-up telephone contact should be made for all participants

the evening and day after bronchoscopy is performed. If there are any related issues pending, additional contact and necessary medical care should be arranged.

Prior to bronchoscopy, a blood sample and oral wash sample will be obtained, as well as a bronchoscope flush before and after scope placement. During bronchoscopy, five protected brushing samples and bronchoalveolar lavage (BAL) samples will be obtained. All samples will be sent to San Francisco, either to the Ansel or Boushey Lab. Immunophenotyping blood and BAL cell pellet will be sent at room temperature on day of bronchoscopy to Ansel Lab. The remaining bronchoscopy samples are frozen and sent to the Boushey Lab. Details regarding processing and storing of samples are documented in the Microbiome Bronchoscopy MOP located on the AsthmaNet website under Protocols: Microbiome: MOP.

Samples should be labeled with a barcode label generated through the AsthmaNet Biological Sample Tracking module. The labels must be affixed to the correct tube type. Sample labels and their associated sample type follow:

Sample Type	**Barcode Prefix**	**Label*#**
Oral Wash	3WASH	Laser Cryo-Tags 1.50” x 0.75”
Bronchoscope Flush (before anesthesia)	3FLSH1	Laser Cryo-Tags 1.50” x 0.75”
Bronchoscope Flush (after anesthesia)	3FLSH2	Laser Cryo-Tags 1.50” x 0.75”
Brushes in RNALater	3BRSHR	Laser Cryo-Tags 1.50” x 0.75”
Brush in Glycerol	3BRSHG	Laser Cryo-Tags 1.50”x0.75”
BAL Fluid	3BAL	Laser Cryo-Tags 1.50” x 0.75”
BAL Cell Pellet	3BALCP	Laser Cryo-Tags ***Or*** Tough-Tags 1.50” x 0.75”
BAL Supernatant	3BALST	Laser Cryo-Tags 1.50” x 0.75”

* White Laser Cryo-Tags, 1.50” x 0.75” DiversifiedBiotech. http://divbio.com/lasercryo-tags150x0751200pk.aspx

\# White Laser Tough-Tags, 1.50” x 0.75” DiversifiedBiotech. http://divbio.com/lasertough-tags150x0751500pk.aspx

Instructions for bronchoscopy sample collection process, labeling, storage and shipment preparation are documented in the Microbiome Bronchoscopy MOP located on the AsthmaNet website under Protocols: Microbiome: MOP. Bronchoscopy samples, with exception to those being sent to the Ansel Lab, will be shipped in batches to the

Boushey Lab, after every 3 subjects have completed the study at a site. These samples must be sent on Monday – Thursday only on dry ice.

Refer to Section 4 in this MOP for details on how to complete bronchoscopy related forms.

Microbiome Bronchoscopy Procedure Documentation (P3_BPD) form
The Microbiome Bronchoscopy Procedure Documentation (P3_BPD) form was created to document the pre-procedure, procedure and post-procedure participant status and procedure details.

Supplies Provided to Sites
Tubes for brushings which contain RNALater, FACS buffer and Streck Cell Preservative vials will be shipped to each site from San Francisco site. Inform Snehal Nariya (snehal.nariya@ucsf.edu) if your bronchoscopy brush supplies are getting low. Inform Laura Christian (laura.christian@ucsf.edu) if your FACS buffer or Streck Cell Preservative vials are getting low. Allow at least 2 weeks for delivery.

See Blood Draw (Immunophenotyping) in this section for further details on blood collected prior to bronchoscopy and associated supplies.

Saline bags, pre-filled saline syringes and lidocaine will be shipped to each site from the DCC. Inform Ron Zimmerman (rzimmerm@phs.psu.edu) if any of these supplies are getting low and allow at least 2 weeks for delivery.

Exacerbations induced by bronchoscopy (asthmatics only)
In rare cases, fiberoptic bronchoscopy may induce an asthma exacerbation. All cases of bronchoscopy-induced exacerbations will be treated with prednisone, 40mg po daily for 5 days. Should an exacerbation occur after bronchoscopy, a two-week recovery period will be imposed following the completion of prednisone therapy. Bronchoscopy will not be performed at Visit 5 if the participant experienced an exacerbation after bronchoscopy at Visit 2.

Exacerbations requiring systemic corticosteroid treatment (asthmatics only)
Should a participant experience an exacerbation which requires systemic corticosteroid treatment during the randomized treatment period, a two-week recovery period will be imposed following the completion of treatment. No visits should take place during treatment or this recovery period. Bronchoscopy will not be performed at Visit 5.

Rescheduling Bronchoscopy at Visit 5
If a participant is ineligible for bronchoscopy at Visit 5 because of low FEV1 and/or low oxygen saturation only, the visit should be rescheduled.

2.12 Certification

Study Coordinators and Technicians
Coordinators who carry out Microbiome study visits must be certified to do so. That is, personnel who complete pregnancy tests (PREG_TEST form) or any of the protocol-specific Microbiome forms (designated by a P3 prefix in the form name) must possess Microbiome protocol certification, as well as certification in Human Subjects Protection Training, HIPAA and Good Clinical Practice. Note that protocol-specific forms includes completion of the Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK).

To obtain Microbiome coordinator certification, clinic personnel must complete the following steps:

- Thoroughly read the Microbiome protocol and this Manual of Operations.

- Pass the Microbiome coordinator certification exam. This exam can be found on the AsthmaNet secure website in the Certification: Microbiome folder. Exams should be completed, scanned into a pdf file, and e-mailed to the AsthmaNet-Certification alias. Include 'Microbiome Exam' and your performance site number on the subject line of the e-mail message to ensure efficient processing and routing at the DCC.

Any individual who performs spirometry, sputum induction or methacholine challenge as part of a Microbiome visit must be AsthmaNet certified in these procedures or be supervised by a certified technician, as applicable. Certification for these procedures is tracked independently of Microbiome study certification. It is acceptable for these procedures to be performed during the Microbiome study by technicians who possess only individual procedure certification and not Microbiome protocol certification, but it is preferred that technicians review the protocol and take the certification exam, as well. If a technician is only certified in spirometry and not in the Microbiome protocol, a Microbiome-certified coordinator must complete the Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK) to qualify participants for spirometry and methacholine challenge testing.

Protocol deviations will be assigned when an uncertified individual performs protocol-related tasks or carries out procedures for which he/she is uncertified. Protocol violations will be assigned if this persists at a given site over a period of time.

The quality of AsthmaNet data is tracked and reported on a regular basis to the individual performance sites, clinical center partnerships, the AsthmaNet Quality Control Committee (QCC), and to the Data and Safety Monitoring Board (DSMB). It is possible to become decertified in some of the procedures (e.g., spirometry, sputum induction) if lack of quality becomes an issue and the study data begins to be affected adversely. The DCC will contact individuals who are in danger of becoming decertified to discuss the situation before they are decertified formally in the certification tracking system.

Licensed Medical Practitioners (LMPs)

Physicians who are listed on the local IRB application as 'key personnel' must take and pass the Microbiome physician certification exam before interacting with study participants. The physician exam is located on the secure website in folder Certification: Microbiome.

Non-physician LMPs, such as nurse practitioners and physician's assistants, may perform physical exams for the Microbiome study (see the Physical Exams discussion in this section for details). These individuals are not required to take either version of the Microbiome exam. Note that certification requirements for non-physician LMPs will vary from study to study.

Data Entry Personnel

Individuals who are only providing data entry support for the Microbiome study and are not collecting data or performing study procedures do not have to meet any specific AsthmaNet certification requirements. However, it should be ensured that local institutional requirements for these individuals (e.g., HIPAA, GCP, and Human Subjects Protection) have been met and are clearly documented on-site. This documentation may be subject to audit during an AsthmaNet site visit.

2.13 Cold History

Respiratory tract infections (i.e., “colds”) are known to be associated with worsening asthma symptoms and asthma exacerbations.

Visit 1
Administer Cold History (COLD_HX)

The Cold History (COLD_HX) form was created by AsthmaNet researchers. This form collects baseline information on the frequency, severity, and effects on asthma of colds the participant experienced in the past year.

The cold history is obtained by participant interview. Read each question to the participant in a consistent, even tone, exactly as written on the forms. Provide clarification when asked.

See Section 10 of the AsthmaNet General Manual of Operations for further details regarding the completion of the COLD_HX form.

2.14 Concomitant Medications

Participants in AsthmaNet protocols are likely to be taking medications for asthma and allergy-related symptoms, both over-the-counter and prescription. It is important to document the medications a participant is taking, or begins to take, throughout the study to ensure that he/she is not taking medications that are excluded during the trial because they may confound the study results. Further, it is important to document any non-study asthma medications the participant begins using during the trial, as such use may indicate that the participant has experienced, or is experiencing, a significant asthma exacerbation.

The Microbiome trial will employ the two standard concomitant medications forms: Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) and Concomitant Medications for Non-Asthma Drugs (CMED_NON)**.**

Medications taken for treatment of adverse events, both asthma-related and those unrelated to asthma, should be recorded on the CMED form. Medications taken for treatment of asthma/allergy symptoms, other than dispensed study medications, should also be recorded on this form.

Medications not taken for asthma, allergies or adverse events should be recorded on the CMED_NON form. Examples include multivitamins and herbs the participant is taking for health maintenance and maintenance drugs taken for a pre-existing condition (e.g., Paxil for depression). Other non-asthma, non-allergy drugs the participant takes chronically, such as oral contraceptives, should also be recorded on this form.

Study medications, including open-label Flovent® and Ventolin® rescue medication (i.e., albuterol) and blinded Diskus inhalers, should not be regarded as concomitant medications and should not be recorded on CMED or CMED_NON. Prednisone taken to treat an asthma exacerbation or other adverse event *should* be recorded on the CMED form as a concomitant medication and linked to the appropriate adverse event on the Clinical Adverse Events (AECLIN) form. Non-study inhaled corticosteroids (e.g, Advair, Flovent, etc.) are considered concomitant medications and should be recorded on the CMED form if they are prescribed during the study (note that these drugs are excluded during the study and their use should be avoided if at all possible).

The following classes of drugs/solutions/products do not need to be recorded on a participant's CMED or CMED_NON form:

- Anesthesia medications administered during surgery and outpatient procedures
- Sedatives used prior to and during procedures
- Novacaine and other dental anesthetics
- Solutions/drugs taken prior to specialized procedures [e.g., Golytely (Colye, Nulytely), phospho-soda, and sodium phosphate tablets (Osmo-Prep, Visicol) taken prior to colonoscopy, Glucola taken during an oral glucose tolerance test]

- Iodine dye and other contrast materials used for MRIs and other procedures

Visit 0
Record concomitant medications the participant has taken since signing the informed consent on the appropriate concomitant medications (CMED, CMED_NON) form

Thorough questioning about medication use during the initial study visit will prevent the presentation of unexpected information when it is time to randomize a participant. It also will help to prevent misinterpretation of medications reported at subsequent contacts, particularly if the participant interacts with a different coordinator.

During the first visit, prompt participants with the following questions:

- What over-the-counter medications do you typically take during a given month, including continuous use and as-needed medications, such as laxatives, antacids, stool softeners, ibuprofen, etc.? Inquire about the participant's use of vitamins and herbal remedies. Use of certain herbs, such as St. John's wort or valerian, during study participation should be discouraged.
- What prescription medications do you typically take during a given month, including continuous use and as-needed medications?
- What over-the-counter medications do you typically pack when you go on vacation or away for business? What prescription medications?
- What over-the-counter medications do you keep in your desk drawer or purse? What prescription medications?

If the participant has taken any medications for asthma or allergies or adverse events that have occurred since he/she signed the informed consent (original signature date), record them on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form. Record medications taken on the day of Visit 1, even if the participant has agreed to stop taking them after completing the visit. List the consent date as the start date for the medication (i.e., when the use of the medication became concomitant with study participation) if the participant started taking the drug prior to his/her original consent signature date.

Any medications that were used to treat conditions <u>other than</u> asthma, allergies or adverse events since the participant signed the informed consent should be recorded on the Concomitant Medications for Non-Asthma Drugs (CMED_NON) form. This includes substances like multivitamins, vitamin D and calcium supplements, and herbs the participant is taking for health maintenance. It also includes maintenance drugs for a pre-existing condition (e.g., Paxil for depression or insulin for diabetes) and other drugs the participant takes chronically, such as oral contraceptives.

Probing for medication use during Visit 0 affords an opportunity to recognize clinically significant medical problems early in the study. For example, a participant may take several medications to treat hypertension. The participant's condition may be deemed

unstable and poorly controlled, therefore, ineligible on the basis of the information collected for the concomitant medications form. If a participant is taking medications for a condition that may exclude him/her from study participation, first check the Microbiome Exclusionary Medical Conditions (P3_EXCLMED) reference card. If the applicable condition is not listed specifically, contact the DCC for guidance.

When scheduling Visit 0, the potential participant should be asked to bring all over-the-counter and prescribed medications and supplements he/she is currently taking to the visit. Alternatively, the participant may write down the names of the medications and supplements and the date he/she started taking each medication and bring this list to the visit.

Note that participants must wash out of inhaled and oral corticosteroids, antiobiotics, etc. for a period of time prior to Visit 1. See the Eligibility Criteria discussion in this section for more details. Some institutions require that participants read and sign the study informed consent document prior to washing out of medications for purposes of study enrollment.

Visits 1-6 (Visits 3-6 asthmatic only)
Follow up medication use from the previous visit and record any new concomitant medications (CMED, CMED_NON)

Each time the clinic has contact with a participant, whether for a scheduled visit or phone contact, impromptu visit, or unexpected phone call, information on concomitant medications should be collected. During these contacts, the concomitant medication information obtained during previous contacts should be updated. If the participant discontinued a medication that he/she was taking, update the stop date on the CMED or CMED_NON form, as appropriate. Probe the participant for any new medications that may have been taken and record these on the appropriate form for the next visit. If the participant began taking a new medication for a condition or disease that existed prior to study enrollment at Visit 0 and no adverse event (i.e., worsening of the condition) is associated with the change in medication, record this information on the CMED_NON form. If the participant has not taken any new medications for asthma, allergy or an adverse event, mark the ‘None’ box on the CMED form for the applicable visit.

Visit 6 (asthmatics only) and other early termination visits
Medications that are still in use at the time of the final study visit or contact should be coded as ‘ongoing at final visit’ on the CMED form. On the CMED_NON form these are coded as ‘ongoing at end of study.’ During the participant’s final visit or contact with the clinical site, finalize his/her CMED and CMED_NON forms. All CMED forms for a given individual should be forwarded to the DCC following his/her study termination.

2.15 Contact Information

Visit 0
Administer Adult Participant Contact Information (CONTACT_ADULT) form

The Adult Participant Contact Information (CONTACT_ADULT) form is completed by the participant. Its purpose is to collect pertinent participant identification information such as full name, address, and telephone number, as well as alternative ways to contact the participant through work, family, or friends.

- This form serves as source documentation proving the existence of the participant. It **must** be completed.
- It is important to obtain complete and accurate phone number information for the participant during Visit 0. They will need to be contacted via phone following bronchoscopy, if they miss a visit or for the phone contact as part of the Microbiome trial.

Store the CONTACT_ADULT form in the participant's study folder; do not forward it to the DCC. This form contains the participant's name, address, and other identifying information. A protocol violation may be assigned if this form is misdirected to the DCC or another off-site group affiliated with AsthmaNet (e.g., sputum lab).

Visit 0
Review and distribute Clinic Contact Information handout (P3_CLINFO)

The Microbiome Clinic Contact Information (P3_CLINFO) handout is completed by the study coordinator who is in charge of the Microbiome study at the performance site. This document provides participants relevant information so that they can easily contact their study coordinator via phone, pager, or e-mail during the time between Visit 0 when the Phadiatop blood sample is drawn and the time when results are available and their study eligibility is known.

Because it will take approximately one week for each participant's screen data and blood sample to be processed and for his/her eligibility for continuation in the screening process to be verified, it is possible that a participant's situation may change during that time. If an individual changes his/her mind and no longer wishes to be considered for participation in the Microbiome study, he/she should be instructed to contact the study coordinator as soon as possible to withdraw consent. Expedited contact allows for a Microbiome Termination of Study Participation (P3_TERM_ A or P3_TERM_C) form to be completed and data entered, and for the participant's status to be updated in the Microbiome database. For further information regarding termination procedures, see the Withdrawal discussion in this section. For further details on completion of the P3_TERM_ A or P3_TERM_C form, see Section 4 of this manual.

The P3_CLINFO handout and its purpose should be reviewed with the participant near the end of Visit 0. As this form is the only study-related item the participant has in his/her possession at the end of the screening visit, he/she should be instructed to keep it in a safe place where it can easily be located for later reference.

2.16 Daily Activities Handout (asthmatics only)

Visit 2
Complete and distribute Daily Activities Handout (P3_DAILYACT1)

Near the end of Visit 2, review the summary handout “Microbiome Daily Activities (Visits 2-4)” (P3_DAILYACT1). This handout summarizes what the study participants must carry out each day of the treatment period until Visit 5. The High Rescue Inhaler Use value from the Participant ID Card (P3_ID) should be completed on the blank space provided on the handout. This will help the participant identify the onset of a significant exacerbation. Review the high rescue use value on the handout and confirm that the participant understands how to monitor his/her asthma.

Visit 5
Complete and distribute Daily Activities Handout (P3_DAILYACT2)

Remove the “Microbiome Daily Activities (Visits 2-4)” handout (P3_DAILYACT1) from the participant’s folder and discard it. Obtain a copy of the “Microbiome Daily Activities (Visit 5)” (P3_DAILYACT2) handout. This reference lists what the participant must carry out each day between Visit 5 and 6. The High Rescue Inhaler Use value from the Participant ID Card (P3_ID) should be completed on the blank space provided on the handout. This will help the participant identify the onset of a significant exacerbation. Review the high rescue use value on the handout and confirm that the participant understands how to monitor his/her asthma.

2.17 Dosing Compliance (asthmatics only)

Visits 3-5
Check compliance with study medication (P3_COMPLY)

The Diskus® contains a counter that shows the number of puffs remaining (out of a total of 60 puffs in a new Diskus®). The counter will be used to assess the participant's compliance with dosing from the Scheduled Diskus® during the treatment period. Participants are instructed to take 1 puff BID from the Diskus® during this period.

The number of scheduled puffs should include all doses the participant should have taken since leaving the last clinic visit.

Compliance with dosing from the Diskus® is documented on the Microbiome Compliance Checklist (P3_COMPLY) and entered into the study database.

Example Diskus® Compliance Calculations
The following chart shows the number of Scheduled Diskus® puffs a participant should have taken between Visit 3 and Visit 4 (ideal 3-week interval). Visit 3 took place on 1/06/2013 and Visit 4 is taking place on 1/27/2013. The Diskus returned by the participant had a counter value of 23.

Date	1/27 Visit day	1/26	1/25	1/24	1/23	1/22	1/21	1/20	1/19	1/18	1/17
Scheduled Puffs	1 – regular AM dose	2	2	2	2	2	2	2	2	2	2

Date	1/16	1/15	1/14	1/13	1/12	1/11	1/10	1/09	1/08	1/07	1/06 Visit Day
Scheduled Puffs	2	2	2	2	2	2	2	2	2	2	1 – regular PM dose

Compliance assessment (follow P3_COMPLY, Questions #1a-1d):

1a. Number of scheduled puffs (from above table): 42

1b. Number of remaining puffs (from Diskus counter): 23

1c. Number of puffs taken:

The number of puffs taken is equivalent to the number of puffs packaged in the Diskus® – the number of puffs remaining in the Diskus®.

The number of puffs taken is equivalent to 60 – 23 = 37.

1d. Percent compliance = # puffs taken / # puffs scheduled x 100
= 37 / 42 x 100
= 88.1%.

∴ Because the participant's compliance percentage exceeds the 75% goal laid out for the study, the participant is doing a good job of dosing with his or her Scheduled Diskus®. He or she should be praised and encouraged to continue being diligent with taking study medications according to protocol.

Visit 3 Compliance
If Visit 3 and participant demonstrates < 75% compliance with study medication, re-emphasize the importance of maintaining daily dosing schedule and reschedule Visit 3 in 10-14 days.

At rescheduled Visit 3, calculate number of puffs taken using Q1b on previous Visit 3 P3_COMPLY form:

Number of puffs taken = Q1b (previous Visit 3 P3_COMPLY) –
Q1b (current Visit 3 P3_COMPLY)

Compliance Calculation when two Diskuses returned
In some instances, a participant may return two Diskuses at a study visit. In these cases, the number of puffs taken is equivalent to 120 – (remaining puffs on Diskus 1 + remaining puffs on Diskus 2), as reflected on the Diskus counters.

2.18 Electrocardiogram (ECG)

Visit 1
Perform Electrocardiogram Test

A standard 12 lead electrocardiogram (ECG) is required at Visit 1 for those participants greater than 45 years of age (46 or older). If the ECG is taken on a "strip" machine, representative tracings from each of the leads should be cut and placed on a single sheet of 8 1/2 by 11 paper. In this case a rhythm strip, representative of the average rhythm over the recording period and containing approximately 5 seconds of ECG tracing should be placed at the bottom of the sheet. Physician evaluation of the ECG is recorded in Q1010 on Eligibility Checklist 2 (P3_ELIG2A or P3_ELIG2C).

Subjects must be completely at rest for at least five minutes before the ECG is performed. Follow the order of procedures on Visit Checklist 1 (P3_VISIT1). The subject must not exert himself or herself in any way (e.g., walking/running up stairs, spirometry, etc.) for a minimum of five minutes prior to the test.

This ECG is a screening tool to identify subjects who have clinically significant cardiac abnormalities (e.g., severe or unstable coronary artery disease, recent history of myocardial infarction (within 6 months of Visit 1), arrhythmias, etc.) and should not participate in Microbiome.

A physician should interpret the ECG for evidence of any conditions that would exclude the subject from participating in Microbiome, including evidence of arrhythmia or ischemia. The physician must provide source documentation (signature and date) below Q1010 on the P3_ELIG2A or P3_ELIG2C form indicating that he or she reviewed the ECG.

ECG reports should be stored in the subject’s study folder at the clinical center; do not submit these reports to the DCC.

2.19 Eligibility Criteria

Visit 0
Complete Eligibility Checklist 0 (P3_ELIG0A or P3_ELIG0C)

Visit 0 is a relatively short, pre-screen visit. Basic eligibility criteria are checked without having to take a thorough medical history. If the participant meets all of the eligibility criteria on Microbiome Eligibility Checklist 0 (P3_ELIG0A for asthmatics or P3_ELIG0C for controls), then he/she is eligible to have a blood sample drawn for allergy determination. Allergy eligibility status for each asthmatic participant will be available on the AsthmaNet secure website under Reports > Participant Status Reports > Microbiome.

If the participant is ineligible based on his/her allergy status, the participant should be notified by phone that he/she is ineligible and a Microbiome Termination of Study Participation (P3_TERM_A or P3_TERM_C) form should be completed.

Participants who pass all the eligibility checks on P3_ELIG0A or P3_ELIG0C and give a blood sample for allergy determination are formally enrolled in the Microbiome study. Data for these participants should be entered into the Microbiome database and forwarded to the DCC.

Participants who do not meet all of the eligibility checks on P3_ELIG0A or P3_ELIG0C are not eligible for study enrollment. Forms that were completed at Visit 0 should not be entered into the study database or forwarded to the DCC; they should be filed in the participant's study folder at the performance site.

Participants should review the data recorded on P3_ELIG0A or P3_ELIG0C and initial/date the source documentation box on page 3 of the form.

Visit 1
Complete Eligibility Checklist 1 (P3_ELIG1A or P3_ELIG1C)
Complete Eligibility Checklist 2 (P3_ELIG2A or P3_ELIG2C)

At Visit 1, participants will have a thorough medical history taken and will undergo a comprehensive physical examination. Findings from these procedures can affect the participant's continued study eligibility. Basic eligibility criteria and eligibility criteria related to the participant's medical condition and medical history are recorded on Eligibility Checklist 2 (P3_ELIG2A for asthmatics and P3_ELIG2C for controls).

If the participant remains eligible at Visit 1 following his/her exam and medical history assessment, he/she will perform spirometry and methacholine challenge. Eligibility criteria related to baseline FEV_1 and PC_{20} are documented on Eligibility Checklist 2 (P3_ELIG2A or P3_ELIG2C). Participants who remain eligible following completion of P3_ELIG2A or P3_ELIG2C will perform sputum induction and provide blood samples for lab testing for the assessment of additional eligibility criteria.

Note that asthmatic participants who do not meet PC_{20} criteria ($PC_{20} \leq 8$mg/ml) will need to stop Visit 1 following methacholine challenge, and a continuation visit will need to be scheduled to attempt reversibility. Continuation visit should be scheduled within 24-48 hours. If the asthmatic participant's FEV_1 improves at least 12% in response to 4 puffs of albuterol, the participant is eligible to continue with Visit 1 procedures.

Participants should review the data recorded on P3_ELIG1A or P3_ELIG1C and P3_ELIG2A or P3_ELIG2C and initial/date the source documentation box on page 1 of the form.

Visit 2

Complete Eligibility Checklist 3 (P3_ELIG3A or P3_ELIG3C)

Eligibility criteria that are assessed at Visit 2 are recorded on Eligibility Checklist 3 (P3_ELIG3A for asthmatics or P3_ELIG3C for controls). Participants who return for Visit 2 will undergo spirometry and reversibility testing. P3_ELIG3A is divided into sections, where Section 1 assesses basic criteria that apply to all participants. If an asthmatic participant remains eligible following completion of Section 1, he/she will proceed with bronchoscopy. Section 2 of P3_ELIG3A will be completed following bronchoscopy, documenting the participant's ability to use the Diskus properly and other general eligibility questions. Asthmatic participants who pass all of the eligibility checks on P3_ELIG3A are eligibile to be randomized. If a control participant remains eligible following completion of P3_ELIG3C, he/she will proceed with bronchoscopy and be terminated following bronchoscopy.

Note: Nightshift workers and others with altered schedules

Microbiome has no specific exclusion for nightshift workers and individuals with other altered day/night schedules. Individuals working the 11 PM to 7 AM shift or the 12 AM to 8 AM shift may be screened and enrolled at the local investigator's discretion. These participants should follow normal AM and PM daily procedures

Visit 0 Inclusion Criteria: All Participants

- Ability to provide informed consent, as evidenced by the signing of a copy of the Microbiome study consent form approved by the study institution's Committee on Human Participants' Research (i.e., Institutional Review Board).

  The informed consent document must be signed on or before the Visit 0 date. See the discussion of Informed Consent in this section for further details.

  This criterion is documented on P3_ELIG0A or P3_ELIG0C.

- Male or female, between age 18 and 60, inclusive.

This criterion is documented on P3_ELIG0A or P3_ELIG0C.

- Willingness to undergo fiberoptic bronchoscopy with endobronchial brushings and bronchial lavage.

  This criterion is documented on P3_ELIG0A or P3_ELIG0C.

- Willingness to give blood for safety variable measurements.

  This criterion is documented on P3_ELIG0A or P3_ELIG0C.

Visit 0 Inclusion Criteria: Asthmatics only

- Physician-diagnosed asthma at least 12 months ago.

  Participant report is sufficient. Medical records and prescriptions for asthma medications are not required, but are helpful if the performance site has routine access to them.

  This criterion is documented on P3_ELIG0A.

Visit 0 Exclusion Criteria: All Participants

- Chronic diseases (other than asthma) that in the opinion of the local investigator would prevent participation in the trial or put the participant at risk by participating, based on self-report at Visit 0.

  In particular, individuals with an established diagnosis of vocal cord dysfunction or chronic diseases of the lung (other than asthma; e.g., emphysema, chronic bronchitis, pulmonary embolism, malignancy, cystic fibrosis, etc.), kidney, heart, liver, endocrine or nervous system, or immunodeficiency will be excluded.

  Note that the majority of the following conditions are exclusionary only if deemed clinically unstable or contraindicated for the protocol in the judgment of the local investigator and the principal investigator for the protocol. If a potential participant's eligibility is in question, contact the Microbiome scientific coordinator at the DCC for assistance.

  At the beginning of Visit 0, this criterion will be assessed by participant self-report. If he/she is eligible to continue on to Visit 1, a comprehensive physical exam and medical history will be taken at Visit 1 to confirm his/her eligibility.

  Exclusionary conditions include, but are not limited to:

  - Addison's disease
  - AIDS
  - Bleeding disorder (history of)
  - Cardiac arrhythmias (clinically significant)
  - Cardiac ischemia

- o Congenital anomaly, including growth abnormalities (clinically significant)
- o Congestive heart failure
- o Coronary artery disease (unstable or severe)
- o Cushing's disease
- o Diabetes mellitus (poorly controlled)
- o Dyspnea by any cause other than asthma
- o Eating disorder (e.g., anorexia or bulimia – active disease only)
- o Hematologic disease (unstable, e.g., severe anemia)
- o Hepatic disease[1]
- o Hypertension (poorly controlled)
- o Hyperthyroidism[2]
- o Immunologic compromise[3]
- o Chronic kidney disease (e.g., glomerulonephritis, polycystic kidney disease, etc.)
- o Lactation
- o Lidocaine allergy
- o Lung disease other than asthma (e.g., COPD, emphysema, chronic bronchitis, pulmonary embolism, malignancy, cystic fibrosis, among others)
- o Lupus (active disease, requiring immunosuppressants)
- o Any malignancy other than basal cell skin cancers
- o Mental illness (uncontrolled)[4]
- o Mental retardation
- o Morbid obesity (BMI ≥ 35)
- o Neurologic disease (including epilepsy requiring treatment)
- o Peptic ulcer disease (active)
- o Pregnancy
- o Renal insufficiency (creatinine > 1.2 mg/dl)
- o Schizophrenia
- o Skeletal disorders, including osteoporosis and rheumatoid arthritis[5]
- o Sleep apnea (untreated)[6]
- o Sleep disorder (history of)[7]
- o Substance abuse (including active drug or alcohol abuse)
- o Tachyarrhythmia (atrial or ventricular, history of)
- o Tuberculosis (active disease excluded; history of positive skin test with negative chest X-ray allowed)

[1] Nonactive hepatitis B/C is allowable; active hepatitis (including antigen positivity or disease requiring treatment) is exclusionary.
[2] Controlled hypothyroidism is allowable.
[3] Resulting in prior infections and/or susceptibility to new infections.
[4] Anxiety, depression, or bipolar disease well-controlled on allowed medications are allowable conditions for the Microbiome trial.
[5] Participants who have rheumatoid arthritis and are on excluded medications should not be screened; osteoarthritis is an allowable condition for the Microbiome trial. Scoliosis, degenerative disc disease, and spinal stenosis are not exclusionary.
[6] Individuals with an OSA diagnosis who are receiving treatment with CPAP, BiPAP, or APAP are eligible.
[7] Occasional insomnia is allowable.

- Urinary retention (active symptoms within last 6 months)
- Vocal cord dysfunction (diagnosis of)

These illnesses are listed on the Microbiome Exclusionary Medical Conditions (P3_EXCLMED) reference card.

This criterion is documented on P3_ELIG0A, P3_ELIG0C, P3_ELIG1A and P3_ELIG1C.

- History of atrial or ventricular tachyarrhythmia.

  This criterion is documented on P3_ELIG0A and P3_ELIG0C.

- History of a bleeding disorder.

  This criterion is documented on P3_ELIG0A and P3_ELIG0C.

- History of an upper respiratory infection in past 6 weeks.

  A respiratory tract infection is defined as a cough, runny nose plus or minus fever, or sore throat that is not related to allergen exposure. This criterion is evaluated by participant self-report; no specific medications need to have been taken to meet this criterion. At all subsequent visits, the occurrence of a recent infection should be documented on the Clinical Adverse Events (AECLIN) form.

  If the participant experiences a respiratory infection between Visits 0 and 2, the participant will be ineligible for Microbiome. At Visits 4 and 5, a respiratory infection within 4 weeks of visit will require the visit to be rescheduled 4 weeks from onset of symptoms. At visits 3 and 6, the visit may proceed with spirometry testing and other study procedures as deemed appropriate by the study physician.

  This criterion is documented on P3_ELIG0A and P3_ELIG0C.

- History of sinusitis or bronchitis with purulent nasal discharge or sputum in past 3 months.

  This criterion is documented on P3_ELIG0A and P3_ELIG0C.

- History of thick or discolored post-nasal drip or nasal discharge associated with facial pain, facial pressure, or maxillary tooth pain causing moderate or severe discomfort on more than 7 days during the past 6 weeks

  This criterion is documented on P3_ELIG0A and P3_ELIG0C.

- A change in bowel function (e.g., diarrheal illness) in past 4 weeks

  This criterion is documented on P3_ELIG0A and P3_ELIG0C.

- Need for the use of any of the drugs listed in Table 1 (that follows); inability to go off these drugs for the required washout periods prior to Visit 1 and for the duration of the Microbiome study. The Microbiome Exclusionary Drugs (P3_EXCLDRUG) reference card contains a summary of this table.

  Excluded drugs/substances on P3_EXCLDRUG must be washed out prior to Visit 1, and the participant must refrain from using them for the duration of the trial. If a participant is taking one or more of these medications at the time of Visit 1, the indication for the drug should be discussed with the local investigator to determine if it is safe for him/her to go off the drug to participate in the trial starting with Visit 1.

  It is important to note that <u>any and all changes</u> in a participant's medications must be approved by a study physician and documented in the participant's clinic notes.

  This criterion is documented on P3_ELIG0A and P3_ELIG0C.

Table 1. Drugs to be withheld throughout the study.

Excluded Drug	Generic Names (may not be inclusive)	Trade Names (may not be inclusive)	Washout Prior to Visit 1
Steroid Medications			
Oral or intravenous steroids for any reason		Medrol, Prednisone	6 months*
Inhaled steroids except study drug (Flovent®)	beclomethasone, budesonide, ciclesonide, flunisolide, fluticasone, mometasone, triamcinolone acetonide	Advair, Aerobid, Alvesco, Asmanex, Azmacort, Dulera, Flovent®, Pulmicort, QVAR, Symbicort	6 months
Nonsteroidal Antiinflammatory Medications			
Leukotriene modifiers	montelukast, zafirlukast, zileuton	Accolate, Singulair, Zyflo	6 months
Cromolyn/Nedocromil for asthma	cromolyn, nedocromil	Intal, Tilade	6 months

* Washout 6 months for use as a long-term asthma controller, 3 months for treatment of an asthma exacerbation

Excluded Drug	Generic Names (may not be inclusive)	Trade Names (may not be inclusive)	Washout Prior to Visit 1
Bronchodilators			
Oral β-agonists	albuterol, metaproterenol, terbutaline	Alupent, Brethine, Bricanyl, Metaprel, Proventil, Repetabs, Ventolin, Volmax	1 week
Short-acting inhaled β-agonists	epinephrine	Bronkaid Mist, Duo-Medihaler, Medihaler-Epi, Primatene Mist	6 hours
Intermediate-acting inhaled β-agonists (except study RESCUE drug)	albuterol, bitolterol, albuterol, metaproterenol, pirbuterol, terbutaline	Alupent, Brethaire, Brethine, Bronkometer, Maxair, Metaprel, Proventil, Tornalate, Ventolin, Ventolin	6 hours
Long-acting inhaled β-agonists	formoterol, salmeterol	Foradil, Serevent	24 hours
Short-acting anticholinergics	atropine, ipratropium bromide, pirenzepine, scopolamine	Atrohist, Atrovent, Bellatal, Combivent, Donnatal, Scopoderm, Transderm-Scop	6 hours
Long-acting anticholinergics	tiotropium	Spiriva	72 hours
Xanthine Derivatives			
Short-acting theophylline	theophylline	Aminophylline, Slo-Phyllin	6 months
Long-acting theophylline	theophylline	Slo-bid, Theo-Dur	6 months
Ultra long-acting theophylline	theophylline	Theo-24, Uniphyl	6 months
Anti-IgE Therapy			
	omalizumab	Xolair	6 months

Excluded Drug	Generic Names (may not be inclusive)	Trade Names (may not be inclusive)	Washout Prior to Visit 1
Cardiac Drugs			
Alpha-beta blockers	labetalol	Normodyne	2 weeks
Beta blockers	acebutolol, atenolol, betaxolol, bisoprolol, carteolol, metoprolol, nadolol, penbutolol, pindolol, propranolol, timolol	Blocadren, Cartrol, Corgard, Inderal, Kerlone, Levatol, Lopressor, Sectral, Tenormin, Visken, Zebeta	2 weeks
Anticoagulants and Antiplatelets	warfarin, clopidogrel	Coumadin, Plavix	2 weeks
Psych or CNS-Related Drugs			
Monoamine oxidase (MAO) inhibitors	harmaline, iproclozide, iproniazid, isocarboxazid, nialamide, phenelzine, selegiline, toloxatone, tranylcypromine	Nardil, Parnate	4 weeks
Antibiotics			
Any antibiotic except for topical	azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, troleandomycin	Biaxin, Dynabac, Rulid, Surlid, TAO, Zithromax, Zitromax	3 months

Table 2 contains drugs and substances that are allowed during the study, but must be withheld for specified periods of time prior to visits 1-6[8].

Table 2. Drugs/substances to be withheld prior to Visits 1-6*.

Drug/Substance	Trade Names (may not be inclusive)	Washout Prior to Visits
Albuterol (study RESCUE inhaler)	Ventolin	6 hours
Oral Antihistamines (chlorpheniramine, desloratadine, diphenhydramine, fexofenadine, loratadine and others)	Allegra, Allegra-D, Benadryl, Chlor-Trimeton, Clarinex, Claritin and others	48 hours
Nasal Antihistamines (azelastine nasal, olopatadine, levocabastine)	Astelin, Astepro, Patanase, Livostin	6 hours
Ophthalmic Antihistamines (azelastine ophthalmic, emedastine difumarate, epinastine ophthalmic, ketotifen fumarate, olopatadine ophthalmic)	Alaway, Elestat, Emadine, Opitvar, Pataday, Patanol, Zaditor	6 hours
Oral Decongestants (pseudoephedrine and others)	Sudafed and others	48 hours
Nasal Decongestants (oxymetazoline and others)	Afrin and others	6 hours
Methylxanthine-containing food or beverages (caffeinated colas, coffee, tea)	Coke, Barq's Rootbeer, Mello-Yellow, Mountain Dew, Pepsi, Red Bull	6 hours
Methylxanthine-containing medications	Anacin, Darvon, Esgic, Excedrin, No-Doz, Norgesic, Vivarin	6 hours
Alcohol-containing foods or beverages		6 hours

Drugs/substances to be withheld prior to Visits 2 and 5 (in addition to above).

Drug/Substance	**Trade Names (may not be inclusive)**	**Washout Prior to Visits 2, 5**
Nonsteroidal anti-inflammatory drugs or NSAIDs (aspirin, ibuprofen and others)	Advil, Bayer, Ecotrin, Motrin	1 week

[8] These drugs/substances are allowed between visits, but not prior to pulmonary function testing. See Spirometry discussion.

A participant who takes probiotics is eligible; however, he/she should have been taking probiotics regularly for the past 6 months.

- Antibiotic use within past 3 months.

  This criterion is documented on P3_ELIG0A or P3_ELIG0C.

- Use of 10 or more doses of a nasal corticosteroid in past 3 months.

  This criterion is documented on P3_ ELIG0A or P3_ELIG0C.

- Smokeless tobacco product (e.g., chew, snuff) in past year.

  This criterion is documented on P3_ELIG0A or P3_ELIG0C.

- Smoking of any substance (cigarettes, a pipe, cigar, marijuana, other illegal drugs, etc.) in the past year (12 months).

  This criterion is documented on P3_ELIG0A or P3_ELIG0C.

  Note: Participants should not use smokeless tobacco products (e.g., chew, snuff etc.) for the duration of the Microbiome study.

- Lifetime smoking history of 5 or more pack-years.

  The pack-year limit applies regardless of when an individual stopped smoking.

  Definition of pack-year: A participant smoked for one pack-year if he/she smoked one pack of cigarettes (i.e., 20 cigarettes) a day for a period of one year. In general, the number of pack-years someone smoked is computed as:

  pack-years = #packs/day * #years smoked that quantity

  A participant with a 10-pack-year history could have smoked one pack of cigarettes per day over 10 years or two packs a day for 5 years, or many other combinations of packs/day and durations.

  If a participant smoked an odd number of cigarettes per day, or had a history of smoking variable amounts of cigarettes per day over time, the resulting number of pack-years should be estimated to one decimal place for each part of the calculation.

  For example, suppose a participant smoked an average of 8 cigarettes per day for 6 years, and 3 cigarettes per day for 3 years, eventually quitting. His/her pack-year history would be computed as:

  (8/20) * 6 + (3/20) * 3 = 2.4 + 0.5 = 2.9 pack-years

This criterion is documented on P3_ELIG0A or P3_ELIG0C.

Note: Pack-year history for asthmatics is quantified on the Adult Asthma and Allergy History (ASTHMA_HX_ADULT) form completed at Visit 1.

- Pregnancy or lactation at Visit 0.

  If the participant is eligible to continue and is a woman of child-bearing potential, she will undergo a urine pregnancy test at Visit 1 and 2. Asthmatic participants will also undergo pregnancy testing at Visit 4 and 5. For additional details, see the Pregnancy Test discussion in this section.

  This criterion is documented on P3_ELIG0A or P3_ELIG0C.

Visit 0 Exclusion Criteria: Asthmatics only:

- Plans to move away from the clinical site in the upcoming 3 months such that a participant's ability to complete the study will be jeopardized.

  If a participant is planning to move in the near future to a location that would preclude his/her completion of the study at the original performance site or at another AsthmaNet Microbiome performance site, then he/she should not be enrolled. This concern should be discussed with students who tend to relocate during the summer months to determine if they will be able to complete all study visits at the local site or make alternate arrangements. Only asthmatic participants who have a high likelihood of completing the entire study (6 visits) should be enrolled.

  This criterion is documented on P3_ELIG0A.

- ED visit or hospitalization for asthma within past 6 months.

  This criterion is documented on P3_ELIG0A.

- More than 2 asthma exacerbations requiring systemic corticosteroid treatment within past 6 months.

  Participants who have experienced two significant asthma exacerbations requiring systemic corticosteroid treatment within the past 6 months of Visit 0 should not complete the visit at this time. These individuals should defer Visit 0 until the full 6 months have passed and their asthma is stable.

  Systemic corticosteroids include oral (e.g., prednisone), injectable (IM), and intravenous (IV) steroids.

  This criterion is documented on P3_ELIG0A.

- Asthma exacerbation requiring systemic corticosteroid treatment in past 3 months.

  Participants who have experienced a significant asthma exacerbation requiring systemic corticosteroid treatment within 3 months of Visit 0 should not complete the visit at this time. These individuals should defer Visit 0 until the full 3 months have passed and their asthma is stable.

  Systemic corticosteroids include oral (e.g., prednisone), injectable (IM), and intravenous (IV) steroids.

  This criterion is documented on P3_ELIG0A.

- Use of long-term asthma controller medication (inhaled or oral corticosteroid, leukotriene modifier, cromolyn, or theophylline) within past 6 months.

  This criterion is documented on P3_ELIG0A.

- Pregnancy or lactation or plans to become pregnant in the next 3 months.

  If the participant is a woman of child-bearing potential, she will undergo a urine pregnancy test at Visit 1, 2, 4 and 5. For additional details, see the Pregnancy Test discussion in this section.

  This criterion is documented on P3_ELIG0A.

- If potentially able to bear children, not using an acceptable form of birth control.

  Acceptable forms of birth control include:

  - Birth control patches (Ortho Evra™)
  - NuvaRing®
  - Oral contraceptives
  - Norplant®
  - Depo-Provera®
  - IUD
  - IUS
  - Single and double barrier methods (e.g., condom, spermicidal foam)
  - Surgical sterilization (i.e., hysterectomy, tubal ligation, or vasectomy in monogamous partner)
  - Post-menopausal (at least 1 year since last menses)
  - Abstinence

  This list is summarized on the Birth Control Methods (BIRTH_CTRL) reference card.

  A history of infertility may <u>not</u> be used as a substitute for appropriate birth control.

This criterion is documented on P3_ELIG0A.

- ACQ score ≤ 1.5

  This criterion is documented on P3_ELIG0A.

Visit 0 Exclusion Criteria: Controls only

- Physician-diagnosed asthma.

  If the control participant has received a physician diagnosis of asthma, he/she is not eligible.

  This criterion is documented on P3_ELIG0C.

Visit 1 Exclusion Criteria: All Participants

- Corticosteroid use (except for topical) since Visit 0.

  This criterion is documented on P3_ELIG1A or P3_ELIG1C.

- Antibiotic use (except for topical) since Visit 0.

  This criterion is documented on P3_ELIG1A or P3_ELIG1C.

- Respiratory infection since Visit 0.

  This criterion is documented on P3_ELIG1A or P3_ELIG1C.

- Treatment with any excluded medication (P3_EXCLDRUG).

  This criterion is documented on P3_ELIG1A or P3_ELIG1C.

- Use of any prescription or over-the-counter medication other than those listed on the Microbiome Allowed Medications (P3_MEDALLOW) reference card.

  Chronic use of any medications other than RESCUE beta-agonist except:

  - acetaminophen
  - analgesics for acute/chronic pain management (with MD discretion)
  - antianxiety agents/anxiolytics (e.g., diazepam, chlordiazepoxide, alprazolam, lorazepam, gabapentin, buspirone) at a stable dose
  - antibiotics (topical only)
  - anti-cholesterol medications (e.g., Lopid, statin medications), except cholestipol and cholestyramine
  - specific antidepressants at a stable dose

    - Selective Serotonin Reuptake Inhibitors (SSRI) (e.g., alaproclate, etoperidone, citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline, zimelidine)
    - Selective Serotonin Norepinephrine Reuptake Inhibitors (SSNRI) (e.g. desvenlafaxine, duloxetine, venlafaxine)
    - Non-SSRI/SSNRI antidepressants (except MAOI class drugs) (e.g. amitriptyline, amoxapine, bupropion, mirtazapine, nefazodone, trazodone and others)
- antihistamines (e.g. chlorpheniramine (Chlor-Trimeton), desloratadine (Clarinex), diphenhydramine (Benadryl), fexofenadine (Allegra, Allegra-D), loratadine (Claritin), and others)
- specific antihypertensive medications
    - alpha blockers (e.g. doxazosin, prazosin, terazosin)
    - angiotensin converting enzyme (ACE) inhibitors (e.g. benazepril, captopril, enalapril, fosinopril, lisinopril, quinapril, ramipril)
    - angiotensin receptor blockers (Sartans) (e.g. candesartan, eprosartan, irbesartan, losartan, olmesartan, telmisartan, valsartan)
    - calcium channel blockers (e.g. amlodipine, diltiazem, felodipine, isradipine, nicardipine, nifedipine, verapamil)
    - diuretics (e.g. amiloride, bumetanide, chlorothiazide, chlorthalidone, furosemide, hydrochlorothiazide, indapamide, methyclothiazide, metolazone, spironolactone, triameterene)
    - mineralocorticoid receptor antagonists (e.g. eplerenone)
    - sympathetic nerve inhibitors (e.g. clonidine, guanabenz, guanfacine, methyldopa)
- antitussives (OTC only) (e.g. dextromethorphan)
- bisphosphonates (e.g. alendronate (Fosamax), ibandronate (Boniva), zoledronic acid (Zometa))
- calcium-based antacids (e.g. TUMS®)
- calcium supplements
- CNS stimulants/appetite suppressants (e.g. lisdexamfetamine, methylphenidate, hydrochloride, amphetamine preps, sibutramine)
- Cox-2 drugs (e.g. celecoxib (Celebrex), rofecoxib (Vioxx) and valdecoxib (Bextra))
- decongestants (e.g. pseudoephedrine (Sudafed), oxymetazoline (Afrin), and others)
- Depo-Provera®
- oral diabetes medications (for treatment of stable, controlled diabetes)
- erectile dysfunction medications (e.g. sildenafil, tadalafil, vardenafil)
- estrogen/progesterone replacement therapy for postmenopausal women
- expectorants (OTC only) (e.g. guaifenesin)
- eye preparations for allergic eye symptoms (topical) (e.g. antihistamines, NSAIDS, antiallergic compounds)
- H_2 blockers (e.g. ranitidine, cimetidine, famotidine, nizatidine) for GERD
- hair growth preparations (e.g. finasteride (Propecia®))
- hemorrhoid treatments
- herpes medications (e.g. acyclovir (Zovirax), valacyclovir (Valtrex))

- insulin (for treatment of stable, controlled diabetes)
- laxatives
- Librax
- lithium
- migraine analgesics/preventatives (e.g. butalbital, Midrin, sumatriptan, topiramate)
- nasal antiallergic spray (Cromolyn/Atrovent)
- nasal saline spray
- non-steroidal anti-inflammatory medications (e.g. aspirin, ibuprofen, naproxen, ketoprofen)
- Norplant®
- oral contraceptives
- proton pump inhibitors (e.g. omeprazole (Prilosec), lansoprazole (Prevacid), esomeprazole (Nexium)) for GERD
- psyllium
- sleep aids used PRN
- stool softeners
- study medications
- thyroid replacement medication (e.g. Levothroid, Levoxyl, Synthroid)
- tretinoin (Retin-A) for acne
- vitamins, minerals
- Low potency topical corticosteroids (BID)
  - aciometasone dipropionate
  - desonide
  - dexamethasone
  - dexamethasone sodium phosphate
  - fluocinolone acetonide
  - hydrocortisone
  - hydrocortisone acetate
- Medium potency topical corticosteroids (BID)
  - betamethasone benzoate
  - betamethasone dipropionate
  - betamethasone valerate
  - clocortolone pivalate
  - desoximetasone
  - diflorasone .05%
  - fluocinolone acetonide
  - fluocinonide .05%
  - flurandrenolide
  - fluticasone propionate
  - hydrocortisone butyrate
  - hydrocortisone valerate
  - mometasone furoate
  - triamcinolone acetonide

This list is summarized on the Microbiome Allowed Medications (P3_MEDALLOW) reference card.

In general, a participant is ineligible if he/she chronically uses any medication other than rescue beta-agonist, study drug, and those in the preceding list. If a participant's use of a specific allowed medication is chronic, a complete clinical assessment should be performed to ensure the participant's safety and his/her ability to complete the entire study. Care should be taken to evaluate any

underlying conditions the participant may be treating with these medications, in the event that he/she may have an exclusionary medical condition.

If a participant is taking a medication that does not appear in the above list, but also does not appear on the Microbiome Exclusionary Drugs (P3_EXCLDRUG) reference card, first consult the local investigator. If the local investigator feels the participant should be considered eligible, then contact the Microbiome scientific coordinator at the DCC with the details. She will contact the lead study investigators and will document the final decision on the participant's suitability for the study.

This criterion is documented on P3_ELIG1A or P3_ELIG1C.

- Chronic diseases (other than asthma, including morbid obesity) that in the opinion of the local investigator would prevent participation in the trial or put the participant at risk by participating, based on the comprehensive physical exam and medical history taken at Visit 1.

  In particular, individuals with an established diagnosis of vocal cord dysfunction or chronic diseases of the lung (other than asthma; e.g., emphysema, chronic bronchitis, pulmonary embolism, malignancy, cystic fibrosis, etc.), kidney, heart, liver, endocrine or nervous system, or immunodeficiency will be excluded.

  Note that the majority of the following conditions are exclusionary only if deemed clinically unstable or contraindicated for the protocol in the judgment of the local investigator and the principal investigator for the protocol. If a potential participant's eligibility is in question, contact the Microbiome scientific coordinator at the DCC for assistance.

  Exclusionary conditions are listed above, but may not be all inclusive.

  These illnesses are listed on the Microbiome Exclusionary Medical Conditions (P3_EXCLMED) reference card.

  This criterion is documented on P3_ELIG2A or P3_ELIG2C.

- Draining purulent discharge visible in pharynx during physical examination.

  This criterion is documented on P3_ELIG2A or P3_ELIG2C.

- ECG evidence of cardiac arrhythmia or ischemia (for those > 45 years of age).

  This criterion is documented on P3_ELIG2A or P3_ELIG2C.

- Any condition or compliance issue which, in the opinion of the investigator, might interfere with study participation.

After the physician interacts with the participant at Visit 1, and the results of the physical exam and medical history are known, it may become apparent that the participant is not an ideal candidate for the Microbiome study for a variety of reasons. If this is the case, the participant should be terminated from the study.

This criterion is documented on P3_ELIG2A or P3_ELIG2C.

Visit 1 Inclusion Criteria: Asthmatics only

- PC_{20} ≤ 8 mg/ml or FEV_1 improvement ≥ 12% in response to four puffs of albuterol.

  At Visit 1, asthmatic participants who do not demonstrate PC_{20} ≤ 8 mg/ml will be required to undergo reversibility testing. During this test, participants perform baseline spirometry followed by the administration of 4 puffs of albuterol and another spirometry session 10-15 minutes later. See the Spirometry discussion in this section and the Spirometry Manual of Operations in appendix 1 of the AsthmaNet General Manual of Operations for further details on the reversibility testing procedures.

  For purposes of eligibility assessment, reversibility is calculated on the basis of the baseline spirometry results (recorded on the Spirometry Testing (SPIRO) form) and the post 4 puffs spirometry session (recorded on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form). Reversal is the relative change in FEV_1 expressed as a percentage.

  Sample reversal calculations:

  - % reversal:

    To calculate the participant's % reversal with 4 puffs of albuterol, take the difference in raw FEV_1 values (in liters) (post FEV_1 value – pre FEV_1 value) and divide by the pre FEV_1 value. Multiply the result by 100.

    Example:

    Pre-test FEV_1 (from Q1030 SPIRO form): 3.24 liters
    Post-test FEV_1 (from Q1030 PALB4_SPIRO form): 3.80 liters

    Reversal % = (3.80 – 3.24) / 3.24 * 100 = 17.28%

  If the participant's reversal % is ≥12% (without rounding), he/she meets the criterion. The participant in the example meets the criterion.

  This criterion is documented on P3_ELIG2A.

Visit 1 Exclusion Criteria: Asthmatics only

- Significant asthma exacerbation since Visit 0.
  This criterion is documented on P3_ELIG1A.

- Negative Phadiatop result.
  This criterion is documented on P3_ELIG1A.

Visit 1 Exclusion Criteria: Controls only

- Eczema (rash in crook of elbow or bend of knee).
  This criterion is documented on P3_ELIG1C.

- Anaphylaxis to food or stinging insects.
  This criterion is documented on P3_ELIG1C.

- Hives/urticarial to food or stinging insects.
  This criterion is documented on P3_ELIG1C.

- FEV_1 < 80% predicted
  This criterion is documented on P3_ELIG2C.

- FVC < 80% predicted
  This criterion is documented on P3_ELIG2C.

- PC_{20} ≤ 16 mg/ml
  This criterion is documented on P3_ELIG2C.

Visit 2 Exclusion Criteria: All participants

- Blood creatinine ≥ 1.3 mg/dL.
  This criterion is documented on P3_ELIG3A or P3_ELIG3C.

- Treatment with any corticosteroid (except for topical) since Visit 1.
  Systemic corticosteroids include oral (e.g., prednisone), injectable (IM), and intravenous (IV) steroids.

  Asthmatic participants who have experienced a significant asthma exacerbation requiring treatment with systemic corticosteroids since Visit 1 are ineligible to continue in the trial. A Microbiome Termination of Study Participation

(P3_TERM_A) form should be completed. The participant may be considered for possible re-enrollment, starting at Visit 0, after the exacerbation resolves and the participant meets enrollment criteria. When scheduling Visit 0, keep in mind that the participant may not have more than 2 asthma exacerbations requiring systemic corticosteroid treatment within past 6 months or an asthma exacerbation requiring systemic corticosteroid treatment in past 3 months before Visit 0.

This criterion is documented on P3_ELIG3A or P3_ELIG3C.

- Respiratory infection since Visit 1.

  This criterion is documented on P3_ELIG3A or P3_ELIG3C.

- Treatment with any antibiotic (except for topical) since Visit 1.

  This criterion is documented on P3_ELIG3A or P3_ELIG3C.

- Treatment with any excluded medication (P3_EXCLDRUG) since Visit 1.

  This criterion is documented on P3_ELIG3A or P3_ELIG3C.

- Participant wishes to withdraw consent.
  This criterion is documented on P3_ELIG3A or P3_ELIG3C.

- New information that makes the participant ineligible according to the eligibility criteria.

  This criterion is documented on P3_ELIG3A or P3_ELIG3C.

Visit 2 Exclusion Criteria: Asthmatic Participants <u>only</u>

- Any condition or compliance issue which, in the opinion of the investigator, might interfere with study participation.

  After the physician interacts with the participant at Visit 1, and the results of the physical exam and medical history are known, it may become apparent that the participant is not an ideal candidate for the Microbiome study for a variety of reasons. If this is the case, the participant should be terminated from the study.

  This criterion is documented on P3_ELIG3A.

- Significant asthma exacerbation since Visit 1.

  This criterion is documented on P3_ELIG3A.

- Occurrence of an immediate bronchoscopy-induced asthma exacerbation requiring oral corticosteroid treatment

This criterion is documented on P3_ELIG3A.

- Inability of the participant to use a Diskus inhaler properly.

  This criterion will be evaluated objectively for all participants using the MDI Inhalation Technique Checklist (Without Spacer) (TECH_DISKUS). Participants must achieve a perfect score of ten (which evaluates two separate inhalations) to pass the performance check. Participants should dose from the assessment Diskus provided by the DCC for this assessment. The Diskus has a fluorescent label that reads 'Diskus Inhalation Technique Inhaler (Contains Placebo).'

  Checklist(s) should be filed in the participant's study folder at the performance site; do not forward them to the DCC. See section 5 of this manual and the Inhalation Technique Assessment discussion in this section for further details.

  This criterion is documented on P3_ELIG3A in section 2 of the form.

Visit 2 Exclusion Criteria: Controls only

- FEV1 improvement ≥ 12% in response to four puffs of albuterol.

  For purposes of eligibility assessment, reversibility is calculated on the basis of the baseline spirometry results (recorded on the Spirometry Testing (SPIRO) form) and the post 4 puffs spirometry session (recorded on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form). Reversal is the relative change in FEV_1 expressed as a percentage.

  Sample reversal calculations:

  - % reversal:

    To calculate the participant's % reversal with 4 puffs of albuterol, take the difference in raw FEV_1 values (in liters) (post FEV_1 value – pre FEV_1 value) and divide by the pre FEV_1 value. Multiply the result by 100.

    Example:

    Pre-test FEV_1 (from Q1030 SPIRO form): 3.24 liters
    Post-test FEV_1 (from Q1030 PALB4_SPIRO form): 3.80 liters

    Reversal % = (3.80 – 3.24) / 3.24 * 100 = 17.28%

  If the participant's reversal % is ≥12% (without rounding), he/she does not meet the eligibility criterion and must be terminated. The participant in the example does not meet the criterion.

This criterion is documented on P3_ELIG3C.

2.20 Genetics Blood Draw

Visit 1

Obtain blood sample for DNA extraction and genetic analysis (three 10 ml purple-top tubes) (optional)
Complete Genetic Analysis Blood Draw (GABLOOD) form
Enter genetics sample information into Genetics Tracking module, if applicable
Record genetic sample information on log (GEN_SAMP_LOG), if applicable

Before drawing blood for genetic analysis, verify that the participant has given consent to participate in the genetic analysis component of the Microbiome study. The genetic analysis blood draw is optional; as stated in the consent, participants can refuse this blood draw and still participate in every other aspect of the Microbiome study. The genetic analysis participation rate for each clinical center partnership and performance site will be summarized on the Microbiome Accrual Report.

The genetic analysis blood draw is scheduled for Visit 1 in the Microbiome protocol; however, blood may also be drawn at Visit 2. See below for details on managing data in this case.

AsthmaNet genetics procedures are described in Appendix 4 of the AsthmaNet General Manual of Operations. The standard blood sample for genetic analysis purposes for adults consists of three purple-top 10 ml vacutainers. Make certain that all tubes are as full as possible to ensure sufficient DNA for future genetic analyses. If a participant cannot provide three full purple-top vacutainers of blood, collect as much blood as possible and submit it to the Arizona Genetics Lab in Tucson for DNA extraction and storage.

Blood tubes collected for genetic analysis should be scanned into the AsthmaNet Genetics Tracking module immediately after they are drawn. The scan date is saved in the database and must be interpretable as the blood draw date. This information is forwarded to the Arizona Genetics Lab electronically and is needed for their tracking database and possible future sample submissions to the Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC). Discrepancies between the scan date in the database and the blood draw date written on the blood tubes will be noted by the lab and reported to the DCC.

Information regarding the genetics blood drawn for a given participant must be entered onto the AsthmaNet Genetics Sample Log (GEN_SAMP_LOG) just prior to refrigerating the samples. This log tracks the collection date and time, refrigeration date and time and the volume of blood collected in each tube. The log collects information needed for BioLINCC purposes.

Complete the Genetic Analysis Blood Draw (GABLOOD) form for all participants, regardless of whether or not they consent to provide a genetics blood sample. For those who elect to provide a blood sample, this form records information about their

level of consent for future genetic analyses, as well as the total volume of blood drawn. See Section 10 and Appendix 4 of the AsthmaNet General Manual of Operations for specific information on completing the GABLOOD form. Note that the participant must review the form and complete the source documentation information (initials and date), even if he/she did not provide a blood sample.

Note: If a participant consents to provide a genetics blood sample, but the sample is not obtained at Visit 1 for whatever reason, the blood draw may be delayed to any subsequent protocol visit. If the genetics blood draw is deferred until Visit 2 (or any other future visit), then the Visit 1 packet GABLOOD form should be marked missing. The GABLOOD form should be completed and data entered as a single form for the visit at which the blood draw takes place (e.g., Visit 2). If the blood draw is attempted at Visit 1 but is unsuccessful, and the participant is unwilling to have another draw attempted at a future visit, then the GABLOOD form should be completed and data entered as part of the Visit 1 packet. In that case, Q1000 and Q1010 should be completed, indicating that a blood sample was not obtained, and the participant should provide source documentation. All individuals who make it past Visit 1 in the study must have a GABLOOD form present in the database.

2.21 Home Environment Questionnaire

Visit 1
Administer Home Environment Questionnaire (HEQ)

The Home Environment Questionnaire (HEQ) was developed by AsthmaNet. This questionnaire collects information about characteristics of the participant's home in general, his/her bedroom, his/her pets, and exposure to others' pets. Information regarding exposure to potential allergens that might affect the participant's asthma is collected in detail.

The participant completes this questionnaire. The coordinator should provide assistance for any questions when requested.

When the participant returns the questionnaire, the coordinator should review it thoroughly to be sure all questions have been answered to the best of the participant's ability. If he/she would rather not answer certain questions, they may be left blank. The participant should initial and date the source documentation box on the last page of the form when he/she is finished.

2.22 Household Socio-Economic Information Form

Visit 1
Administer Household Socio-Economic Information form (HOUSEHOLD_SEI)

Socio-economic status (SES) and health outcomes tend to be positively correlated (i.e., the higher the SES, the better the health outcome in terms of morbidity and mortality). Dr. Sheldon Cohen, affiliated with the Pittsburgh clinical center partnership, is an expert in this field and provided assistance for AsthmaNet to develop a very brief Household Socio-Economic Information (HOUSEHOLD_SEI) form. This form collects the highest level of education attained by members in a participant's household, the combined gross annual income of all members of the household, and the number of individuals supported by the income.

This form is completed by the participant. He/she can decline to answer any question he/she wishes.

2.23 Informed Consent

Visit 0
Acquire signed Microbiome informed consent

Informed consent **must** be obtained before any study information is collected or any study procedures are performed.

The Microbiome consent template explains the procedures and time commitment necessary to participate in the Microbiome trial, should the potential participant be deemed eligible. The AsthmaNet Data and Safety Monitoring Board reviewed and approved the template language which was prepared and submitted to each performance site's Institutional Review Board (IRB) for consideration. Some IRBs require or request changes to the template language which are reviewed by the DCC for consistency with the intent of the original document and completeness in terms of included information. A copy of the IRB approval memo and an IRB-stamped version of the consent document must be forwarded to the DCC prior to the start of recruitment at a given performance site. Each performance site must use its most recent IRB-approved version of the consent document in obtaining consent. The potential study participant must be given the opportunity to read, understand, and sign the consent document before any study-related activities take place.

Guidelines for obtaining consent:

- At the beginning of Visit 0, or prior to scheduling the visit, provide the potential participant a copy of the informed consent document and ask him/her to read it thoroughly. The participant should not sign the form until after you have discussed its contents with him/her.
- Allow ample time for the potential participant to read the informed consent form thoroughly. This will take some time, as the documents are often lengthy and include very detailed information for full disclosure.
- If the potential participant is unable to read the informed consent form or seems to be struggling, offer to read it to him/her or to help him/her with the more difficult sections.
- Be prepared to answer any questions the potential participant may have. If the person does not appear to understand the study or what participation entails, or if he/she has any other doubts about enrolling, do not ask him/her to sign the informed consent form. This person is not eligible to participate in the study.
- Maintain the signed informed consent form in the participant's study folder. To ensure confidentiality, **do not send this form to the DCC**. This document will be reviewed during data quality site visits.

If the participant fails to qualify during the run-in for a reason that can be remedied (e.g., respiratory tract infection, etc.), he/she may be re-enrolled starting at Visit 0 at a later date. During the new Visit 0, the participant should be given a clean copy of the performance site's most current, IRB-approved Microbiome consent document to review and sign. See the Re-Enrollment discussion in this section for further details.

If modifications are made to the Microbiome consent document and approved by the local IRB while a participant is in the study, he/she must be re-consented following local IRB rules. All versions of the Microbiome consent document the participant signed must be retained in his/her Microbiome study folder and are subject to audit.

Local IRB rules and regulations should be followed at all times.

Note: The Microbiome consent template contained language for the Microbiome main study and optional genetic analysis participation. Some IRBs required the language for one or both of the optional sections to be placed into its own consent document. At Visit 0, consent should be sought for both study components, regardless of how they are packaged at a given performance site. All signed documents must be retained in the participant's study folder.

The date the participant signed the Microbiome study consent is recorded and tracked on Microbiome Eligibility Checklist 0 (P3_ELIG0A or P3_ELIG0C). Genetic analysis participation is tracked on the Genetic Analysis Blood Draw (GABLOOD) form which is completed at the blood draw visit.

Visit 0
Administer BioLINCC consent document
Complete BioLINCC Consent Tracking Form (BIOLINCC)

As a network funded by the National Institutes of Health, National Heart, Lung, and Blood Institute (NIH/NHLBI), AsthmaNet is expected to participate in the NHLBI's biobank which is coordinated by the Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC). A biobank is a centralized collection of biological samples and health information that can be used by researchers outside of AsthmaNet for future studies in the areas of asthma and other diseases. At some time in the future, with the acceptance of BioLINCC, leftover samples from the Microbiome study (potentially including sputum supernatant, plasma, serum, DNA, BAL fluid) will be transferred to BioLINCC and made available to other researchers. A participant must be asked to give his/her consent to transfer samples to BioLINCC. Samples for participants who refuse to provide consent will be retained by AsthmaNet. Participation is voluntary. See the AsthmaNet Genetics Procedures and BioLINCC manual in Appendix 4 of the AsthmaNet General Manual of Operations for further details regarding BioLINCC.

At Visit 0, after a participant provides consent to be in the Microbiome trial, he/she must be given the IRB-approved Microbiome BioLINCC consent document to review. If he/she agrees to allow his/her leftover Microbiome samples to be transferred to

BioLINCC, he/she should sign the document and indicate the level of consent he/she is providing. Two levels of consent are possible: 1) allowing consent for all types of analyses, including genetic analyses, on the transferred samples and 2) allowing analyses with the exception of genetic analyses by researchers outside of AsthmaNet. The participant should indicate his/her preference in the consent document, prior to signing it. If the participant consents to participate in BioLINCC for his/her Microbiome samples, then his/her consent document must be retained with the Microbiome study consent document in his/her Microbiome study folder at the performance site. This consent document is also subject to audit during an AsthmaNet data quality site visit.

Every Microbiome participant must have a BioLINCC Consent Tracking Form (BIOLINCC) completed at Visit 0. This form tracks whether or not the participant agreed to donate his/her leftover Microbiome samples to BioLINCC and, if so, what level of consent he/she provided. Information submitted to the DCC on the BIOLINCC form must match the participant's consent document. The BIOLINCC form data will be used to determine which samples are transferred to BioLINCC in the future.

Visit 2, 5

Acquire Signed Bronchoscopy Consent or the bronchoscopy section of Microbiome Consent

At Visit 2 (and Visit 5 for asthmatics), prior to the bronchoscopy procedure, the bronchoscopy procedure and its related risks must be reviewed with the participant one more time. At the centers that have a separate bronchoscopy consent, the participant must sign and date the bronchoscopy consent. At centers that do not have a separate bronchoscopy consent, the bronchoscopy procedure section in the Microbiome consent must be reviewed again with the participant and the participant must initial and date acknowledging that he or she agrees to undergo bronchoscopy procedure at the visit. If the participant does not agree to undergo the procedure, he or she is not eligible to proceed with bronchoscopy. If the participant does not agree to undergo bronchoscopy at Visit 2, he/she is not eligible for the Microbiome study and a Termination of Study Partcipation (P3_TERM_A or P3_TERM_C) form should be completed.

2.24 Inhalation Technique Assessment (asthmatics only)

Visit 1

Assess inhalation technique using the MDI Inhalation Technique Checklist (TECH_MDI_NOSP) and placebo provided. Complete as many TECH_MDI_NOSP forms as necessary and store in participant's folder.

To assure that each participant has met the AsthmaNet standards for MDI use, an MDI Inhalation Technique Checklist (Without Spacer) (TECH_MDI_NOSP) has been developed.

During the technique assessment, ten separate criteria are assessed by observing the participant inhale from a placebo MDI provided by the DCC. The placebo inhaler has a fluorescent label that reads 'Diskus Inhalation Technique Inhaler (Contains Placebo)'. The participant is given one point for each of the following steps that is completed correctly:

1. Removes cap of inhaler.
2. Shakes inhaler up and down.
3. Breathes OUT fully.
4. When breathing out fully, does so away from MDI.
5. Puts mouthpiece in mouth, closes lips around mouthpiece.
6. Activates inhaler by pressing down on canister one time.
7. Breathes IN SLOWLY, filling lungs with medicine.
8. Holds breath for at least 5 seconds (with or without mouthpiece in mouth).
9. Removes mouthpiece from mouth before breathing normally.
10. Breathes normally for at least 30-60 seconds.

After successfully completing one puff/inhalation, the participant must repeat the sequence correctly for a second puff to earn the 11^{th} point and pass the technique assessment.

It is important to remind participants that exactly <u>one actuation</u> from the inhaler is allowed for each inspiration (i.e., no double, triple, etc. actuations for a single inspiration).

Results of the technique assessment are recorded on the TECH_MDI_NOSP checklist and stored in the participant's study folder; do not submit these forms to the DCC.

Visit 2

Assess inhalation technique using the Diskus Inhalation Technique Checklist (TECH_DISKUS) and placebo provided. Complete as many TECH_DISKUS forms as necessary and store in participant's folder.

Because proper medication dosing is crucial for the success of the Microbiome study, each participant must demonstrate that he/she can accurately use a Diskus inhaler. Proper Diskus technique is an eligibility requirement that is assessed at Visit 2 on Microbiome Eligibility Checklist 3 (P3_ELIG3A).

To assure that each participant has met the AsthmaNet standards for Diskus use, a Diskus Inhalation Technique Checklist (TECH_DISKUS) has been developed. Participants are considered eligible at Visit 2 only after they are able to carry out each of the ten steps (corresponding to ten points) listed on the technique checklist. There is no upper limit on the number of test puffs a participant may take to satisfy these requirements.

During the technique assessment, ten separate criteria are assessed by observing the participant inhale from a placebo Diskus provided by the DCC. The placebo inhaler has a fluorescent label that reads 'Diskus Inhalation Technique Inhaler (Contains Placebo)'. The participant is given one point for each of the following steps that is completed correctly:

1. Uses thumb or finger in thumb grip to open device until the mouthpiece appears
2. Keeps Diskus® horizontal prior to step #3 and until step #7 completed
3. Slides lever once until it clicks
4. Breathes OUT fully
5. When breathing out fully (step #4), does so away from Diskus®
6. Puts lips tightly above and below mouthpiece opening
7. Breathes IN QUICKLY, filling lungs with medicine
8. Holds breath for at least five seconds (with or without Diskus® in mouth)
9. Removes Diskus® before breathing normally
10. Closes Diskus® by placing thumb or finger in the thumb grip and sliding it closed

It is important to remind participants that exactly one actuation from the inhaler is allowed for each inspiration (i.e., no double, triple, etc. actuations for a single inspiration).

Results of the technique assessment are recorded on the TECH_DISKUS checklist and stored in the participant's study folder; do not submit these forms to the DCC.

2.25 Medical History

Visit 1

Complete Adult Asthma and Allergy History form (ASTHMA_HX_ADULT) – *asthmatics only*
Complete Prior Asthma/Allergy Treatment form (PRIOR_TRT) – *asthmatics only*
Complete Prior Conditions for All Participants form (PRIOR_COND_ALL)
Complete Prior Conditions for Adult Participants form (PRIOR_COND_ADULT)

A comprehensive medical history is taken at Visit 1. The medical history is broken into three parts recorded on four data collection forms:

1. The Adult Asthma and Allergy History (ASTHMA_HX_ADULT) form is administered to asthmatics and collects information regarding the onset of asthma and family history, recent asthma symptoms and acute episodes of asthma, asthma triggers, allergies, and basic smoking history.

   Note that smoking history is quantified in pack-years. One pack-year is defined as a one-year period when the participant smoked one pack (20 cigarettes per pack) per day. Participants whose smoking history changed over time will have their pack-year history calculated in pieces and summed over the entire history. For example:

   Sam smoked ½ a pack of cigarettes per day (10 cigs per day) while in his last year of college. Following college, he smoked a pack per day (20 cigs per day) for four years, until his employer no longer allowed smoking in the building. At that point he cut back to 5 cigarettes per day (0.25 packs per day) for 6 months while trying to quit. He has been a non-smoker ever since.

   Sam's pack-year history is calculated as follows:

   (1 x .5) + (4 x 1.0) + (.50 x .25) = 4.625 pack-years

   Sam may be eligible for Microbiome, given his current non-smoker status and less than 5 pack-year history. Note that pack-year history is assessed for eligibility on Eligibility Checklist 1 (P3_ELIG0A or P3_ELIG0C). Actual pack-years are recorded on ASTHMA_HX_ADULT for asthmatics and P3_ELIG0C for controls.

2. The Prior Asthma/Allergy Treatment (PRIOR_TRT) form is administered to asthmatics and collects detailed information about the medications the participant used to treat asthma and allergies in the past 12 months. This form also collects non-asthma/allergy use of oral and injectable steroids. Information on this form will be used to determine if the participant meets necessary washouts for spirometry at Visit 1 and for entry into the study according to the eligibility criteria.

3. The Prior Conditions for All Participants (PRIOR_COND_ALL) and Prior Conditions for Adult Participants (PRIOR_COND_ADULT) forms collect detailed information on prior diseases, illnesses, conditions and surgeries the participant has had.

The medical history is administered early in the visit so that eligibility criteria that are easy to confirm can be checked quickly. All portions of the medical history are obtained by participant interview. Read each question to the participant in a consistent, even tone, exactly as written on the forms. Provide clarification when asked.

When available, information contained in medical records should be considered more accurate than participant reporting. If the coordinator chooses to report interview information rather than information from the participant's medical record (when it is available), the affected item(s) should be dated and initialed to document this override. A notation indicating the override should also appear in the clinic notes. This documentation will be necessary when the data are audited during a site visit.

See Section 10 of the AsthmaNet General Manual of Operations for further details regarding the completion of the medical history forms.

2.26 Methacholine Challenge

Methacholine challenges are used in the Microbiome trial to establish a participant's study eligibility (through the PC_{20} criterion evaluated at Visit 1) and to collect an important secondary outcome variable, PC_{20}.

Individuals performing methacholine challenges must be AsthmaNet-certified in this procedure or, at minimum, supervised by AsthmaNet-certified personnel.

To maximize supplies, old (unexpired) stock of methacholine should be used before newer lots.

Participants must pass all of the checks on the Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK) and the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT) before proceeding with the challenge. Note that METHACHK_ADULT Q1050 excludes a participant from performing the challenge only if he/she used systemic corticosteroids for 4 or more days for treatment of an asthma exacerbation; if less systemic steroid was used, or it was used for a different indication, the question should be answered 'No.'

General procedures for carrying out a methacholine challenge can be found in the Methacholine Manual of Operations in Appendix 2 of the AsthmaNet General Manual of Operations.

Post-Methacholine Challenge Procedures
After a methacholine challenge has been completed, the participant should be reversed back to at least 90% of baseline (pre-challenge) lung function with albuterol. Baseline lung function (FEV_1) is obtained from Q1030 on the participant's Spirometry Testing (SPIRO) form completed at the visit.

Standard reversal is two puffs of albuterol if no sputum induction will follow at the visit. At Visits 1 and 4, eligible participants will be proceeding with sputum induction and should be reversed with four puffs of albuterol. Results of standard reversal are recorded on the Methacholine Challenge Testing (METHA) form.

Puffs of albuterol given to reverse the participant from a methacholine challenge should not be counted in the RESCUE Ventolin® puffs the participant records on the Asthma Monitoring Log (P3_ASTHMA_LOG) the evening of the visit.

If a participant requires additional treatment to achieve reversal, this information should be recorded on the Additional Treatment Post Methacholine Challenge Testing (METHA_ADD_TRT) form.

See Section 10 of the AsthmaNet General Manual of Operations for details on the completion of these forms.

Visit 1
Complete Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT)
Perform Methacholine Challenge Testing (METHA)
Complete Additional Treatment Post Methacholine Challenge (METHA_ADD_TRT) form, if needed

Spirometry and methacholine challenge testing are required at Visit 1 on all participants.

Participants must pass all of the checks on the Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK) and the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT) before proceeding with the challenge. Results of the challenge are recorded on the Methacholine Challenge Testing (METHA) form and are referenced on Microbiome Eligibility Checklist 2 (P3_ELIG2A and P3_ELIG2C). The methacholine challenge report generated through the MedGraphics system must be printed and submitted with the data forms.

If an individual does not meet all the criteria on the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT) at Visit 1, he/she cannot proceed with the challenge at the visit. In this case, the visit may be rescheduled if there is a chance the participant will meet the criteria in the near future. If not, the participant is ineligible to continue participation in Microbiome.

Asthmatic participants who qualify for the methacholine challenge but do not meet the PC_{20} criterion for eligibility ($PC_{20} \leq 8$ mg/ml) must undergo reversibility testing. Visit 1 will be stopped following methacholine challenge and the participant will be scheduled for a continuation visit. Continuation visit should be scheduled within 24-48 hours. See the Spirometry discussion in this section for further details. Control participants must have $PC_{20} > 16$ mg/ml to be eligible for Microbiome.

Visit 4
Complete Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT)
Perform Methacholine Challenge Testing (METHA)
Complete Additional Treatment Post Methacholine Challenge (METHA_ADD_TRT) form, if needed

Participants must pass all of the checks on the Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK) and the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT) before proceeding with the challenge. Results of the challenge are recorded on the Methacholine Challenge Testing (METHA) form. The methacholine challenge report generated through the MedGraphics system must be printed and submitted with the data forms.

If an individual does not meet all the criteria on the METHACHK_ADULT form, he/she cannot proceed with the challenge at the visit. Complete the METHACHK_ADULT form

indicating the participant's ineligibility and proceed to the next procedure on the Visit Procedure Checklist. Challenge data will be missing for this participant.

Standard reversal is two puffs of albuterol if no sputum induction will follow the methacholine challenge at the visit. If the participant will be proceeding with qualification for sputum induction, then he/she should be reversed with four puffs of albuterol. Results of standard reversal are recorded on the METHA form.

2.27 Microbiome Exposure Questionnaire

Visit 1
Administer Microbiome Environment Questionnaire (P3_MEQ)

The Microbiome Environment Questionnaire (P3_MEQ) was developed by AsthmaNet. This questionnaire collects information on exposures that may affect the microbiome, or microscopic environment, of the participant's lungs.

The questionnaire is obtained by participant interview. Read each question to the participant in a consistent, even tone, exactly as written on the forms. Provide clarification when asked.

2.28 Missed Visits

A missed visit is defined as one for which the participant is unavailable to undergo any clinic procedures for purposes of obtaining important outcome data for analysis. If spirometry, methacholine challenge, and/or sputum procedures are attempted during a visit, the visit is not considered missed, even if not all procedures are completed. Spirometry is important to the Microbiome study because it is necessary for a full assessment of significant asthma exacerbation criteria.

Ideally all visits for a participant should occur at the same time of day (+/- 3 hours) as measured by the time that baseline spirometry takes place during a visit. When this is not possible, it is desirable for all visits to fall within a 4-hour window. Do not skip a visit if it is not possible to maintain these goals. Consistency in spacing of visits is more important for the collection of outcome data. If a participant cannot be seen within the 3-hour time window, contact the Microbiome Scientific Coordinator at the DCC to discuss the allowance of an exception. Visits that take place outside the 3-hour window from the time of baseline spirometry at Visit 1 without a pre-approved exception will be assigned protocol deviations.

If it is not possible to schedule a visit within the regular visit window, schedule it in the extended window, if possible. If a participant cannot be seen within the extended windows, contact the Microbiome Scientific Coordinator at the DCC to discuss alternate arrangements. See the Visit Windows discussion in this section for further details.

For Visits 3-5, if a participant cannot come to the clinic at all within the regular or extended windows and no suitable alternate arrangements can be made, the visit will be considered missed. Arrangements should be made to send new study medications to the participant and to provide him/her a new Asthma Monitoring Log (P3_ASTHMA_LOG).

Visits 0-2, 6 (Visit 6 asthmatics only)
These visits are mandatory. Eligibility assessments take place at Visits 0, 1 and 2. Bronchoscopy, the integral procedure in this study, takes place at Visit 2. For asthmatics, randomization takes place at Visit 2 and termination procedures take place at Visit 6. Contact the Microbiome Scientific Coordinator at the DCC if scheduling issues arise for these visits.

Visits 3-5 (asthmatics only)
It is not ideal for these visits to be missed. Data collected at visits 4 and 5 are especially critical to the trial's success. Contact the Microbiome Scientific Coordinator at the DCC to discuss possible options to prevent missed data for these visits.

If either Visit 3 or 4 must be missed, arrangements should be made to get a new supply of study drugs to the participant before he/she runs out of his/her Diskus inhaler. A new Diskus inhaler number should be generated through the Microbiome Randomization

Module using the number of the missed visit, and a Microbiome Scheduled Medications (P3_MED) form should be completed and data entered as a single form. When the Diskus inhalers are collected, a Microbiome Compliance Checklist (P3_COMPLY) form should be completed for both Diskus inhalers. In this case, the number of puffs taken is equivalent to 120 – (remaining puffs on Diskus 1 + remaining puffs on Diskus 2), as reflected on the Diskus counters.

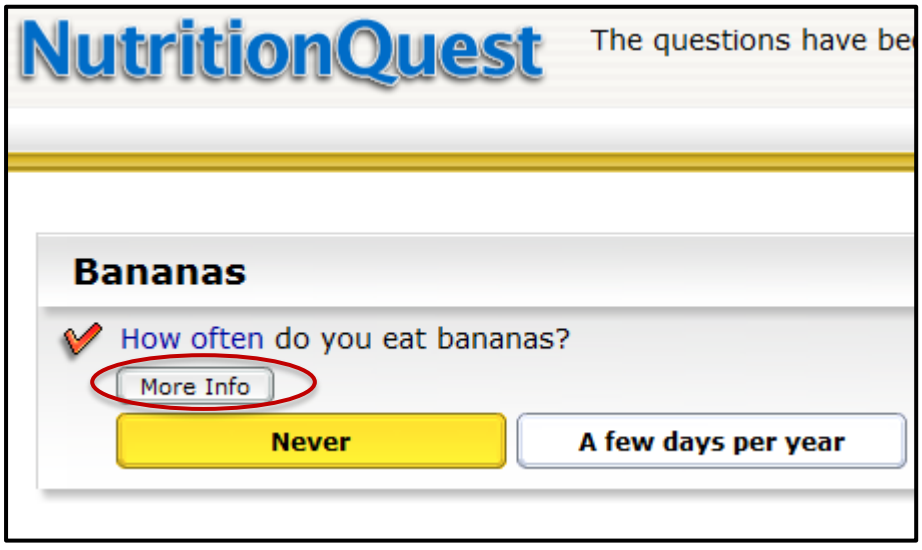
2.29 Nutrition Questionnaire

Visit 1
Administer Block Brief Food Questionnaire

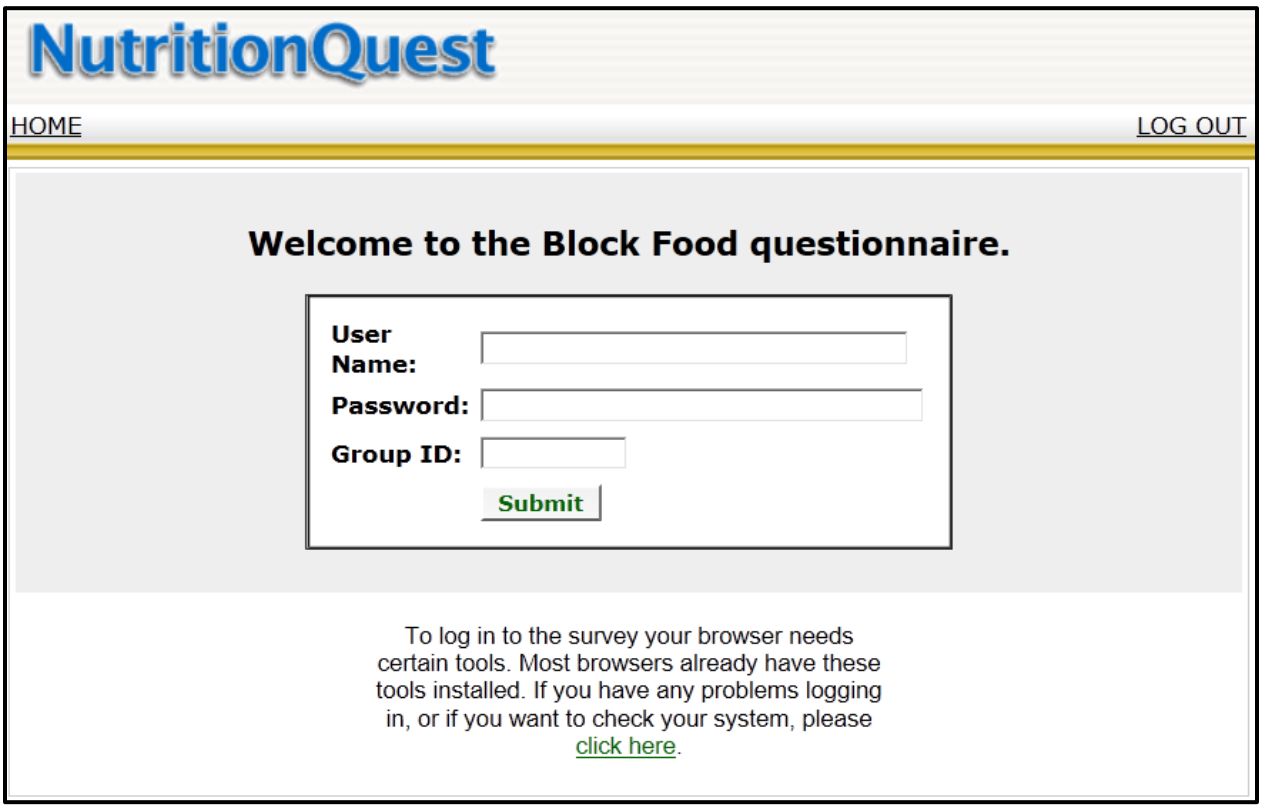
The Block Brief Food Questionnaire was developed under the guidance of Dr. Gladys Block.

The questionnaire will be used for the purposes of quantifying dietary intake. The questionnaire contains a food list of about 70 food items, developed from the NHANES III dietary recall data, and takes 15-20 minutes to complete. It was designed to provide estimates of usual and customary dietary intake. The nutrient database was developed from the USDA Nutrient Database for Standard Reference. Individual portion size is asked, and pictures are provided.

In administering the questionnaire, request that the participant complete the entire questionnaire and provide answers as completely and as accurately as possible. The questionnaire is self-administered and participant completed. No stated or implied time limit should be set. If the participant requests help with or clarification of any question, the Study Coordinator should instruct the participant to reread the instructions, click the **More Info** button as shown below, and to give the best answer possible to each question. The Study Coordinator should not provide an answer to any question. Providing guidance may bias the participant's responses.

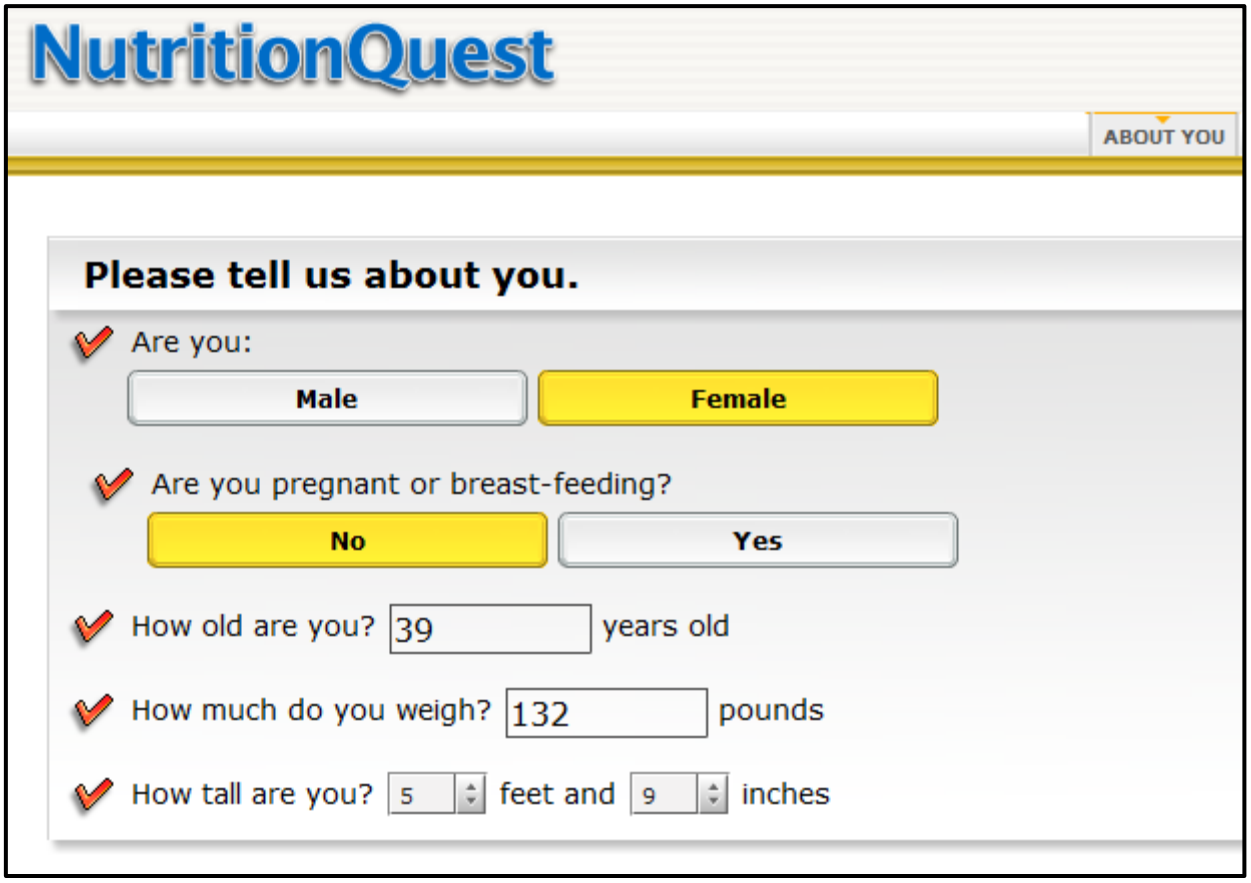


The questionnaire will be administered via NutritionQuest's online system. It can be accessed at the following URL: https://www.nutritionquest.com/login/. The webpage will look as follows:



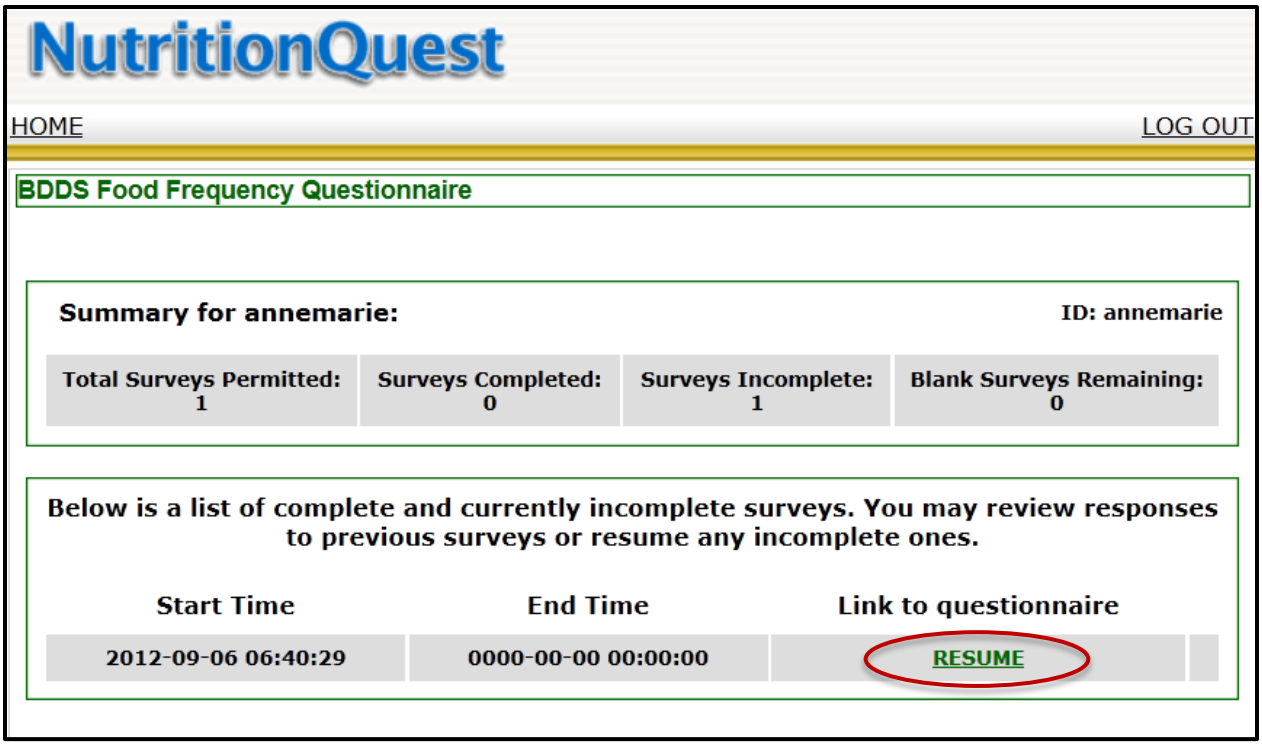
Each site will be provided a "Login List", which is a list of participant IDs, user names and unique passwords for your site. **Be sure to keep the Login List secure.** When a participant is ready to complete the Block Food Questionnaire, locate the participant ID on the "Login List" to identify their user name and password. Use this user name and password to log the participant into NutritionQuest to complete the questionnaire. The "Group ID" for Microbiome is 566. **NOTE: It is extremely important that you use the user name and password assigned to the corresponding participant ID on the Login List, because the provided user name links to the appropriate participant ID.** Participant ID will be stored in the data we receive, and is based on the user name entered at login. If the incorrect user name is used, the data will be associated with the wrong participant. **Also, the user name and password must be typed in *exactly* as provided.**

The screen below will follow the introduction screen:



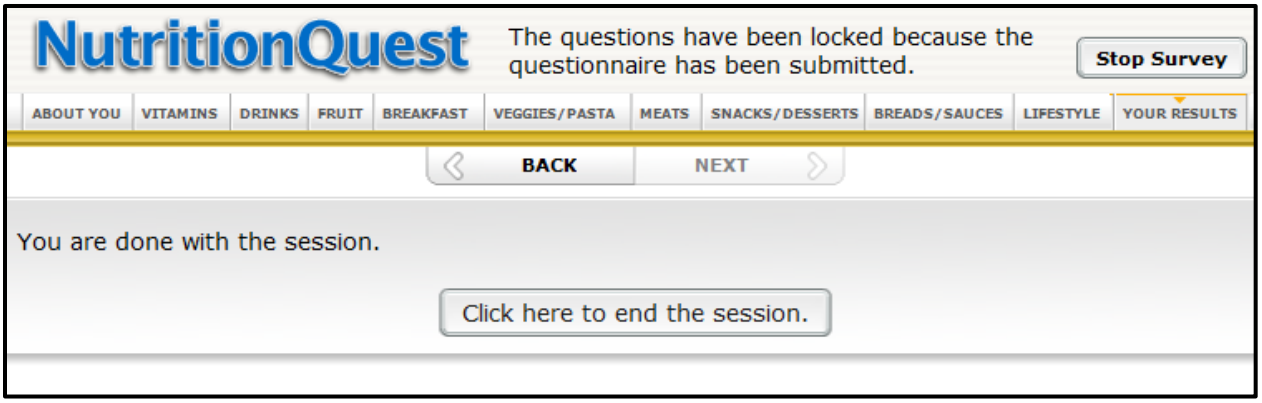
On this screen, enter the participant's gender, if female whether pregnant or breastfeeding (should be no, since females pregnant or breastfeeding are ineligible), age at Visit 1, and weight and height as recorded on the BODYMEAS_ADULT form and click **NEXT**. The participant can then be left on his/her own to complete the questionnaire. The participant should have the NutritionQuest Instructions for Participant (P3_NUTRITION) handout on hand for reference while completing the questionnaire.

If the participant needs to stop temporarily while completing the survey, he/she can click the **STOP SURVEY** button in the upper right corner of the screen. The screen below will appear:

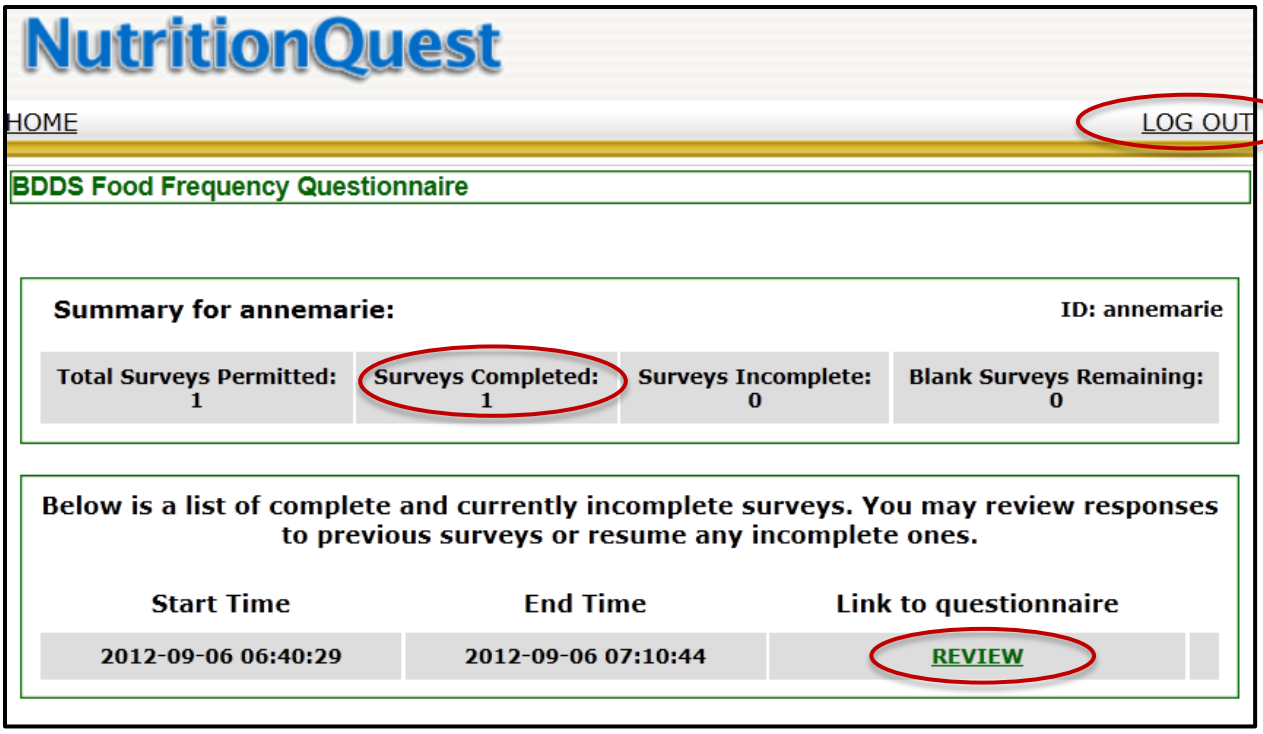


To continue with the survey, the **RESUME** button should be clicked.

At the end of the survey, the following screen will appear:



The participant should click **Click here to end the session** and let you know that he/she is finished. If the participant has completed the questionnaire, you should see that the survey is completed and **REVIEW** on the following screen:



The participant can then be logged out by clicking **LOG OUT** in the upper right corner.

2.30 Participant Assignment Log and Protocol Enrollment

A Participant Assignment Log (P3_LOG) has been developed for Microbiome for each performance site. This log includes columns for unique participant ID numbers, participant initials, participant's name, and assigned Diskus numbers.

Participant ID numbers are preprinted on P3_LOG and are comprised of 7 digits:

- The first digit is the number of the AsthmaNet protocol. For the Microbiome protocol the first digit is 3.
- The next 3 digits are the AsthmaNet performance site identifier (111=Brigham & Women's Hospital, 121=Northwestern, 131=National Jewish – Adult, 142=University of Wisconsin – Adult, 151=University of Pittsburgh - Adult, 161=Washington University-Adult, 171=University of California (SF)-Adult, 182=Duke, 191=Wake Forest)
- The last 3 digits constitute the participant identification (ID) number that is unique within the performance site. Participant IDs start with 001 and increase sequentially for the number of participants who are screened for the Microbiome protocol at Visit 0 at a given site.

To assign an individual a participant ID number, select the next available blank entry on the Microbiome Participant Assignment Log. This number will be the primary participant identifier used during the Microbiome study; it should be used in all communications with the DCC. The participant ID number also should be used to label the participant's Microbiome study folder at the performance site.

Once issued, a participant ID number cannot be re-assigned to any other person.

If a participant re-enrolls at Visit 0, a new participant ID number should be assigned. See the Re-Enrollment discussion in this section for further details.

In order to maintain participants' confidentiality, do NOT use participants' names in any communications with the DCC, either written or oral. Provide only participant ID numbers and initials.

The Participant Assignment Log (P3_LOG) is a confidential document because it ties a participant ID number to a name. This document is required when it is necessary to verify a participant's actual treatment assignment, either during or after the study. For this reason, this log should be stored in a secure location and retained indefinitely at the performance site following the close of the study.

Visit 0
Assign participant ID number (P3_LOG)

Immediately following assignment of the participant's ID number on the Microbiome Participant Assignment Log (P3_LOG), the protocol enrollment module should be accessed to enroll the participant formally in the Microbiome database. Close attention should be paid when entering the participant's information to ensure that the correct ID is entered. If a participant is enrolled mistakenly under an incorrect participant ID, the DCC should be contacted immediately for assistance in correcting the error.

Visit 2 (asthmatics only)
Log drug assignment (P3_LOG)

Visits 3-4 (asthmatics only)
Log drug assignment (P3_LOG)

After accessing the randomization module at each visit to obtain a new Diskus number for the participant, the Diskus number must be logged on P3_LOG. This log provides a single reference for all of an individual's Diskus inhalers over the life of the study. If a backup Diskus is assigned for a participant, it should be listed under the original Diskus for the appropriate visit on the log.

2.31 Participant Identification Card (asthmatics only)

The Microbiome Participant Identification Card (P3_ID) provides a quick reference for the participant to use to monitor his/her asthma. It includes baseline rescue use information for determining when an individual may be experiencing an asthma exacerbation. The ID card also contains instructions for treatment of asthma attacks by physicians and emergency department personnel who may not be familiar with the Microbiome study. The ID card should be carried by the participant at all times in a wallet or purse that is readily accessible.

Visit 2
Complete and distribute Participant ID Card (P3_ID)

Print a Microbiome Participant Identification (ID) Card (P3_ID). Write the participant's name, Microbiome protocol ID number, and the names and phone numbers of study personnel on the card. The participant may enter the name and number of his/her primary physician, if applicable. All information should be written in dark ink.

Fill in the participant's Baseline PEF and High Rescue Inhaler Use value in the spaces provided on the front of the ID card:

- The Baseline PEF is calculated by multiplying FEF Max absolute on the Visit 1 spirometry report (baseline measure prior to methacholine challenge) by 60.
- The High Rescue Inhaler Use value is calculated by adding 8 to the value recorded in Q1000 on the P3_ASTHMA_LOG form at Visit 2.

If the participant has had an increase in symptoms of cough, chest tightness, and/or wheezing and used at least the number of puffs per day denoted by the High Rescue Inhaler Use value, for a two day period then he/she has met significant asthma exacerbation conditions. This value aids the participant in recognizing when he/she needs to be seen for additional treatment of his/her asthma.

Review the contents of the ID card with the participant and explain the use of the card. Stress to the participant that the Ventolin® (RESCUE) inhaler is the first-line treatment for asthma symptoms. If no relief is achieved, the participant should contact performance site personnel to determine whether he/she should come to the clinical site or go to the emergency department for care.

Review when and where emergency care should be sought. Remind the participant that he/she should seek care from study personnel, if possible. However, participants should never delay seeking care if study personnel cannot be reached.

Treatment procedures have been developed with the utmost regard for participant safety. Instruct the participant to contact study personnel if he/she receives emergency

treatment outside the study. Document medications, procedures, and other treatments the participant received.

2.32 Phone Contact (asthmatics only)

Visit 3 (week 5)
The Microbiome protocol designates 3 weeks between Visits 3 and 4. To ensure that the participant is taking his/her study medications and to address the participant's concerns regarding his/her asthma control, formal phone contacts should be scheduled between these visits, approximately 1 ½ - 2 weeks following Visit 3.

Phone contacts are documented on the Microbiome Phone Contact Form (P3_PHONE_CONTACT). Completed forms should be stored in the participant's study folder at the performance site; do not forward these forms to the DCC. Phone contact documentation is participant to audit during an AsthmaNet site visit.

Phone contacts should be scheduled according to the dates provided on the participant's Visit Scheduler Report generated at Visit 2. If multiple attempts are made to contact the participant within the range of dates given on the report and no contact is made, the coordinator should continue to try to get in touch with the participant until his/her next scheduled visit. Document all contact attempts on P3_PHONE_CONTACT.

Refer to Section 4 for more details on how to complete this form.

2.33 Physical Exams

Adult physical exams are documented on administrative forms that are not entered into the study database. Comprehensive exams are documented on the Adult Long Physical Exam (LEXAM_ADULT) form and brief exams are documented on the Adult Short Physical Exam (SEXAM_ADULT) form. These forms should be completed at the applicable visits and stored in the participant's study folder at the performance site. These forms are subject to audit during an AsthmaNet site visit.

The short physical exam includes measures of resting blood pressure, pulse rate, and body temperature, as well as results of pulmonary auscultation. Short exams can be performed by study coordinators, registered nurses, physician assistants, and other individuals who are appropriately trained in these procedures and certified in the Microbiome protocol.

The long physical exam includes the measurements made during a short physical exam, as well as documentation of the presence/absence of oral candidiasis and physical findings. A licensed medical practitioner (LMP) must complete the physical findings and pulmonary auscultation portions of the long exam. A LMP is defined as a physician (MD/DO), physician assistant (PA), or nurse practitioner; a registered nurse does not qualify as a LMP. If a non-physician LMP completes a required long exam at the beginning of the study, the participant still must have interaction with a physician during the visit.

In addition to regular physical exams, additional physical measurements including height and weight, and waist, hip and neck circumference, are taken at various points during each study. These measurements are documented on the Adult Body Measurements (BODYMEAS_ADULT) form and entered into the AsthmaNet database. Body measurements can be made by study coordinators, registered nurses, physician assistants, and other individuals who are appropriately trained in these procedures and certified in the Microbiome protocol.

Visit 1
Perform long physical exam (LEXAM_ADULT)
Examine pharynx for thick strands of draining purulent discharge

A long physical exam is required at Visit 1 in order to ensure that it is safe and appropriate for each participant to enroll in the Microbiome study. Follow the order of procedures on the appropriate visit procedure checklist.

For the Microbiome trial, participants must have interaction with a physician at Visit 1, even if the physician is not performing the long exam.

Draining purulent discharge is indicative of sinusitis which is an exclusionary criterion. The eligibility question corresponding to this physical evaluation is on Eligibility Checklist 2 (P3_ELIG2A and P3_ELIG2C).

The LMP conducting the long physical exam should sign, date and note the time in the gray box on the LEXAM_ADULT form as source documentation.

Visits 2, 4, 5, 6
Perform short physical exam (SEXAM_ADULT)

A brief physical exam is conducted at Visits 2, 4, 5 and 6.

The person conducting the physical exam should sign, date and note the time in the gray box on the SEXAM_ADULT form as source documentation.

Visits 1, 6
Complete Adult Body Measurements form (BODYMEAS_ADULT)

Follow the instructions on the form for making the various measurements. Note that height is captured on the BODYMEAS_ADULT form for everyone at Visit 1 and Visit 6. Individuals who are less than 21 years of age will have their heights updated at every visit involving a baseline spirometry session until the point when they turn 21. Updated heights are recorded on the Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK) form for these individuals.

2.34 Pregnancy Test

At protocol-defined visits urine samples will be obtained from female participants of child-bearing potential for assessment of pregnancy by the presence of the beta subunit of human chorionic gonadotropin (HCG). Testing will be performed at the performance site during the participant's visit using the HCG combo stick test approved by each institution. The results of the pregnancy test should be recorded on the Urine Pregnancy Test (PREG_TEST) form and the participant should initial and date the source documentation box to acknowledge the results. If a participant is found to be pregnant at any point during the Microbiome study, she must be terminated from study participation immediately.

Visits 1, 2
Complete Urine Pregnancy Test form (PREG_TEST) form for all female participants; administer urine pregnancy test, if necessary

At Visit 1 and 2, the PREG_TEST form is required for all female participants, regardless of their child-bearing potential. A urine pregnancy test must be administered if the participant is deemed to be of child-bearing potential.

Visits 4, 5 (asthmatic participants only)
Complete Urine Pregnancy Test form (PREG_TEST) form for all female participants; administer urine pregnancy test, if necessary

For asthmatic participants, the Urine Pregnancy Test (PREG_TEST) form must be completed and a urine pregnancy test administered, if necessary, at Visits 4 and 5. At Visit 4, no form or pregnancy test is required for female participants who are not eligible to proceed with the methacholine challenge.

At all relevant visits, if the participant is potentially able to bear children by the information supplied on the PREG_TEST form, the pregnancy test must be performed and results reported to the participant and to the DCC. Participants who are post-menopausal (defined as at least one year since last menses) or have undergone a hysterectomy or tubal ligation do not need to be tested. This information is documented on the PREG_TEST form.

Note that a history of infertility does not constitute a valid reason to skip the pregnancy test at a visit, nor does a participant’s insistence that she does not have heterosexual intercourse.

Note that individuals who are transgendered or are transitioning to the opposite gender should be tested for pregnancy in accordance with their biological sex. Biologically female participants who are of child-bearing potential must use birth control and provide urine for pregnancy tests as required by the protocol.

After performing a urine pregnancy test, the participant should be shown the results and asked to initial and date the source documentation box at the bottom of the form as verification that the information on the form is correct and acknowledged by her. ***Source documentation should be completed even if a pregnancy test was not performed at the visit.***

If a participant is considered able to bear children, results of the pregnancy test must be known before she proceeds with the diluent stage of the methacholine challenge at Visit 1 and 4 and bronchoscopy at Visit 2 and 5. Pregnant women should not perform methacholine challenges or bronchoscopy. In addition, pregnant or nursing participants are ineligible for the Microbiome protocol.

If a woman is found to be pregnant at any time during the study, she is ineligible for continued participation. Pregnant women should be terminated from further study participation immediately. Participants who become pregnant during the study should have a Microbiome Termination of Study Participation (P3_TERM_A or P3_TERM_C) form submitted to the DCC as soon as possible. Pregnancy should not be recorded and reported as an adverse event.

See Section 10 of the AsthmaNet General Manual of Operations for further details on the completion of the Urine Pregnancy Test (PREG_TEST) form.

2.35 Randomization (asthmatics only)

Visit 2
Randomize participant, if eligible
Log drug assignment (P3_LOG)

The randomized portion of Microbiome is a parallel arm trial during which each asthmatic participant is randomized at Visit 2 to receive one of the following regimens for 6 weeks:

1. Flovent® Diskus (fluticasone) ICS

OR

2. Placebo Diskus

The goal is to randomize 42 asthmatic participants Network-wide (approximately 5 per clinical performance partnership).

At the end of Visit 2, if an asthmatic participant meets all of the eligibility requirements documented on Microbiome Eligibility Checklist 3 (P3_ELIG3A), he/she is eligible to be randomized. The study coordinator should access the Microbiome Randomization Module on the secure AsthmaNet website and enter the appropriate visit number (i.e., 2), the participant's Microbiome ID number, and the performance site at which the participant is being randomized to complete the randomization in the system. At this point, the system is assigning the participant to the regimen that he/she will receive for the rest of the study. A Diskus inhaler from the designated performance site that matches the person's randomized arm will be assigned. The assigned Diskus number should be recorded on the Microbiome Participant Assignment Log (P3_LOG) in the V2 column.

It should be noted that participants can be randomized in the Microbiome randomization module at Visit 2 only if <u>all</u> of the following criteria are met:

1) The participant's Microbiome ID number is enrolled in the Microbiome protocol.
2) The participant's Visit 0 packet, including Visit 0 eligibility data, has been entered at the performance site (only first entry required). The Visit 0 eligibility forms (P3_ELIG0A) must indicate that the participant is eligible (P3_ELIG0A Q1350=1).
3) The participant's Visit 1 packet, including Visit 1 eligibility data, has been entered at the performance site (only first entry required). The Visit 1 eligibility forms (P3_ELIG1A, P3_ELIG2A) must indicate that the participant is eligible (P3_ELIG1A Q1070=1 and P3_ELIG2A Q1080=1).
4) No Microbiome Termination of Study Participation (P3_TERM_A) form has been entered for the participant.

See Section 3 of this manual for details on accessing and interacting with the Microbiome Randomization Module.

Note that treatment assignments in the Microbiome study are double-blind. That is, neither the participant, nor performance site personnel, will be aware of the contents of the participant's Diskus from Visit 2 through 5. The majority of DCC personnel are also blinded to the treatment assignments while the study is ongoing.

Visits 3-4

Generate new drug assignment number via Randomization Module (may occur up to 5 calendar days ahead of a visit)
Log drug assignment (P3_LOG)

At Visits 3-4, clinic personnel must utilize the Microbiome Randomization Module to generate a new Diskus number from which the participant will take his/her daily doses until the next regularly scheduled visit. To prepare for an upcoming visit, the Diskus inhaler number may be generated up to five calendar days ahead of a visit. If the randomization module is accessed to produce a Diskus number more than five calendar days before the visit is completed, a protocol violation may be assigned, depending on the circumstances.

The study coordinator should access the Microbiome Randomization Module on the secure AsthmaNet website and enter the applicable visit number from a dropdown menu, the participant's Microbiome ID number, and the performance site at which he/she is being seen for the visit. The randomization module will display the participant's new Diskus number corresponding to his/her assigned treatment arm. The resulting Diskus number should be recorded on the Microbiome Participant Assignment Log (P3_LOG) under the appropriate visit number.

It should be noted that the following criteria must be met at Visits 3-4 before a Diskus inhaler number will be displayed:

1) The participant must have been randomized via the Microbiome Randomization Module at Visit 2.
2) The participant must not have been terminated from the study (i.e., no P3_TERM_A form has been entered).

Backup Diskus inhalers

If a participant loses his/her Diskus inhaler between visits, then he/she will require the assignment of a new (backup) Diskus inhaler. To generate a new Diskus inhaler number, the study coordinator should access the Microbiome Randomization Module on the secure AsthmaNet website and enter the applicable visit number (i.e., the same visit number for which the previous (lost) Diskus inhaler number was generated) from a dropdown menu, the participant's Microbiome ID number, and the performance site at which he/she is being seen for the visit. The randomization module will recognize that the participant has already had a Diskus assigned for this visit number and will provide

a warning message giving the coordinator a chance to cancel out of the module if a mistake has been made. If the coordinator chooses to generate a new Diskus inhaler number, it will be displayed.

Backup Diskus inhaler numbers should be recorded on the Microbiome Participant Assignment Log (P3_LOG) under the appropriate visit number. A Microbiome Scheduled Medications (P3_MED) form should be completed and data entered any time a backup Diskus inhaler is dispensed to a study participant. See the Study Medications discussion in this section and Section 4 for further details.

Backup randomization procedures

In the rare event that the Microbiome Randomization Module is unavailable during any visit when it is required (i.e., visits 2-4), clinic personnel must contact the DCC for assistance. During week days (Monday through Friday) between 8 AM and 5 PM ET, calls should be made to the AsthmaNet main line at 717-531-3663. After-hours coverage will be provided from 5 – 8 PM ET Monday through Friday as well.

It is extremely important that Diskus inhalers are assigned using the Microbiome Randomization Module. Randomly choosing an available Diskus inhaler at Visits 3-4 and assigning it to a participant in lieu of the randomization module is inappropriate, as it may not contain the participant's assigned treatment regimen. If an incorrect study Diskus is dispensed to a participant, a protocol violation will be assigned.

2.36 Recruitment

Microbiome visits will commence on September 4, 2012. Nine clinical center partnerships will recruit for Microbiome.

A recruitment period of 12 months has been established for Microbiome. Each clinical center partnership should strive to maintain Visit 0 screen percentages of 50% female participants and at least 33% minority participants over the recruitment period.

The gender and minority status of individuals screened at Visit 0, individuals enrolled in the run-in period, and individuals randomized in Microbiome will be summarized by clinical center partnership on the Microbiome accrual report. This report will be available on the secure AsthmaNet website in the Reports: Accrual: Microbiome folder shortly after visits begin.

Target sample sizes for each partnership are based on the number of participants who are successfully entered into the run-in, and subsequently randomized in the Microbiome trial. Each of the nine clinical center partnerships is expected to randomize approximately 5 asthmatic participants and enroll approximately 5 control participants for a Network total of 42 asthmatic and 42 control participants. It is expected that as many as 20 participants (approximately 10 asthmatics and 10 controls per partnership) may need to be screened at Visit 0 at each site in order to randomize up to 5 asthmatic participants and to enroll up to 5 control participants.

Approximate Microbiome Timelines

September 4, 2012:	First participant screened at Visit 0
September 19, 2012:	First participant randomized at Visit 2
September 1, 2013:	Final screening visit (Visit 0)
September 15, 2013:	Final randomization visit (Visit 2)
October 31, 2013:	Final participant visit (Visit 6)

2.37 Re-Enrollment

Participants who do not successfully complete the Microbiome run-in for reasons that may be overcome with time or additional training (e.g., use of excluded medications, significant exacerbation experienced during the run-in for reasons that may be remedied, respiratory infection, etc.) may be suitable candidates to re-enroll in Microbiome for a second attempt. Randomized participants who drop out early may not re-enroll in the trial.

Visit 0 Failures
Participants who do not qualify for the Microbiome study at Visit 0 for reasons that may be overcome with time (e.g., insufficient medication washout, respiratory tract infection in past 6 weeks, etc.) may be invited to repeat Visit 0 at a later date. Data collected during the unsuccessful Visit 0 should not be entered into the AsthmaNet database and forms should not be forwarded to the DCC regardless of whether the participant will re-enroll in the study or not. The Visit 0 packet should be stored at the performance site in a section of folders denoted as 'Microbiome Visit 0 Failures.'

Participants who present at the performance site for a second attempt at Visit 0 should repeat all of the Visit 0 procedures as listed on Microbiome Visit Procedure Checklist 0 (P3_VISIT0). A new visit packet should be completed.

When re-enrollment occurs, the following procedures apply:

- The participant must be given a new participant ID number from the Participant Assignment Log (P3_LOG). See the Participant Assignment Log discussion in this section and Section 4 for further details. This new ID will need to be linked to the participant through the protocol enrollment process before data can be entered into the Microbiome database. For information on the protocol enrollment process, refer to Section 7 of the AsthmaNet General Manual of Operations.

- The participant must read and sign new copies of the Microbiome and BioLINCC informed consent documents. The documents signed at the initial enrollment should reside in the folder created for the participant's original ID number. The new signed consent documents should reside in the participant's current study folder. Informed consent documents should not be updated with a new signature and date, as this practice violates institutional procedures at some of the performance sites.

- The Adult Participant Contact Information (CONTACT_ADULT) form should be reviewed and updated by the participant. A photocopy should be made and stored with the participant's original Visit 0 packet. The original form with updates should be stored in his/her new study folder.

- A new Visit 0 packet with the participant's new ID number should be completed and submitted to the DCC if the participant is now eligible. Do not attempt to update previously-completed forms with the participant's new information. A new study folder should be created to house the participant's forms under his/her new study ID number.

After a Successful Visit 0 and Prior to Randomization at Visit 2
Once a participant is deemed eligible at Visit 0, he/she is formally enrolled in the Microbiome study. The data collection forms from Visit 0 should be entered into the study database and forwarded to the DCC.

If a participant withdraws consent or is deemed ineligible during the run-in, then he/she must be formally terminated from the study. A Microbiome Termination of Study Participation (P3_TERM_A or P3_TERM_C) form should be completed and entered into the database. All of the forms completed at the termination visit should be entered into the AsthmaNet database and sent to the DCC. If any blood or sputum samples were collected during the visit, they should be sent to the appropriate labs according to the instructions in this manual. Such participants should not be invited to re-enroll unless their reason for withdrawing or being withdrawn was such that there is a very high probability that re-entry will result in randomization and full participation in Microbiome.

Note that participants who are withdrawn from the run-in due to asthma exacerbation may be re-enrolled one time at the discretion of the local investigator. When scheduling Visit 0, keep in mind that the participant may not have more than 2 asthma exacerbations requiring systemic corticosteroid treatment within past 6 months or an asthma exacerbation requiring systemic corticosteroid treatment in past 3 months before Visit 0. If the participant experiences a second exacerbation during re-enrollment, he/she is ineligible for further study participation and should not be re-enrolled a third time.

Participants who are good candidates for re-enrollment must re-enter the Microbiome study starting anew at Visit 0.

The following guidelines apply when the participant is re-enrolled:

- The participant must be given a new participant ID number from the Participant Assignment Log (P3_LOG). See the Participant Assignment Log discussion in this section and Section 4 for further details. This new ID will need to be linked to the participant through the protocol enrollment process before data can be entered into the Microbiome database. For information on the protocol enrollment process, refer to Section 7 of the AsthmaNet General Manual of Operations.

- The participant must read and sign new copies of the Microbiome and BioLINCC informed consent documents. The documents signed at the initial enrollment should reside in the folder created for the participant's original ID number. The new signed consent documents should reside in the participant's current study

folder. Informed consent documents should not be updated with a new signature and date, as this practice violates institutional procedures at some of the performance sites.

- The Adult Participant Contact Information (CONTACT_ADULT) form should be reviewed and updated by the participant. A photocopy should be made and stored with the participant's original Visit 0 packet. The original form with updates should be stored in his/her new study folder.

- The Adult Asthma and Allergy History (ASTHMA_HX_ADULT) form, Home Environment Questionnaire (HEQ), Microbial Exposure Questionnaire (P3_MEQ), Prior Conditions for Adult Participants (PRIOR_COND_ADULT) form, Prior Conditions for All Participants (PRIOR_COND_ALL) form, and Prior Asthma/Allergy Treatment (PRIOR_TRT) form may be reused if they were completed at Visit 1 during the participant's prior enrollment. These forms must be reviewed with the participant in detail and updated appropriately. The participant's new ID number and visit date must be written on the forms. A photocopy should be made and stored with the Visit 1 packet from the participant's original enrollment. The form with the handwritten updates should be stored in his/her new study folder and send to the DCC.

- Participants who underwent Phadiatop and total IgE testing at Visit 0 prior to their termination may have their previous AsthmaNet test data reused. It is not necessary to repeat the Phadiatop test for these participants.

- All study procedures must be carried out anew, with the exceptions noted above, beginning with Visit 0. Complete and submit new data collection forms for the participant using his/her new participant ID number and current dates.

- The blood draw for genetic analysis is optional in the Microbiome study; however, participants who gave a sample prior to their study termination should be asked to provide a new blood sample upon re-enrollment, if the participant is amenable. New blood, stool, and sputum samples must be obtained at the applicable visits.

- Visit 0 and Visit 1 can take place on the same day. In this case, the ACQ form should be entered with the Visit 0 packet (i.e. set ACQ to missing at Visit 1, and enter with Visit 0 packet).

After Randomization in Microbiome

Participants who withdraw consent after they have been randomized in the Microbiome study at Visit 2 are NOT eligible to re-enroll. Each participant can contribute only one set of data for the analysis.

2.38 Registration

Visit 0 or Earlier
Register participant in AsthmaNet Registry

Before a participant can be enrolled in the Microbiome trial, he/she must be present in the AsthmaNet Registry with 'complete' status. ACRN and CARE Network participants who completed Registry forms in those networks already will have 'complete' status in the AsthmaNet Registry. Any participants from the earlier networks who have 'incomplete' status, or individuals who are new to the NHLBI asthma networks, will need to undergo the full AsthmaNet registration process.

All individuals who are enrolled in the Microbiome trial will need to have AsthmaNet label sheets and reports printed and stored with the AsthmaNet Registry documentation.

Complete Registry procedures are documented in Section 9 of the AsthmaNet General Manual of Operations.

Visit 0
Complete Registry Checklist (REG_CHK)

Follow the procedures for completing the Registry Checklist (REG_CHK) as outlined in Section 9 of the AsthmaNet General Manual of Operations. Attach one of the participant's "Registry Checklist" labels to the gray box at the bottom of the checklist before submitting the form to the DCC. This label contains the participant's AsthmaNet master ID number and serves as a reference during the protocol enrollment process.

Include REG_CHK behind the Visit Procedure Checklist (P3_VISIT0) in the participant's Visit 0 packet.

2.39 Satisfaction Questionnaire

Participant's termination visit
Give participant AsthmaNet Satisfaction Questionnaire (SATQX) with preaddressed, postage-paid envelope

The AsthmaNet Satisfaction Questionnaire (SATQX) is a quality control tool that was developed by the AsthmaNet Quality Control Committee (QCC) to solicit feedback from participants when they leave AsthmaNet studies. The questionnaire is anonymous in that no participant or master ID number or other identifying information is recorded on the form. In addition, the participant returns the form directly to the DCC in a pre-addressed, postage-paid envelope. Performance site staff does not review the data on the form, does not see individual results, and does not data enter the information on the form. Data entry takes place solely at the DCC.

The Satisfaction Questionnaire (SATQX) is posted on the secure AsthmaNet website in the visit packet corresponding to the final study visit for a given protocol. For Microbiome, it is present in the Asthmatic Visit 6 packet and the Control Visit 2 packet. In addition, the questionnaire is also posted appended to the single Microbiome Termination of Study Participation (P3_TERM_A or P3_TERM_C) form for use with participants who terminate from the study before Visit 6 (for asthmatics) or before Visit 2 (for controls).

Postage-paid envelopes that are pre-addressed to the DCC may be obtained from the DCC as supplies are needed. At least one month's lead time should be allowed for shipment and receipt of the envelopes to ensure an adequate supply at the performance site at all times.

Only Microbiome participants who successfully complete Visit 0 should be given a questionnaire at the time of their study termination.

Process: The following steps should be carried out to ensure that all participants who terminate from the Microbiome trial have an equal opportunity to provide feedback on their experiences.

1. Distribute a copy of the questionnaire to any participant who successfully completes Visit 0, then terminates, whether he/she completes the study or terminates early (for his/her own reasons, due to ineligibility, or for other reasons).
2. Download the questionnaire from the secure AsthmaNet website along with the Microbiome Termination of Study Participation (P3_TERM_A or P3_TERM_C) form. Questionnaires in visit packets will have protocol number and site ID pre-completed in the key fields area of the form. Questionnaires appended to single P3_TERM_A or P3_TERM_C forms will have only protocol number completed.

Coordinators should complete the site number before distributing the questionnaire to a participant.

3. Print the questionnaire double-sided and staple the pages together to avoid loss.
4. Complete the participant's final study status in the gray box at the top of page 1 of the form. Individuals who terminate during the pre-randomization phases of the study should be coded as 'Run-in termination.'
5. Give the questionnaire to the participant at the conclusion of his/her final study visit. The participant should be given a pre-addressed, postage-paid envelope with the questionnaire.
6. Instruct the participant to complete the questionnaire, put it in the envelope, seal it, and place it in the US postal mail. If a participant elects to complete the questionnaire at the performance site, clinic personnel should not interact with him/her as the form is completed. In this case, it is preferable for the participant to drop the questionnaire in any postal box himself, but he/she may seal the questionnaire in the envelope and ask clinic personnel to mail it.

Note: If an individual is not present at the time he/she withdraws from the study, and he/she is unwilling to come to the performance site for a final visit, the Satisfaction Questionnaire should be mailed to his/her home address. Include instructions for completion with the questionnaire and prepaid envelope.

The DCC will provide periodic reports of the data from the questionnaire for the QCC and the coordinators/investigators to review. Response rates will be compared across the performance sites to ensure that all sites are participating fully in the survey process.

2.40 Significant Asthma Exacerbation (asthmatics only)

Visit 0-6
Complete Significant Asthma Exacerbation form (P3_SIGEX), if applicable

Definition
Asthma exacerbations are defined by an increase in symptoms of cough, chest tightness, and/or wheezing in association with one or more of the following criteria:

1. An increase in rescue albuterol of ≥ 8 inhalations/day over baseline use for a period of 48 hours or ≥ 16 actuations per 24 hours, with baseline defined as average daily use during the week prior to randomization

   This criterion is assessed through a review of the participant's Asthma Monitoring Log at a given visit.

2. FEV_1 < 50% predicted

   The baseline pre-bronchodilator FEV_1 % predicted value (in liters), Q1030 on the participant's Spirometry Testing (SPIRO) form, should be evaluated at each visit.

3. A fall in FEV1 to < 80% of baseline (visit 1)

   A participant will meet this criterion if he/she experiences pre-bronchodilator FEV_1 values that are < 80% of the Visit 1 baseline FEV_1

4. Receives systemic corticosteroids for an exacerbation

Documentation
When a participant experiences an asthma exacerbation during the Microbiome study, he/she should notify the performance site as soon as possible, preferably within 24 hours. Timely reporting ensures that the exacerbation is documented accurately and that the participant receives appropriate treatment. Once the significant asthma exacerbation has been confirmed, the following forms should be completed:

- Clinical Adverse Events (AECLIN)

  All significant asthma exacerbations should be documented on AECLIN using ICD-9 code 493.92.

  The start date recorded should correspond to the date exacerbation criteria were confirmed. For example, if a participant is deemed a non-responder after 48 hours of escalated albuterol treatment, the date corresponding to the second day of treatment should be recorded as the exacerbation date. If multiple criteria for asthma exacerbation are met, record the earliest date any of the applicable criteria were met.

- Concomitant Medications for Asthma/Allergy and Adverse Events (CMED)

  Any non-study medications used to treat the exacerbation event should be recorded on the CMED form. Examples include oral or parenteral corticosteroids (e.g., rescue prednisone) and nebulized beta-agonist administered in a doctor's office or at the performance site.

  Medications used for treatment of exacerbations and listed on the CMED form should be linked to the exacerbation adverse event recorded on the AECLIN form.

- Microbiome Significant Asthma Exacerbation (P3_SIGEX)

  P3_SIGEX must be completed any time the participant meets the criteria for an asthma exacerbation. This form is always treated as a single form.

  The exacerbation date is recorded in Q1060. It should correspond to the date exacerbation criteria were confirmed for the current event. If multiple criteria for exacerbation are met, record the earliest date any of the applicable criteria were met.

  If a participant meets asthma exacerbation criteria during the run-in (between Visit 0 and Visit 2), P3_SIGEX should be completed as a single form and data entered. Use the number of the last regular visit completed as the visit number on the form. In this situation, the participant is ineligible and must be terminated from further study participation.

  If a participant meets asthma exacerbation criteria during the post-randomization phase of the trial, P3_SIGEX should be completed as a single form and data entered using the visit number of the participant's most recently entered visit packet.

- Serious Adverse Event Reporting Form (SERIOUS)

  If a participant is hospitalized due to a significant asthma exacerbation event, or the event is considered to be life-threatening or meets other criteria in the definition of a serious adverse event (SAE), a SERIOUS form should be completed. SERIOUS forms should be submitted to the DCC within 72 hours of the notification of a SAE. See the Adverse Events discussion in this section for further details.

Significant Asthma Exacerbation Rescue Algorithm

Once an asthma exacerbation has occurred, the participant should contact the study coordinator and/or be evaluated at the performance site or the nearest medical emergency facility as quickly as possible.

Participants who are not responsive to the exacerbation rescue algorithm or who develop asthma exacerbations will be managed according to the following rescue algorithms. Rescue algorithms are based on recommendations from the NAEPP Guidelines for Diagnosis and Management of Asthma and prior ACRN trials.

Albuterol (study RESCUE Ventolin®) and oral prednisone are the principal medications for rescue management. At Visit 1, participants will be dispensed RESCUE Ventolin®. At Visit 2, randomized participants will be given a course of prednisone to keep at home for rescue use, only as directed by a study physician. Participants will be instructed in their use for home management. Oral prednisone will be used if increased albuterol therapy does not resolve the exacerbation. For severe acute episodes of asthma, treatment will be administered according to the best medical judgment of the treating physician.

Home Care

Asthma exacerbations will be recognized by an increase in albuterol (Ventolin®) use or symptoms. Participants will be educated to recognize exacerbations as early as possible to facilitate prompt treatment and to lessen morbidity.

Participants who recognize increased symptoms will use albuterol by MDI, 2-4 puffs, every 20 min up to 60-90 min if needed and then every 4 hours, or less, if needed. Participants will be instructed to use the "Rescue Ventolin®" inhaler for treatment.

If symptoms are not improved after the first 60-90 min of therapy, the participant should contact the investigator, their primary physician or seek care in the emergency department. Failure of albuterol may necessitate the use of oral steroids (see below).

Physician's Office or Emergency Room Treatment

Participants will be assessed by history, physical examination, and by physiological monitoring including spirometry or PEF. If the participant's PEF and/or FEV_1 are less than 25% of predicted or if the participant shows evidence of altered mental status, cyanosis, labored breathing, or use of accessory muscles, sampling of arterial blood for respiratory gas analysis is indicated, with appropriate action taken depending on the results obtained.

When treated in the physician's office or the hospital emergency department, participants should initially be given albuterol by nebulization (0.5 cc of 0.5% solution) every 20 min over the first 60-90 min.

If the PEF increases to >65% of baseline after the first 60-90 min, the participant can be discharged to continue treatment at home. Prednisone may be administered at the discretion of the physician to augment therapy.

If symptoms persist and PEF remains ≤65% of baseline, nebulized albuterol should be continued as often as every as every hour. Oral or parenteral corticosteroids should be considered (40 mg prednisone orally; methylprednisolone 40 mg iv bolus). Monitoring of

PEF or spirometry should continue every hour. Within 4 hours of treatment, a decision should be made regarding participant disposition.

If PEF increases to >65% of baseline within 4 hours, the participant can be discharged to continue treatment at home. Home treatment should include a 5-day course of prednisone (see below).

If PEF remains >40% but ≤65%, an individualized decision should be made to hospitalize the participant for more aggressive therapy or to continue therapy at home with a course of prednisone.

If PEF is ≤ 40% of baseline after repeated albuterol treatments, the participant should be admitted to the hospital unless in the physician's best judgment alternative treatment could suffice.

Prednisone Treatment

In this protocol, prednisone will be used when acute exacerbations occur, whether induced by bronchoscopy or another cause, and cannot be controlled by increased albuterol therapy alone.

The dose of prednisone used during an acute exacerbation shall consist of 40 mg as a single dose every day for 5 days. The decision to initiate or to continue a course of prednisone beyond 5 days is left to the discretion of the physician.

For safety reasons, all participants will be seen within 1 week of the date of an exacerbation, sooner if possible.

Study Participation Following an Asthma Exacerbation during Run-In

Participants experiencing an asthma exacerbation during the run-in period of the study are ineligible to continue in the trial. A Microbiome Termination of Study Participation (P3_TERM_A) form should be completed. See the discussion of Withdrawal Due to Exacerbation in the Withdrawal section for further details.

Study Participation Following an Asthma Exacerbation after Randomization

In rare cases, fiberoptic bronchoscopy may induce an asthma exacerbation. All cases of bronchoscopy-induced exacerbations will be treated with prednisone, 40mg po daily for 5 days. Should an exacerbation occur after bronchoscopy, a two-week recovery period will be imposed following the completion of prednisone therapy. Bronchoscopy will not be performed at Visit 5 if the participant experienced an exacerbation after bronchoscopy at Visit 2.

For exacerbations not induced by bronchoscopy, following clinical assessment and appropriate medical management, regular study visits will continue in accordance with the participant's visit schedule. However, if systemic corticosteroid treatment is required, a two week recovery period will be imposed. See below.

Recovery Period following Systemic Corticosteroid Treatment for Exacerbation after Visit 2

Should a participant receive systemic corticosteroid treatment for an asthma exacerbation after randomization at Visit 2, a two-week recovery period will be imposed following the completion of treatment. No visits should take place during treatment or this recovery period. Bronchoscopy will not be performed at Visit 5.

2.41 Sinonasal Questionnaire

Rhinitis and sinusitis are common in patients with asthma. These conditions represent a disease continuum referred to as sinonasal disease. Because sinonasal disease may lead to poorly controlled asthma, evaluation and documentation of this condition is important for the Microbiome trial.

The Sinonasal Questionnaire (SNQ)[9] is a simple five-item questionnaire that screens for chronic sinonasal disease. Participants are asked how often, on average, over the last 3 months, they have had each of five symptoms. The SNQ has been shown to be sensitive, specific and highly reproducible. The SNQ has been incorporated in an AsthmaNet-formatted form, the Sinonasal Questionnaire (SNQ) form. AsthmaNet received approval for use of the formatted form in Microbiome from Dr. Anne Dixon, one of the instrument's original authors.

Visit 1
Administer Sinonasal Questionnaire (SNQ)

The administration of the SNQ is one of the first procedures performed at the visit. This timing in the visit structure is intentional so that a participant's responses are not affected by other study procedures, such as spirometry. Study coordinators should observe the order of procedures as they are laid out on the visit procedure checklists to ensure that SNQ results are not biased by other study activities.

If a given visit has been partially completed and then rescheduled for a later date because of the participant's time constraints, a new SNQ form must be completed at the beginning of the rescheduled visit. Do **not** allow the participant to refer to or update his/her previously completed questionnaire. Old copies of the questionnaires should be filed in the participant's study folder and clearly marked as such; they should not be entered into the study database or forwarded to the DCC.

The SNQ is completed by the participant. When administering the questionnaire, request that the participant complete the entire 5-question form and provide answers as accurately as possible. Only one box should be checked for each question. No stated or implied time limit should be set. If the participant requests help with or clarification of any question, the study coordinator should instruct the participant to reread the instructions and to give the best answer possible to each question. The study coordinator should not provide an answer to any question. Providing guidance may bias the participant's responses.

Participants should use a black or blue pen to complete the questionnaire. If the participant wishes to change a response, the original response should be crossed out

[9] Dixon AE, Sugar EA, Zinreich SJ, Slavin RG, Corren J, Naclerio RM, Ishii M, Cohen RI, Brown ED, Wise RA, Irvin CG. Criteria to screen for chronic sinonasal disease. Chest 2009; 136:1324-1332.

with a single line and then dated and initialed by the participant. The final response should be circled for clarification. No changes to the participant-completed form may be made by study personnel; changes may only be made by the participant.

When the participant is finished with the questionnaire, collect it and review it for completeness before proceeding with the visit. If a question has been left blank, ask the participant to do his/her best to answer it. The answers to all of the questions are necessary to score the instrument. Check that the participant's responses are clearly marked.

The participant should provide source documentation on the SNQ form by writing his/her initials and the date/time in the source documentation box. Review the source documentation provided by the participant to ensure that the date and time are accurate before collecting the form.

2.42 Spirometry

Spirometry procedures are carried out at all Microbiome visits, with exception to Visit 0. Pulmonary function data are very important, as they confirm the participant's eligibility for the study and provide data for assessment of significant asthma exacerbation criteria.

General Instructions
The Microbiome trial utilizes the MedGraphics spirometry system. The Spirometry Manual of Operations is located in Appendix 1 of the AsthmaNet General Manual of Operations.

Individuals performing spirometry must be AsthmaNet-certified in pulmonary function testing or, at a minimum, observed and supervised by an AsthmaNet-certified technician. If an uncertified individual is performing any spirometry procedures at a visit, a supervisor ID must be recorded on the applicable form(s), including the Spirometry Testing (SPIRO) form and Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form, as applicable at a given visit.

A participant's prior spirometry results should not be reviewed with him/her at the current visit. Knowledge of past test results can influence current expectations and bias the resulting data.

In general, before a participant can proceed with spirometry testing, he/she must meet all of the medication and substance holds specified on the Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK). If a participant has taken any of the listed substances within the specified washout period prior to a visit, he/she generally may not proceed with spirometry testing at the visit. In this case the visit should be rescheduled within the visit window for appropriate washouts to be met.

At Visits 4 and 5, additional washouts are required. If a participant had a respiratory tract infection in the 4 weeks prior to the visit, the visit should be rescheduled 4 weeks from the onset of symptoms. Likewise, if the participant has taken any antibiotic (except for topical) in the 4 weeks prior to the visit, the visit should be rescheduled 4 weeks from the last day of antibiotic use.

Participants who are less than 21 years old (i.e., participants who have not yet had their 21st birthday) will have their height measured and recorded at each visit until they turn 21. Heights for these individuals will be recorded on the P3_PULMONARYCHK form at all visits, with the exception of Visits 1 and 6 when height is recorded for all participants on the Adult Body Measurements (BODYMEAS_ADULT) form. The height value for participants who are under age 21 should be updated in the spirometry software for all sessions. Participants who are at least 21 years old will have their height measured and updated in the spirometry system only twice during the study (at Visit 1 and Visit 6). Once a participant is over the age of 21, he/she should not be re-measured until Visit 6.

The participant's spirometry race/ethnicity designation and gender should be retrieved from his/her AsthmaNet Registry Report. The participant's spirometry race/ethnicity category corresponds to the primary racial designation that he/she supplied in Q1150 on the Registry (REGISTRY) form. Individuals who specified 'American Indian/Alaskan Native' or 'Other' will use Caucasian predicted lung function equations. Always use the spirometry race/ethnicity designation listed on the participant's Registry report in the MedGraphics software. Both race and gender have a large influence on a participant's predicted lung function values.

Individuals who are transgendered or transitioning to the opposite gender should have their biological sex entered into the AsthmaNet Registry (under 'gender'). Biological sex should be entered into the MedGraphics software for purposes of calculating predicted lung function values.

Care must be taken to enter the participant's identification (i.e., participant ID number (e.g., 03100222), initials, etc.) and demographic information into the spirometry software correctly. A technician ID must also be included for each test that is performed. Failure to provide complete and accurate information in the MedGraphics system may result in the assignment of a protocol deviation.

Visit 1, 6 (Visit 6 asthmatics only)
Complete Adult Body Measurement (BODYMEAS_ADULT) form

Visits 1-6
Complete Pulmonary Procedure Checklist (P3_PULMONARYCHK)
Perform Spirometry Testing (SPIRO)

Baseline spirometry at Visit 1 is used to determine study eligibility. These results are recorded on the Spirometry Testing (SPIRO) form and are referenced on Microbiome Eligibility Checklists (P3_ELIG2A, P3_ELIG2C) at this visit.

At Visit 1, asthmatic participants must have an FEV1 that is ≥ 55% predicted to be eligible. Control participants must have an FEV_1 ≥ 80% predicted and FVC ≥ 80% predicted to be eligible. To calculate FVC % predicted, locate FVC and FVC Predicted on the spirometry report (under "FVC absolute" column) and calculate as follows: $\frac{FVC}{FVC\ Predicted} \times 100$.

Baseline spirometry at Visit 1 is used to qualify the participant for methacholine challenge at these visits. If the participant's baseline FEV_1 ≥ 55% of predicted and baseline FEV_1 ≥ 1.0 liter, and all other criteria on the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT) are met, then he/she is eligible to proceed with the challenge at the visit.

At Visit 2, control participants with an FEV_1 < 80% predicted are ineligible.

Visit 1, 2, 5 (Visit 5 asthmatics only)

Administer 4 puffs of albuterol, wait 10-15 minutes, and perform post-bronchodilator testing

Complete Post-Albuterol (4 puffs) Spirometry Testing form (PALB4_SPIRO)

Asthmatic participants who do not meet PC_{20} eligibility requirements ($PC_{20} \leq 8$ mg/ml) at Visit 1 will stop Visit 1 following methacholine challenge and be scheduled for a continuation visit. Continuation visit should be scheduled within 24-48 hours. During the continuation visit, spirometry will be performed and participants will be given 4 puffs of albuterol and allowed to rest for **10-15 minutes**. After the 10-15-minute wait, spirometry should be repeated and the results recorded on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form. If participant reverses ≥ 12%, he/she is eligible to continue with Visit 1. If not, the participant is ineligible for the Microbiome study.

At this continuation visit, new Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK) and Spirometry Testing (SPIRO) forms must be completed with the current date, as well as a Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form (using the same visit number as the prior visit). The P3_PULMONARYCHK, SPIRO, and PALB4_SPIRO forms from the continuation visit should be entered into the Microbiome database as single forms (in addition to the regular packet forms). A single PALB4_RPT should be printed to document the values on the single SPIRO and PALB4_RPT forms. All data collected on the P3_PULMONARYCHK and SPIRO forms for both parts of the visit should be entered into the study database.

Note: When the participant returns for the continuation visit, the first procedure performed is spirometry. Do not have the participant redo previously completed questionnaires at this visit; the questionnaires completed on the original visit date will be submitted with the visit packet.

At Visit 2 (and Visit 5 for randomized asthmatics), participants will be given 4 puffs of albuterol and allowed to rest for **10-15 minutes**. After the 10-15-minute wait, spirometry should be repeated and the results recorded on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form. Control participants who reverse ≥ 12% are ineligible to continue; a Termination of Study Participation (P3_TERM_C) form should be completed. Asthmatic participants with a postbronchodilator $FEV_1 < 70\%$ predicted are ineligible to proceed with bronchoscopy; a Termination of Study Participation (P3_TERM_A) form should be completed.

Asthmatic participants performing reversibility at Visit 1 and control participants performing reversibility at Visit 2 should dose from albuterol (Ventolin®) inhalers taken from bulk supply (log on Microbiome Drug Dispensing Log: Ventolin® (RESCUE) Inhaler (P3_DRG_RESC). Actuators should be sterilized between participants, allowing for multiple participant use.

At Visit 2, asthmatic participants should dose from their albuterol (RESCUE Ventolin®) inhalers for this test. Albuterol puffs taken as part of this visit procedure should not be included in the RESCUE puffs the participant records in his/her Asthma Monitoring Log (P3_ASTHMA_LOG) the evening after the visit.

2.43 Sputum Induction

Visit 1, 4 (Visit 4 asthmatics only)
Perform tongue scraping and oral saline rinse
Complete Sputum Induction Checklist (SPUTUMCHK)
Perform Sputum Induction (SPUTUM)
Complete Additional Treatment Post Sputum Induction (SPUTUM_ADD_TRT), if needed
Enter sputum sample data into Biological Sample Tracking module

Sputum induction provides important secondary outcome variables for the Microbiome study. The Sputum Induction Manual of Operations is located in Appendix 7 of the AsthmaNet General Manual of Operations. Individuals performing sputum induction must be AsthmaNet-certified in this procedure.

Pre-sputum induction spirometry
Individuals who complete the methacholine challenge at Visit 1 and Visit 4 should be reversed with 4 puffs of albuterol as the standard reversal to be qualified for sputum induction at the visit. Results of standard reversal are recorded on the Methacholine Challenge Testing (METHA) form. If the participant requires additional treatment to reverse to ≥ 90% of his/her baseline (pre-challenge) FEV_1 value, these results are recorded on the Additional Treatment Post Methacholine Challenge Testing (METHA_ADD_TRT) form. The final FEV_1 attained after all post-challenge treatment should be used to qualify the participant for sputum induction.

Note that the Methacholine Report obtained through the MedGraphics system does not include FEV_1 % predicted values which are needed to assess the participant for the minimum 50% of predicted value required to proceed with sputum induction at the visit. To compute FEV_1 % predicted values, locate the FEV_1 value (in liters) from the final reversal treatment and divide it by the predicted FEV_1 value (in liters) from the top of the 'FEV1 absolute' column on the Methacholine Report. Multiply the result by 100% and round to the nearest %.

Individuals who do not complete the methacholine challenge at Visit 4 and who are eligible to qualify for sputum induction at the visit (as described above) must undergo reversal with 4 puffs of albuterol to be assessed for procedure eligibility. Results of the reversal testing are recorded on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form. The Spirometry Report generated through the MedGraphics system (Pre/Post report) provides the % predicted value needed to complete the Sputum Induction Checklist (SPUTUMCHK) form and assess the participant for eligibility for the sputum induction procedure.

The FEV_1 value (in liters) after final reversal prior to sputum induction is recorded in Q1030 and the corresponding % predicted value in Q1040 on the SPUTUMCHK form. The % predicted value must be at least 50% for the participant to continue with sputum

induction at the visit.

Tongue scraping and oral saline rinse
Prior to sputum induction, tongue scraping and oral saline rinse will be performed. The purpose is to decrease contamination of the collected sputum by oral-associated microbial flora. This oral specimen does not need to be saved and can be discarded. See Tongue scraping and Oral saline rinse in this section for procedural details.

Sputum processing criteria
In order for the resulting sputum sample to be processed, its volume must be deemed adequate for processing by the technician processing the sputum induction and the duration of the procedure (not including spirometry maneuvers) must be at least 4 minutes. No minimum volume is required for processing.

If the duration of the procedure was less than 4 minutes, the sample must not be processed. No exceptions are allowed.

Sputum Processing
The processing of induced sputum to make sputum slides, pellets, and supernatant is explained in the Sputum Induction Manual of Operations; however, additional steps are necessary for the Microbiome study as indicated below. Samples MUST be processed immediately in order to ensure that the slides are of acceptable quality.

For the Microbiome study, given the sensitivity of the molecular methods to be used to characterize the microbiome, it is important to implement measures, where feasible, to limit the potential for sample contamination from environmental sources. The following measures are strongly recommended, recognizing that site-specific facilities and practices can vary:

- Wipe workbench surfaces and pipettes with 70% ethanol prior to use in processing sputum. Avoid the use of absorbent pads on countertops as they can serve as sources of contamination, particularly if they are not regularly changed. If they must be used, replace with a new pad prior to sputum processing.
- Avoid concurrent processing of other specimens in the immediate area.
- If a certified biosafety hood is available in the vicinity, it is most ideal to carry out steps of the sputum processing that are feasible under the hood (e.g. transferring sputum into tubes, pipetting solutions, etc.). It is recognized that larger equipment needed for the processing may need to remain outside of the hood (e.g. water baths).
- Observe good standard laboratory practices and techniques to minimize inadvertent contamination of pipet tips, tubes, etc.

Also note:

- Only sterile PBS should be used for processing.

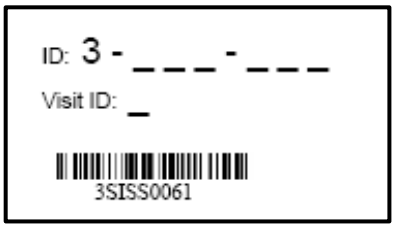
- While performing cell counts and making slides, store remaining sputum sample on ice or at 4°C.

Additional Samples for Microbiome

After Sputolysin-treated sputum (SS) sample has been in 37°C shaking water bath for 15 minutes and before proceeding with cell count, if at least 3 mL of induced sputum was collected (therefore, there is at least 6 mL volume after addition of Sputolysin), take a total of three 0.5 mL aliquots of the Sputolysin-treated sputum (SS) and preserve as follows:

- Add 0.5 mL of SS into each of 2 tubes containing 70% glycerol (provided by UCSF);
- Add 0.5 mL of SS into an empty, sterile microcentrifuge tube (same type tube used for supernatant);
- After labeling, immediately freeze all three aliquots at -80°C before proceeding to next steps in Appendix 7.6.2 (Cell Count).

Label the SS tubes with barcode labels (Cryo-Tag 1.50 x 0.75") generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system. Labels should be placed vertically on tube so that the barcode can be scanned. The length of the label is 1.5" so the label should be placed as high as possible (just under the screw top). For the SS with glycerol sample, place a barcode label that starts with "3SISSG" on it. The sample type associated with this tube in the BST module is "MCBM Sputum Ind SS (Glycerol)". For the SS without glycerol sample, place a barcode label that starts with "3SISS" on it. The sample type associated with this tube in the BST module is "MCBM Sputum Ind SS". The participant's Microbiome ID and visit number should be written in the space provided on the label with an alcohol-proof permanent Sharpie marker. Using an alcohol-proof permanent marker, write the last three digits of the participant's ID and visit number (i.e. V1 or V4) on the caps of the tubes. Sample Microbiome SS with Glycerol and SS without Glycerol barcode labels follow:



Samples should be added to the Biological Sample Tracking (BST) module along with the other sputum samples.

Storage

As indicated below, Microbiome sputum supernatant, pellet and SS samples (with and without glycerol) will be shipped to UCSF after three subjects have completed the

Microbiome study. As a result, sputum samples should be stored by participant rather than by visit as indicated in the AsthmaNet Sputum MOP.

Supernatant and SS samples will be shipped in the same box, so they can be stored in the same box. To help with sorting samples in UCSF, arrange SS samples together in the box.

The first box designated for Microbioime should be labeled as box # 1. When the first box provides storage for three participants, the next box designated for Microbiome should be labeled as box # 2, etc. Each box should have the following information written with permanent Sharpie pen in two places, one on the side of the lower part of the box and one on the lid: study name, sample type (SI-Supernatant & SS), participant ID, city and site name, and box number. For example, if the supernatant was collected at Boston at Brigham & Women's site for the Microbiome study and this is the first box, containing samples for 3-111-001, 3-111-002, and 3-111-003, the information on the box should be as follows:

Microbiome – SI-Supernatant & SS
Subjects – 001, 002, 003
Boston – Brigham & Women's
Box # 1

Sputum shipments to San Francisco

Slides

For the Microbiome trial, all accumulated Visit 1 and Visit 4 sputum slides should be shipped to San Francisco the first Tuesday of each month for overnight receipt. The scheduled shipment dates follow:

2012 October 2
November 6
December 4

2013 January 2*
February 5
March 5
April 2
May 7
June 4
July 2
August 6
September 3
October 1

* Wednesday

If a performance site has a conflict with a particular shipment date, arrangements should be made with San Francisco lab staff to ship the slides on an alternate date; slides should not be held until the next month's shipment date.

Supernatant, pellets, SS and SS in glycerol

For the Microbiome trial, sputum supernatant, pellets, and SS samples (with and without glycerol) will be packed and shipped with bronchoscopy samples to UCSF. This will occur after every 3 subjects have completed the Microbiome study at the site. Sputum pellets should be packaged in a separate Ziploc bag and sputum supernatant and SS samples should be packaged in a separate box. Further shipment details are provided in Section I.9.3 of the Bronchoscopy Manual of Procedures.

2.44 Stool Sample

Visit 1
Distribute stool collection kit and instructions (P3_HSSC)

Stool collection kits will be assembled by UCSF and mailed to the clinical sites. The kits will include:

- Stool collection device
- Stool collection tube
- Ice pack
- Cold shield bubble mailer
- White mailer envelope
- Specimen (biohazard) bag with absorbent material

Important: Absorbent material must be placed inside the specimen biohazard bag. This was not provided by UCSF, and must be purchased and placed in the biohazard bag prior to giving stool kit to participant.

Item	Vendor	Catalog #	# Per Collection
3" X 4" Absorbent Sheet	Fisher Sci	22-130-039	1

Kit and Home Stool Sample Collection (P3_HSSC) handout should be given to participant at Visit 1. Before giving kit to participant at Visit 1, be sure to:

- Purchase and place pre-paid stamp on the white mailer envelope as instructed below;
- Obtain and complete Express Mail label as instructed below, and place in participant's kit;
- On the stool collection tube, cross off name and write the participant's Microbiome ID number on that line using a permanent Sharpie marker, and cross off DOB as well.

The stool sample sought for this research study is a sample from the first bowel movement after waking in the morning. Ideally, the sample should be collected the morning of the Visit 2 bronchoscopy and brought to the clinic. If the participant does not have a bowel movement before coming to the visit, the sample can be collected after arrival, or even after the bronchoscopy, as long as it is collected from the first bowel movement of the day. If the participant does not have a bowel movement the day of bronchoscopy, he/she can collect a sample from the first bowel movement of the following Monday, Tuesday, or Wednesday and mail it to the clinic in the pre-paid bubble-lined mailer provided.

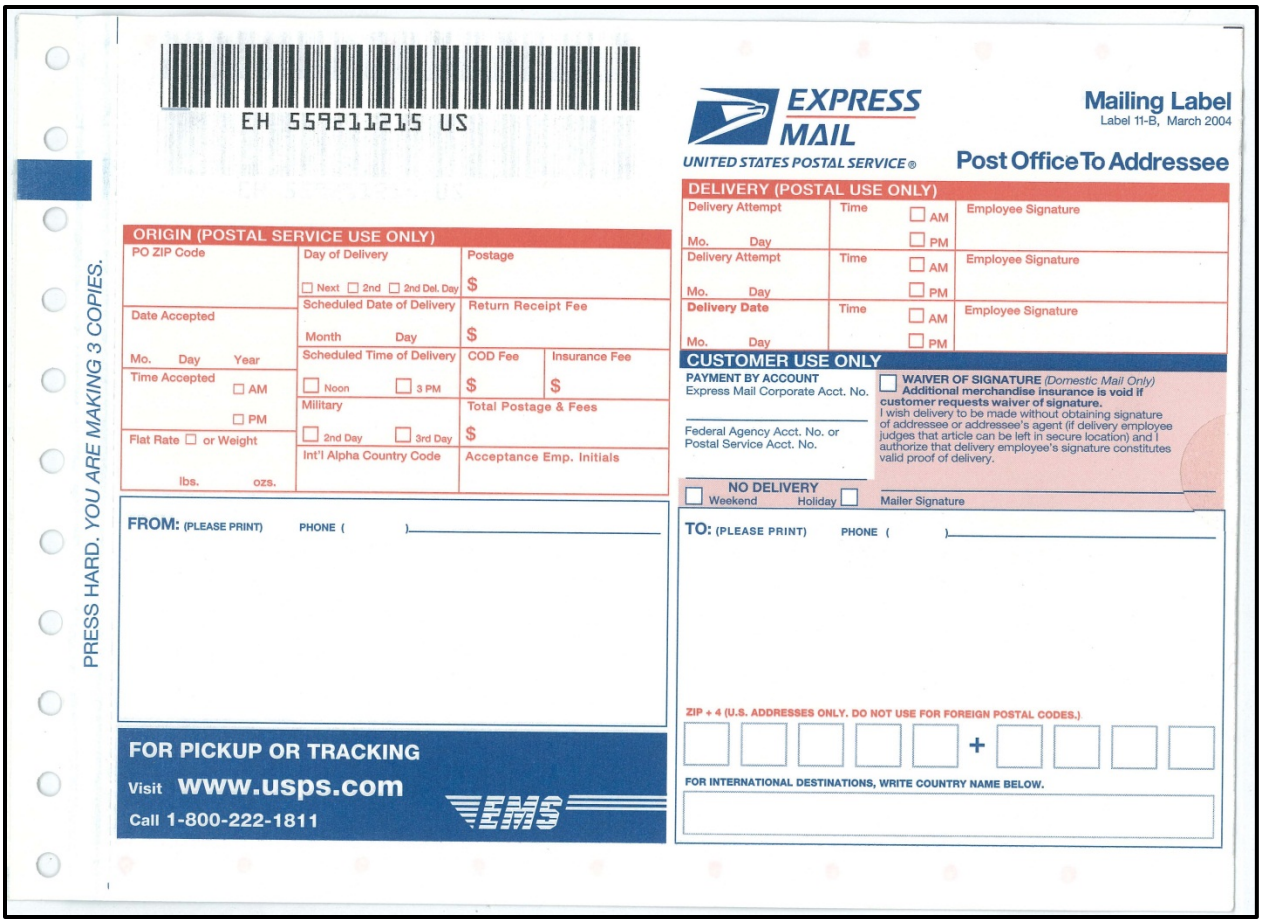
Purchasing Stamp for Mailer Envelope

For stool samples being shipped from participant's home to clinic, USPS Express Mail® will be used. Cost to ship is based on sender zip code and recipient zip code. To check cost to ship in order to pre-pay for shipping:

1. Go to http://postcalc.usps.com.
2. Enter the zip code of the participant in the "From ZIP Code" and the zip code of the clinic in the "To ZIP Code." (Unless the participants are far from the clinic, all should cost $16.05 to ship.)
3. Use Select a Shape and select "Large Envelope."
4. For weight, enter 1 Pounds 0 Ounces and click "Continue."
5. The first row in the table should list "Express Mail®"; this is the amount it will cost to ship from recipient's home to clinic using Express Mail® and the amount you should pay to stamp the bubble mailer. Purchase stamp in the given amount from your mailing department.
6. Before attaching stamp, place a blank address label in the upper-right corner of the bubble mailer. Attach stamp to white bubble mailer over top of the blank label in the upper-right corner. (Mailing label is placed overtop blank label to prevent mailing label from falling off.)

Express Mail® Label

Obtain an Express Mail® address label (on following page) and print the clinic address in the "TO:" box. To protect participant confidentiality, print the clinic address in the "FROM:" box as well **and include the participant ID to whom the sample collection kit is being given**.



Pull out Customer Copy for reference in tracking shipment, if necessary. Place mailing label in kit; participant will place on bubble mailer if he/she does not produce a stool sample the day of bronchoscopy and must ship stool sample after Visit 2.

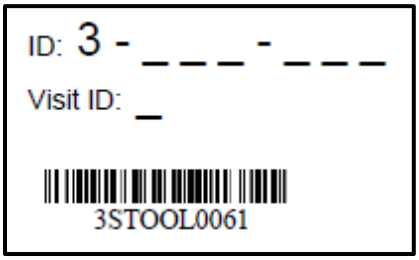
Visit 2
Collect stool sample
Enter stool sample data into Biological Sample Tracking module

Item	Vendor	Catalog #	# Per Collection
White Laser Cryo-Tags barcode label (Cryo-Tags 1.5”x0.75”)	Diversified Biotech	LCRY-1200	1

Upon receipt at the clinic, remove stool collection tube from bubble mailer(s).

Complete an entry for the stool sample on the Microbiome Stool Sample Log (P3_STOOL_SAMP_LOG). Complete the participant's Microbiome ID number, visit number, collection date and date of sample receipt at clinic.

Remove stool collection tube from biohazard bag. Label the stool collection vial with a barcode label (Cryo-Tag 1.50 x 0.75") generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system. The barcode label starts with "3STOOL", representing the sample type (3 for Microbiome protocol and STOOL for stool sample), and is followed by a 4 digit number. The sample type associated with the serum tubes in the BST module is "MCBM Stool." The participant's Microbiome ID number should be written in the space provided on the label, as well as the date of stool sample collection (as provided by participant on stool collection tube). A sample Microbiome stool sample barcode label follows:



Immediately after labeling the stool collection tube, access the BST module and scan the barcodes to insert records for the samples. Input the participant ID information to link the barcodes to the correct Microbiome participant. For details on accessing and interacting with the BST Module in the AsthmaNet Database Application, see the AsthmaNet Computing and Networking Environment details in Section 7 of the AsthmaNet General Manual of Operations.

Store the stool sample at -80° C until the shipment day.

Stool Shipments

Stool samples should be shipped to the Lynch Lab in two batches, the first when half the participants have been enrolled and the second after the final participant has finished the study. Samples should be shipped priority overnight on Monday through Thursday only.

Preparing Samples for Shipment to Lynch Lab

A few days prior to shipment, e-mail the Lynch Lab to notify them of the shipment:

Juliana.Durack@ucsf.edu
Susan.Lynch@ucsf.edu

To create a shipment, scan the barcodes for all samples available to ship into the AsthmaNet BST system. Include a shipment comment detailing the contents of the shipment (i.e., human stool). Each shipment (from each site) will receive a unique shipment ID number. A shipment inventory will be generated that contains: date of

shipment, tracking number, site of origination, shipment ID, and an inventory detailing all the tubes in the shipment with their barcode numbers and participant information (study ID number, initials, visit number and blood draw date). Print the shipment inventory for inclusion in the shipment.

Once the shipment is confirmed in the BST module, an e-mail will automatically be sent to the Lynch lab. The e-mail will include an export file from the database that shows the information from the shipment inventory. A summary of the shipment will be included in the body of the e-mail message.

Packaging Samples for Shipment to Lynch Lab
Before packaging available samples for shipment, they must be scanned into the BST system and an inventory of the shipment generated as described above. After the samples have been scanned and the shipment has been confirmed by the performance site, the samples should be packaged for shipment. The following materials are required:

Item	Vendor	Catalog #	# Per Shipment
Insulated Shipper Container (e.g. ThermoSafe)	Fisher Sci.		
Dry Ice			
Biohazard specimen bags 6”x10” (Fisherbrand)	Fisher Sci.	19-075-388D	2
Ziploc bag			
Packaging tape	Staples	380107	
Exempt Human Specimen labels Therapak 2.5”x2”	Fisher Sci.	22-130-070	1
Dry Ice labels (DRY ICE – UN 1845)	Air Sea Containers http://www.airseacontainers.com 1-866-272-9880	Dry Ice UN 1845 Label, Roll of 500 (No product number)	1
Shipment inventory from BST			1

Assembly instructions:

1. Place about 10 lbs. dry ice in the Styrofoam container.
2. Place the stool samples in a biohazard bag and seal bag.

3. Securely tape the bag closed with packaging tape, and pack in the Styrofoam container.
4. Place the shipment inventory in a Ziploc bag and insert into container.
5. Seal the shipping container.
6. Attach one large “Exempt Human Specimen” sticker to the shipping container.
7. Attach dry ice label “DRY ICE – UN 1845” to the container. Mark the approximate weight of dry ice in kg for each shipment.
8. Address the shipment to:

   Juliana Durack
   UCSF
   513 Parnassus Ave, S-363
   San Francisco, CA 94143

   Phone: (415) 476-6423
   Fax: (415) 476-0659

9. Specify priority overnight shipment for AM receipt.

Supplies Provided to Sites

Stool collection kits are arranged by San Francisco site. Inform Kelly Norsworthy (kelly.norsworthy@ucsf.edu) and Kelsey Wollen (kelsey.wollen@ucsf.edu) if your supplies are getting low and allow at least 2 weeks for delivery.

2.45 Study Handout Folder

At the end of Visit 1, asthmatic Microbiome study participants will be given several handouts related to study procedures. Each handout contributes to increased adherence in areas such as dosing with study medications, and monitoring for significant exacerbation. Participants should be given an AsthmaNet folder to use for carrying and storing the handouts. The participant should store the study folder in a convenient location, as it will serve as a reference throughout his/her Microbiome participation. The folder should be brought to each study visit so that clinical personnel can review and/or update handouts, as necessary.

Microbiome Study Handout Folder Contents – Asthmatics

- Microbiome Daily Activities (P3_DAILYACT1) – distributed at Visit 2
- Microbiome Daily Activities (P3_DAILYACT2) – distributed at Visit 5

"Daily Activities" handouts contain simple summaries of the activities the participant should carry out each day during the Microbiome study. Other handouts provide details on the execution of these activities. The "Daily Activities" handouts also list the participant's high rescue inhaler use reference values. They prompt the participant to contact clinical personnel when they may be experiencing a asthma exacerbation. See the Daily Activities Handout discussion in this section for further details.

- If Your Asthma Gets Worse (P3_ASWORSE)
- Participant Identification Card (P3_ID)

These references facilitate the identification and treatment of asthma exacerbations according to the protocol, both by the participant and by healthcare providers. The P3_ASWORSE handout is introduced at Visit 1 and reviewed at subsequent visits, as needed. P3_ID is introduced at Visit 2. The ID card contains reference values for defining exacerbations and instructions for emergency treatment. It should be carried in the participant's wallet so that it is available at all times. See the Participant Identification Card, and Significant Asthma Exacerbation discussions in this section for further details.

- How to Use Your Metered Dose Inhaler (HTMDI)

This is a standard handout that provides information on MDI closed-mouth inhalation technique and instructions for cleaning the inhaler. It is introduced at Visit 1.

- How to Use Your Diskus Inhaler (HTDISKUS)

This is a standard handout that provides information on how to use the Diskus and Diskus closed-mouth inhalation technique. It is introduced at Visit 2.

- Home Stool Sample Collection Instructions (P3_HSSC)

This handout instructs the participant on how to perform home stool sample collection and how to ship to the clinical center. It is handed out at Visit 1.

- Microbiome Visit Preparation Checklist (P3_VISPRP)

This handout is a tool for improving the participant's adherence with respect to keeping scheduled visits and preparing for the visits appropriately. The handout should be photocopied/printed two-sided. The P3_VISPRP handout includes a checklist on one side that itemizes the medications and other study materials the participant should bring to each visit. The participant should check off each item as he/she prepares for each visit to ensure that nothing is overlooked. If clinical personnel notice that the participant is not using the checklist, and he/she is not always prepared for visits, use of the checklist should be reinforced. This handout is introduced at Visit 1.

- Microbiome Visit Scheduler Report

A copy of the current Visit Scheduler Report should be included in the participant's handout folder for personal reference. Old versions should be discarded to avoid confusion. See the Visit Schedule discussion in this section for further details.

Microbiome Study Handouts – Controls

- Microbiome Visit Preparation Checklist (P3_VISPRP)

This handout is a tool for improving the participant's adherence with preparing for the visits appropriately. This handout is introduced at Visit 1.

- Microbiome Visit Scheduler Report

A copy of the current Visit Scheduler Report should be given to the participant for personal reference. See the Visit Schedule discussion in this section for further details.

2.46 Study Medications (asthmatics only)

During the Microbiome trial, participants will receive the following study medications:

- fluticasone DPI (Flovent® Diskus; 250 mcg per puff), an inhaled corticosteroid (ICS), or its placebo to be used 1 puff BID every day through the treatment period (Visits 2-5).

  fluticasone DPI for randomization period is labeled 'Flovent 250 mcg/puff or Placebo 60 count Diskus Inhaler' throughout the trial. It is an orange Diskus. Labels for these inhalers are white.

- fluticasone DPI (Flovent® Diskus; 250 mcg per puff), an inhaled corticosteroid (ICS), to be used 1 puff BID every day between Visits 5 and 6.

  fluticasone DPI for open-label post-V5 period is labeled 'Flovent 250 mcg/puff 60 count Diskus Inhaler'. It is an orange Diskus. Labels for these inhalers are blue. This is an open-label medication that will be dispensed from bulk supplies provided by the DCC.

- albuterol rescue drug (Ventolin®), an inhaled beta-agonist to be used as-needed throughout the Microbiome trial, starting at Visit 1, to treat asthma symptoms.

  Ventolin® rescue drug will be labeled 'Albuterol (RESCUE) Inhaler.' It is supplied with a blue plastic actuator with a blue strapcap. Labels for these inhalers are red. Ventolin® will be dispensed from bulk supplies provided by the DCC.

- rescue prednisone, an oral steroid to be used only in emergencies and under the direction of clinical staff to treat an asthma exacerbation. Rescue prednisone will be obtained through the individual performance site pharmacies and dispensed to each participant following randomization at Visit 2. See the Significant Asthma Exacerbation discussion in this section for details.

Procedures related to drug dispensation and participant education on use of these medications are given under the appropriate visits below. See Section 5 of this manual for detailed drug-related instructions.

Visit 1
Log/dispense Ventolin® (RESCUE) inhaler (P3_DRG_RESC)

Visits 2-5
Log/dispense Ventolin® (RESCUE) inhaler, if needed (P3_DRG_RESC)

Every participant should receive a new albuterol rescue inhaler (labeled 'Ventolin (RESCUE) Inhaler') at Visit 1. At all other visits through Visit 5, the participant's rescue use should be reviewed and a new inhaler dispensed, if necessary. The participant

must be assured an adequate supply of rescue medication throughout the trial. When dispensing a rescue inhaler to a participant, the next available row on the Microbiome Drug Dispensing Log: Ventolin® (RESCUE) Inhaler (P3_DRG_RESC) should be completed by the person dispensing the medication. See Sections 4 and 5 for details on completion of this log.

Visit 1
Instruct participant on use of Ventolin® (RESCUE) inhaler (HTMDI, P3_ASWORSE)

Review the closed-mouth inhalation technique described in the "How to Use Your Metered Dose Inhaler (MDI)" (HTMDI) handout and demonstrate the method. The participant will have an opportunity to practice his/her inhalation technique with a placebo inhaler at this visit. Clinical staff will assess the participant's technique via the MDI Inhalation Technique Checklist (Without Spacer) (TECH_MDI_NOSP). See the Inhalation Technique Assessment discussion in this section for further details.

Show the participant the label on the albuterol inhaler and explain that the RESCUE inhaler is to be used on an as-needed basis whenever he/she experiences asthma symptoms and needs relief. Emphasize that the albuterol RESCUE inhaler should be carried with the participant at all times and that he/she should not use other inhalers for rescue medication.

Review the "If Your Asthma Gets Worse" (P3_ASWORSE) handout with the participant. Ensure that the participant knows his/her reference rescue use value and how to identify a potential exacerbation event. Ensure that he/she understands how to initiate treatment and how to contact the clinical site in an emergency. Further details regarding exacerbations can be found in the Significant Asthma Exacerbation discussion in this section.

Visit 2-6 and any time a Ventolin® inhaler is collected
Collect/log Ventolin® (RESCUE) inhaler (P3_DRG_RESC)

Used rescue inhalers must be collected and accounted for. When collecting a rescue inhaler from a participant, its row on the Microbiome Drug Dispensing Log: Ventolin® (RESCUE) Inhaler (P3_DRG_RESC) should be identified and updated by the person collecting the medication. See Sections 4 and 5 for details on completion of this log.

Visit 2
Randomize participant, if eligible
Log drug assignment (P3_LOG)
Log/Dispense study medication (P3_DRG_SCH, P3_DRG_SCH_ALL)
Confirm medication dispensation (P3_MED)

Eligible asthmatic participants are randomized at Visit 2. The Microbiome Randomization Module presents the participant's assigned Diskus inhaler number. This number is logged on the Microbiome Drug Dispensing Log: Post-Randomization Study

Medication (P3_DRG_SCH) and on the Participant Assignment Log (P3_LOG). The person dispensing the Diskus inhaler should complete this information. See the Randomization discussion in this section and Section 3 of this manual for details on the Randomization Module.

Each time a post-randomization Diskus is dispensed, a Microbiome Scheduled Medication (P3_MED) form must be completed to confirm the dispensation in the Microbiome database. See Sections 4 and 5 of this manual for details on the completion of this form and related logs (P3_DRG_SCH).

Visit 2
Instruct participant on use of Diskus inhaler (HTDISKUS, P3_DAILYACT1)

Review the "Microbiome Daily Activities (Visit 2)" (P3_DAILYACT1) handout with the participant. This handout covers dosing with the Diskus inhaler that should take place twice a day, every day, until Visit 5.

Visit 2
Log/dispense rescue prednisone (P3_DRG_PRED)

At some point during the study, the participant may experience a significant asthma exacerbation and may require therapy in addition to his/her albuterol [Ventolin® (RESCUE)] inhaler. Prednisone will be used when, in the judgment of the investigator, an acute exacerbation cannot be controlled by albuterol. Prednisone will be used at the investigator's discretion and under his/her direction only.

To ensure prompt treatment should an exacerbation occur at home, each participant will be given one 5-day course of oral prednisone to store at home. At Visit 2, clinical personnel should dispense a course consisting of 40 mg as a single dose every day for 5 days. This medication should be dispensed in childproof packaging.

Prednisone should be obtained from the local clinical pharmacy for dispensation as a rescue medication; it will not be supplied by AsthmaNet. Proper labeling is the responsibility of the performance site.

The decision to initiate or to continue a course a prednisone beyond 5 days is left to the discretion of the investigator.

When dispensing rescue prednisone to a participant at Visit 2, the next available row on the Microbiome Drug Dispensing Log: Rescue Prednisone Tablets (P3_DRG_ PRED) should be completed by the person dispensing the medication. See Sections 4 and 5 for details on completion of this log.

Visit 3-4
Generate new drug assignment number via Randomization Module
Log drug assignment (P3_LOG)

Log/dispense study medication (P3_DRG_SCH, P3_DRG_SCH_ALL)
Confirm medication dispensation (P3_MED)

At Visits 3-4, the Microbiome Randomization Module is accessed in order to generate a new Diskus inhaler number for the participant. The module may be accessed up to 5 calendar days in advance of the visit to generate the inhaler number and prepare for the visit. This inhaler number is logged on Microbiome Drug Dispensing Log: Post-Randomization Study Medications (P3_DRG_SCH) and on the Participant Assignment Log (P3_LOG). The person dispensing the inhaler should complete this information. See the Randomization discussion in this section and Section 3 of this manual for more details on the Randomization Module.

Each time a post-randomization Diskus inhaler is dispensed, a Microbiome Scheduled Medications (P3_MED) form must be completed to confirm the dispensation in the Microbiome database. See Sections 4 and 5 of this manual for details on the completion of this form and related logs.

Visit 3-5
Collect/Log study medication (P3_DRG_SCH, P3_DRG_SCH_ALL)

At each scheduled post-randomization visit the participant returns a Diskus inhaler to the performance site. The person collecting the Diskus inhaler should update the appropriate row on the Microbiome Drug Dispensing Log: Post-Randomization Study Medication (P3_DRG_SCH) with information on the collection. See Sections 4 and 5 of this manual for details on the completion of this log.

Visit 5
Log/dispense Flovent (P3_DRG_FLO)

At Visit 5, an open-label Flovent Diskus inhaler will be dispensed for use between Visits 5 and 6. See Sections 4 and 5 of this manual for details on the completion of this log (P3_DRG_FLO).

Visit 6
Collect/log Flovent (P3_DRG_FLO)

At Visit 6, the participant will return their open-label Flovent Diskus inhaler to the performance site. The person collecting the Diskus inhaler should update the appropriate row on the Microbiome Drug Dispensing Log: Flovent Inhaler (P3_DRG_FLO) with information on the collection. See Sections 4 and 5 of this manual for details on the completion of this log.

Rescheduling V4 or V5 due to Respiratory Infection or Antibiotic use
If a participant has a respiratory tract infection in the 4 weeks prior to either V4 or V5, the visit should be rescheduled 4 weeks from the onset of symptoms. Likewise, if the participant has taken any antibiotic (except for topical) in the 4 weeks prior to either V4

or V5, the visit should be rescheduled 4 weeks from the last day of antibiotic use. If a visit must be rescheduled, ensure that the participant has enough drug to make it to the rescheduled visit date. If not, the participant's Diskus should be collected and a new Diskus dispensed using the Backup Diskus procedures found in the Randomization discussion in this section. When the Diskus is collected, a Microbiome Compliance Checklist (P3_COMPLY) form should be completed and entered into the Microbiome database as a single form (with visit number of the last regular visit and current date) to assess compliance from prior visit to current date. Likewise, when the new Diskus is dispensed, a Microbiome Scheduled Medication (P3_MED) form should be completed and entered into the Microbiome database as a single form (with visit number of the last regular visit and current date). When the participant returns for the rescheduled visit, the Compliance Checklist should evaluate participant's compliance from the time the new Diskus was dispensed to the rescheduled visit date.

Visit Windows Exceptions and Extended Visit Windows

When visit window exceptions are granted, or the participant requires visit(s) outside the extended lower V3 window and/or extended upper V4 window (which may cause the number of days between V3 and V4 to exceed 30 days*), verify that the participant has enough study drug to make it to his/her next study visit. If not, the participant should be supplied another Diskus inhaler. A new Diskus inhaler number should be generated through the Microbiome Randomization Module using the number of the last (or current*) visit, and a Microbiome Scheduled Medications (P3_MED) form should be completed and data entered as a single form. When the Diskus inhalers are collected, a Microbiome Compliance Checklist (P3_COMPLY) form should be completed for both Diskus inhalers. In this case, the number of puffs taken is equivalent to 120 – (remaining puffs on Diskus 1 + remaining puffs on Diskus 2), as reflected on the Diskus counters.

* When the participant requires visit(s) outside the extended lower V3 window and/or extended upper V4 window, two Diskuses may need to be given at V3.

2.47 Study Treatment Questionnaires (asthmatics only)

Visit 5 (or last post-randomization contact at Visits 3-5)
Have participant complete Participant Study Treatment Questionnaire (P3_PARTTXQX)
Complete Coordinator Study Treatment Questionnaire (P3_CTXQX)

The study treatment questionnaires are used to assess how well the masking of the scheduled inhaler was carried out. The Participant Study Treatment Questionnaire (P3_PARTTXQX) was developed to evaluate the blind from the participant's perspective. The Coordinator Study Treatment Questionnaire (P3_CTXQX) was developed to evaluate the blind from the study coordinator's perspective. These questionnaires are completed at the participant's final post-randomization study visit. Questions on the forms address the treatment the participant or study coordinator thought the participant received since starting blinded, randomized treatment at Visit 2.

If a participant withdraws from the study following randomization and prior to Visit 5, both questionnaires should be completed at the time of the participant's final contact with the performance site. If the final contact is by phone, the coordinator may administer the P3_PARTTXQX questionnaire over the phone. In this case, no source documentation will be recorded.

Participant Study Treatment Questionnaire
Near the conclusion of Visit 5, the participant should complete a Participant Study Treatment Questionnaire (P3_PARTTXQX). This form is designed to determine how well the blind on the Microbiome Diskus inhaler performed with respect to the participant's perceptions of the study medication (i.e., active fluticasone versus placebo) he/she received since randomization at Visit 2. Clinical personnel should explain the purpose of the questionnaire to the participant and confirm that the participant understands that the form references only the medication taken from his/her study Diskus.

This questionnaire is participant-completed. It is relatively short and should take no longer than five minutes to complete. Study personnel should not help the participant to answer questions on the questionnaire, as such assistance could influence the participant's responses and result in bias. Participants should be asked to answer all questions to the best of their ability; they should not leave any blank. When the form is complete, the participant should initial and date the source documentation box on page 2.

Coordinator Study Treatment Questionnaire
Near the end of Visit 5, the study coordinator who was primarily responsible for the participant's Microbiome study visits since randomization at Visit 2 should complete a Coordinator Study Treatment Questionnaire (P3_CTXQX). This form is designed to determine how well the blind on the Microbiome Diskus inhaler performed with respect to the coordinator's perceptions of the study medication (i.e., active fluticasone versus placebo) the participant received since randomization. The coordinator should

complete this form before reviewing the participant's questionnaire (P3_PARTTXQX) and before entering the participant's form into the study database. The participant should not review the coordinator's form, and the coordinator should not discuss his/her perceptions of the study treatment with the participant.

When the P3_CTXQX form is complete, the coordinator should initial and date the source documentation box at the bottom of the page. If the primary study coordinator in charge of the participant's visits is unavailable during Visit 5 or the participant's early withdrawal visit, the P3_CTXQX form should be completed as soon as possible on his/her return to the performance site, preferably within 1 week of the visit. Only one coordinator should complete the form, and only one form should be submitted per participant.

If a randomized participant is lost to follow-up or withdraws early and is unavailable to complete the P3_PARTTXQX form, the study coordinator still should complete a P3_CTXQX form, as long as the participant had at least one follow-up visit during the double-blind treatment period. In this case the P3_CTXQX form should be submitted as a single form, along with any other information that might be available.

See Section 4 in this manual for further details regarding the completion of the P3_CTXQX and P3_PARTTXQX forms.

2.48 Tongue scraping and Oral Saline Rinse

Visits 1, 4
Perform tongue scraping and oral saline rinse

This oral preparation will be performed immediately before sputum induction.

Item	Vendor	Catalog #	# Per Collection
Breath Rx tongue scrapers (3 scrapers per pack) http://www.smilox.com/breathrx-tonguecleaner.cfm	www.smilox.com		1
BD POSIFLUSH™ NORMAL SALINE SYRINGES (pre-filled 10 mL)	Supplied by DCC		1
Paper cup			1

Step 1: Tongue Scraping

1) Have the participant extend his/her tongue, and hold tip gently with sterile gauze pad.
2) Using moderate pressure, rapidly scrape the tongue from back to front 6 times to accumulate a buildup of debris on the tongue scraper.
3) Discard tongue scraper.
4) Repeat steps 1 and 2, using a new tongue scraper, 2 more times.

Step 2: Oral Rinse

1) Have participant clear his/her mouth and spit secretions into the cup and then ACTIVELY swish and gargle 10 mL of sterile saline (from syringe pre-filled with 10 mL sterile saline) for 20 seconds. Use a stopwatch to time the swish/gargle. Do not include time the patient pauses to rest.
2) At end of swish/gargle, instruct participant to gently spit oral rinse into the cup.
3) Discard cup and its contents.
4) Proceed with preparing participant for sputum induction.

Visits 2, 5
Perform tongue scraping and oral saline rinse (oral wash)
Enter oral wash sample data into Biological Sample Tracking module

For additional details on tongue scraping and oral saline rinse prior to bronchoscopy, see Microbiome Bronchoscopy Manual of Procedures.

Step 1: Tongue Scraping

1) Have the participant extend his/her tongue, and hold tip gently with sterile gauze pad.
2) Using moderate pressure, rapidly scrape the tongue from back to front 6 times to accumulate a buildup of debris on the tongue scraper.
3) Swirl the tongue scraper in the ORAL specimen container containing 20 mL of RNALater to remove tongue debris. Discard tongue scraper. If necessary to facilitate transfer of material from the scraper, use a sterile syringe to aspirate fluid from the container and flush over the scraper.
4) Repeat steps 1, 2 and 3, using a new tongue scraper, 2 more times.
5) Please keep lid of specimen container closed as much as possible to reduce environmental contamination.

Step 2: Oral Rinse

1) Have participant clear his/her mouth and spit secretions into the ORAL specimen container and then ACTIVELY swish and gargle 10 mL of sterile saline (from syringe pre-filled with 10 mL sterile saline) for 20 seconds. Use a stopwatch to time the swish/gargle. Do not include time the patient pauses to rest.
2) At end of swish/gargle, instruct participant to gently spit oral rinse into the ORAL specimen container.
3) Cap and gently invert the oral rinse specimen container 10 times to thoroughly mix. Place on ice.
4) Under a biosafety hood, transfer the contents (should have approximately 30 mL total volume) equally into two, 15-ml conical tubes for more secure storage. (see also Section I.8.B)
5) Store at 4° C for 24 hours or at least overnight.
6) Move samples to -80° C freezer until ready to ship.

Labeling and shipping details for oral wash sample can be found in Microbiome Bronchoscopy Manual of Procedures.

2.49 Transfer Participants

Transfer participants are defined as individuals who are enrolled in a trial and successfully complete at least one study visit at one performance site, then transfer to another performance site for a set number of visits or for the remainder of their study participation. General database procedures related to transfer participants are outlined in Section 7.5.2 of the AsthmaNet General Manual of Operations. Microbiome-specific considerations follow.

- Participant Assignment Log: Complete the participant ID number and other information on the Participant Assignment Log (P3_LOG) (Not Pre-Filled) version. Maintain this log with the site-specific Microbiome log. The participant should retain his/her original ID that was assigned at the originating site.

- Randomization: In the Microbiome Randomization Module, enter the participant ID and select the location where the randomization is taking place (i.e., 'new' site). If the enrollment site is chosen by mistake, the Randomization Module will return Diskus inhaler numbers that are physically located at the transfer participant's enrollment site, not the site of the current visit. If this occurs, the DCC should be contacted immediately.

- Study ID Card: A new study ID card should be distributed to the participant (with updated study personnel and primary physician information completed, as necessary).

- Baseline Rescue Use Values: The originating site should provide the new performance site a photocopy of the Visit 2 Asthma Monitoring Log (P3_ASTHMA_LOG). In addition, the originating site should confirm the participant's baseline (Visit 1) FEV_1 value.

- Physical Measurements: For participants $\geq$ 21 years old, the new performance site may use the Participant Data module to view the Adult Body Measurements (BODYMEAS_ADULT) form completed at Visit 1. The height and weight recorded on this form should be referenced when entering participant characteristics into the MedGraphics PC.

- Physical Exams: The originating site should send copies of the short and long physical exam forms (LEXAM_ADULT, SEXAM_ADULT) to the new site.

- Genetics Blood: If the genetics blood draw was deferred to a later visit and has not yet been completed, the originating site should notify the new site. The new

site should confirm the participant's consent for participating in the genetics blood draw based on his/her responses on the local consent documents.

- Visit Schedule: The originating site should supply the new site a copy of the most recently generated Visit Scheduler Report.

- Prednisone Supply: If the participant has been randomized, the new site should verify that he/she has a supply of rescue prednisone on hand. If he/she does not, a new supply should be dispensed.

2.50 Visit Scheduler

Visits 1, 2, 5
Run Microbiome visit scheduler
Review planned visit schedule

A visit scheduler program has been included on the AsthmaNet secure website to allow clinical personnel to create a Visit Scheduler Report for a given participant's Microbiome study visits. The visit scheduler is run at Visits 1, 2 and 5. The visit scheduler at Visit 1 creates the participant's schedule, based on the Visit 1 date, for Visit 2. The Visit 2 scheduler creates the participant's schedule, based on the Visit 2 date, for Visits 3-5 (randomized treatment period). The Visit 5 scheduler creates the participant's schedule, based on the Visit 5 date, for Visit 6.

The visit scheduler has been created in three pieces to adjust the dates for each participant such that there is the appropriate spacing between visits, per protocol. Visit Scheduler Reports should be run near the end of the applicable visits (1, 2, 5) and reviewed with the participant. Reports are customized for each participant in that his/her actual visit dates (1, 2, 5) are entered so that ideal dates and visit windows for all subsequent visits can be calculated and displayed on the report.

IMPORTANT: Bronchoscopy visits (Visits 2 and 5) should <u>not</u> be scheduled on Fridays.

If clinical staff and the participant desire to see the ideal schedule for all subsequent visits at the time of Visit 1, the following procedures should be followed. First, generate the participant's Visit 1 report by entering the participant's Visit 1 date. Next, review the Visit 1 report and find the ideal date for the participant's Visit 2. Enter this Visit 2 date into the Visit 2 portion of the scheduler to generate a second report. Next, review the Visit 2 report and find the ideal date for the participant's Visit 5. Enter this Visit 5 date into the Visit 5 portion of the scheduler to generate a third report. The three reports combined show the participant's complete schedule for the study. Be sure to explain to the participant that alterations to this schedule will be necessary if his/her visits occur earlier or later than the ideal date on the reports.

Instructions for accessing and generating the Microbiome Visit Scheduler Reports on the AsthmaNet secure website can be found in Section 3 of this manual.

A copy of the Microbiome Visit Scheduler Reports should be included in the participant's study handout folder for personal reference. An additional copy should be placed in the participant's study folder at the performance site. As Visit Scheduler Reports are updated at appropriate visits, be sure to discard outdated copies.

2.51 Visit Windows

Table 5 summarizes the regular and extended windows allowed by protocol around the ideal visit date for each of the Microbiome study visits. The run-in is 7-10 days long, followed by the randomization period which is ideally 6 weeks long. An open-label treatment period (7-10 days) follows the randomized treatment period.

Visits should be scheduled on the ideal date whenever possible. When this is not possible, the regular windows should be used. The extended windows should be used only to accommodate extenuating circumstances when a visit will otherwise be missed. When extreme scheduling conflicts arise, the Microbiome scientific coordinator at the DCC should be consulted before scheduling the visits to ensure that analysis- and drug-related repercussions of any mistimed visits have been considered.

Note that in addition to the visit windows, the time of day of the visits should also be considered. Because of the circadian variability associated with lung function, all subsequent visits should be scheduled such that baseline spirometry at the visit occurs within +/-3 hours of baseline spirometry at Visit 1. If a participant cannot be scheduled in the spirometry windows, contact the scientific coordinator at the DCC to seek an exception.

Scheduling Visit 1
No formal visit windows have been established for scheduling Visit 1; however, the window between Visit 0 and Visit 1 should be no longer than 4 weeks. Also, before Visit 1 can occur, Phadiatop results must be received.

Table 5. Regular and Extended Windows for Microbiome Study Visits

Visit Number	Study Week	Regular Window (days) Lower	Regular Window (days) Upper	Extended Window (days) Lower	Extended Window (days) Upper
1	0				
2 (randomization)	1		+3		
3	3	-3	+3	-5*	+5
Phone Contact	5	-3	+3	-5	+5
4	6	--	+3	--	+4*
5	7	--	+3	--	+5
6	8-9	-3	+3	-5	+5

* At the extreme extended lower V3 window (-5) AND the extreme extended upper V4 window (+4), the participant will have enough drug between V3 and V4. However, if an exception is granted to conduct one of these visits outside the extended window, the participant may need an additional Diskus to have adequate supply between V3 and V4.

Visit 2 marks the end of the Microbiome run-in period.

Visit 5 marks the end of the randomized treatment period. Ideally, the treatment period will be 6 weeks.

Ideal visit dates and regular and extended visit windows have been programmed into the Microbiome Visit Scheduler Reports for ease of scheduling participant visits. See the Visit Schedule discussion in this section and Section 3 for further details on these reports.

If a participant routinely fails to keep scheduled visits, he/she should be counseled by the performance site coordinator. If the problem persists, the local investigator should talk with the participant. Participants who have unusual scheduling conflicts may not be good prospects for randomization, as most of the Microbiome study visits cannot be missed.

2.52 Withdrawals

Early Study Withdrawal
Complete Microbiome Termination of Study Participation form (P3_TERM_A or P3_TERM_C)

Participants have the right to withdraw consent for study participation at any time and for any reason. In the case of a serious adverse event, either due to an asthma exacerbation or another medical condition, the study investigator may determine that it is in the best interest of the participant to discontinue participation in the trial.

When a participant is withdrawn from the study or withdraws consent after completing Visit 1 successfully, a Microbiome Termination of Study Participation (P3_TERM_A or P3_TERM_C) form should be completed, entered into the database, and submitted to the DCC as soon as possible. Note that any AsthmaNet investigator at the performance site may approve and sign off on the P3_TERM_A OR P3_TERM_C form.

In addition to the P3_TERM_A or P3_TERM_C form, participants who are withdrawn from Microbiome should be asked to complete an AsthmaNet Satisfaction Questionnaire (SATQX). This questionnaire is optional and anonymous in that no participant ID number or other identifying information is recorded on the form. The participant should be given a pre-addressed, postage-paid envelope in which to return the questionnaire directly to the DCC. The Satisfaction Questionnaire is posted on the secure AsthmaNet website appended to the single P3_TERM_A and P3_TERM_C forms and as part of the Visit 6 packet. See the Satisfaction Questionnaire discussion in this section for instructions on the administration of the Satisfaction Questionnaire (SATQX).

The specific termination procedures that should be followed are dependent on when in the trial the participant terminates his/her participation. See below for additional details.

Withdrawals during the Pre-Randomization Phase (Run-in or Visit 0-2)
The primary purpose of the run-in phase (Visit 0-2) is to identify an appropriate group of asthmatic participants for randomization in the Microbiome trial. This phase gives clinic personnel an opportunity to review eligibility criteria and adherence to study procedures for each participant before he/she is randomized. For the Microbiome study it is extremely important to gauge the participant's ability to maintain high levels of compliance. Participants who cannot accommodate the date/time of the visits, who take exclusionary medications, who fail to track their rescue use on the Asthma Monitoring Log are non-compliant. These participants should not be randomized at Visit 2, as their lack of adherence can affect the results of the study adversely and may jeopardize their safety if they cannot recognize asthma exacerbation conditions. Thus, the run-in phase is the optimal times to identify and withdraw inappropriate participants.

When an asthmatic or control participant is withdrawn from the run-in or withdraws consent prior to Visit 2, a Microbiome Termination of Study Participation (P3_TERM_A or P3_TERM_C) form should be submitted to the DCC along with any study data that have been collected. The number of the last completed visit should be indicated as the visit number on the P3_TERM_A or P3_TERM_C form (Visit 0 or 1).

In addition to the P3_TERM_A or P3_TERM_C form, participants who are withdrawn after successfully completing Visit 0 and prior to Visit 2 should also be asked to complete an AsthmaNet Satisfaction Questionnaire (SATQX). The participant's status at the time of termination should be completed by the coordinator at the top of the form as 'Run-in termination.'

Any blood, urine or sputum samples that are collected during the participant's termination visit should be forwarded to the appropriate labs as outlined in this manual.

Minimum data packet requirements for *asthmatic* individuals terminated at Visit 2 include:

- Pulmonary Procedure Checklist (P3_PULMONARYCHK)
- Eligibility Checklist 3 (P3_ELIG3A)

Minimum data packet requirements for *control* individuals terminated at Visit 2 include:

- Pulmonary Procedure Checklist (P3_PULMONARYCHK)
- Eligibility Checklist 3 (P3_ELIG3C)
- Termination of Study Participation (P3_TERM_C)

Early Withdrawals after Randomization

The intention-to-treat principle applies to the Microbiome study. Once a participant has been randomized, all efforts must be made to follow the participant and to collect data on his/her progress for the duration of the study. This principle applies even for participants who are discovered to be ineligible (unless the reason for ineligibility presents a safety concern) or who fail to comply with study procedures following randomization. Once a participant leaves the performance site with his/her randomly assigned Diskus inhaler at Visit 2, he/she *must* be followed. Any losses in participant follow-up can lead to bias in the study results. Participant withdrawal during the post-randomization period is permissible only in the following situations:

- Withdrawn Consent (i.e., participant refusal to continue)
- Pregnancy
- Serious Adverse Event or Severe Asthma Exacerbation

  A serious adverse event, either unrelated to asthma or due to a significant asthma exacerbation, may prompt the study investigator to terminate the participant from further study participation because it is in the participant's best interest for safety reasons.
- Loss to Follow-up

Participants who cannot be contacted for an extended period of time qualify as lost to follow-up. Clinic staff should continue to attempt to contact the participant until the time he/she would have completed the trial. At this point, a Microbiome Termination of Study Participation (P3_TERM_A) form should be completed, entered into the database, and sent to the DCC.

Once randomized, participants cannot be terminated from the study solely for non-compliance with attendance at study visits, dosing with study medications, or any other form of non-compliance. Non-compliance may be stated as a secondary reason for participant termination on the P3_TERM_A form; it may not be used as the primary reason for termination.

Withdrawal at a regular visit (3 – 5)
If a randomized participant withdraws consent during a post-randomization visit, any data already collected at that visit should be reported on the data collection forms and forwarded to the DCC. A Microbiome Termination of Study Participation (P3_TERM_A) form should be submitted. The participant should be asked to complete the Microbiome Participant Study Treatment Questionnaire (P3_PARTTXQX) and the coordinator should complete the Microbiome Coordinator Study Treatment Questionnaire (P3_CTXQX). If termination is occurring at visits 3-5, these forms should be submitted as single forms with the current visit number on them. The participant should be given an AsthmaNet Satisfaction Questionnaire (SATQX) with pre-addressed, postage-paid envelope to complete and return at his/her leisure.

Any bronchoscopy or sputum samples that are collected during the participant's termination visit should be forwarded to the appropriate labs as outlined in this manual.

Withdrawal between regular visits (3 – 5)
If a randomized participant withdraws consent by contacting performance site personnel between visits, the Microbiome Termination of Study Participation (P3_TERM_A) form should be completed with the visit number of the last visit the participant completed. If the participant returns completed Asthma Monitoring Logs (P3_ASTHMA_LOG), these data should be entered and submitted to the DCC, as well. A Microbiome Participant Study Treatment Questionnaire (P3_PARTTXQX) may be administered over the phone, if the participant is agreeable. No source documentation will be available on the form in this case. A Microbiome Coordinator Study Treatment Questionnaire (P3_CTXQX) should be completed as well. The participant should be mailed an AsthmaNet Satisfaction Questionnaire (SATQX) with return envelope and instructions for completion.

Arrangements must be made to have the participant ship his/her study medications back to the site. Compliance should be estimated as best possible from the returned Diskus and recorded on the Microbiome Compliance Checklist (P3_COMPLY).

Withdrawal Due to Exacerbation

Participants who have a significant asthma exacerbation during the run-in period (pre-randomization) will be terminated from study enrollment and managed as clinically-indicated, with treatment based on clinical standard and initiated by/in accordance with the participant's usual asthma care provider. The participant may be re-screened at Visit 0 for entry into the study at the discretion of the local investigator. Eligibility criteria, such as no asthma exacerbation requiring systemic corticosteroid treatment in past 3 months and no more than 2 asthma exacerbations requiring systemic corticosteroid treatment in past 6 months will need to be met at re-enrollment. See the Re-Enrollment discussion in this section for further details.

Once randomization has occurred at Visit 2, intention-to-treat principles apply. Should a participant receive systemic corticosteroid treatment for an asthma exacerbation after randomization at Visit 2, a two-week recovery period will be imposed following the completion of treatment. No visits should take place during treatment or this recovery period. Bronchoscopy should not be performed at Visit 5. Should asthma exacerbations become too severe following randomization, the principal investigator or site director of the participant's performance site may at any time elect to drop him/her from further study participation for the participant's safety. Study termination procedures will be completed. Any complication resulting from an asthma exacerbation (pneumothorax, pneumomediastinum, etc.) will be recorded as an adverse event in addition to the significant asthma exacerbation event itself.

See the Significant Asthma Exacerbation discussion in this section for details on forms completion and rescue algorithm.

General Note:

After a participant has been terminated from the Microbiome trial, no additional data and/or specimens may be collected from the participant with the exception of the AsthmaNet Satisfaction Questionnaire (SATQX) referenced above. If any procedures are performed and/or specimens are collected after the participant's termination date, a protocol violation will be assigned.

It should be noted that the above rule applies only to procedure-related data and specimen collection. For example, when induced sputum is collected, the results of slide reading are not known immediately. The SPUTLAB form may be completed after the participant's termination date, but the sputum induction procedure itself may not be completed after the termination date (i.e., the SPUTUM and SPUTUM_ADD_TRT forms may not be dated after the termination date).

4 STUDY FORMS AND INSTRUCTIONS

This section provides information about two types of forms: data collection forms and administrative forms. Data collection forms are used to collect data from or about the participant. These forms are entered into the AsthmaNet database and submitted to the DCC. Administrative forms facilitate the processing of the participant and the visit flow by the performance sites and the DCC. Administrative forms are not entered into the AsthmaNet database and they are not submitted to the DCC in most cases.

These instructions are divided into two parts—instructions for data collection forms followed by instructions for administrative forms. The instructions for both parts are in alphabetical order based on the full form name found in the header of the form. Forms with a 'P3' prefix are specific to the Microbiome protocol.

For each form, the following information is provided: the purpose of the form, who completes the form, when the form should be completed, for which track the form should be completed (when necessary), and form instructions. Most forms have a comments section (Q6000) at the bottom of the form. The coordinator can record additional comments or information related to the form in this section. This information is entered into the AsthmaNet database management system. If you are unable to find the specific information needed to complete a form, please contact the Microbiome Primary or Secondary Data Manager at (717) 531-3663.

Some forms are only completed for one of the Microbiome protocol tracks (Asthmatic or Control). The Asthmatic track refers to allergic asthmatic participants enrolled into the Microbiome protocol. The Control track refers to both allergic non-asthmatic and non-allergic, non-asthmatic participants enrolled into the Microbiome protocol.

4.1 List of Forms Contained in the Visit Packets:

Form Name	Form Code	Refer to AsthmaNet General MOP (Section 10) for Instructions
Visit 0 Packet – Asthmatic Track : P3_VISIT0		
BioLINCC Consent Tracking Form	BIOLINCC	*
Asthma Control Questionnaire	ACQ	*
Microbiome Eligibility Checklist 0A	P3_ELIG0A	
Visit 1 Packet – Asthmatic Track: P3_VISIT1		
Asthma Control Questionnaire	ACQ	*
Sinonasal Questionnaire	SNQ	*
Microbiome Eligibility Checklist 1A	P3_ELIG1A	
Adult Asthma and Allergy History	ASTHMA_HX_ADULT	*
Cold History Questionnaire	COLD_HX	*
Prior Asthma/Allergy Treatment	PRIOR_TRT	*
Prior Conditions for All Participants	PRIOR_COND_ALL	*
Prior Conditions for Adult Participants	PRIOR_COND_ADULT	*
Adult Body Measurements	BODYMEAS_ADULT	*
Microbiome Pulmonary Procedure Checklist	P3_PULMONARYCHK	
Spirometry Testing	SPIRO	*†
Adult Methacholine Challenge Testing Checklist	METHACHK_ADULT	*†
Methacholine Challenge Testing	METHA	*
Micriobiome Eligibility Checklist 2A	P3_ELIG2A	
Sputum Induction Checklist	SPUTUMCHK	*
Sputum Induction	SPUTUM	*
Microbiome Laboratory Results	P3_LAB	
Genetic Analysis Blood Draw	GABLOOD	*†
Household Socio-Economic Information	HOUSEHOLD_SEI	*
Home Environment Questionnaire	HEQ	*
Microbial Exposure Questionnaire	P3_MEQ	
Visit 2 Packet – Asthmatic Track: P3_VISIT2A		
Asthma Control Questionnaire	ACQ	*

Microbiome Pulmonary Procedure Checklist	P3_PULMONARYCHK	
Spirometry Testing	SPIRO	*†
Post-Albuterol (4 puffs) Spirometry Testing	PALB4_SPIRO	*†
Micriobiome Eligibility Checklist 3A	P3_ELIG3A	
Microbiome Bronchoscopy Checklist Asthmatic	P3_BRONCHCHK_A	
Microbiome Asthma Monitoring Log	P3_ASTHMA_LOG	
Microbiome Scheduled Medication	P3_MED	
Microbiome Bronchoscopy Procedure Documentation	P3_BPD	
Visit 3 Packet – Asthmatic Track: P3_VISIT3		
Asthma Control Questionnaire	ACQ	*
Microbiome Compliance Checklist	P3_COMPLY	
Microiome Pulmonary Procedure Checklist	P3_PULMONARYCHK	
Spirometry Testing	SPIRO	* †
Microbiome Asthma Monitoring Log	P3_ASTHMA_LOG	
Microbiome Scheduled Medication	P3_MED	
Visit 3A Packet - Asthmatic: P3_VISIT3A		
Microbiome Compliance Checklist	P3_COMPLY	
Visit 4 Packet – Asthmatic: P3_VISIT4		
Asthma Control Questionnaire	ACQ	*
Microbiome Compliance Checklist	P3_COMPLY	
Microbiome Pulmonary Procedure Checklist	P3_PULMONARYCHK	
Spirometry Testing	SPIRO	*†
Adult Methacholine Challenge Testing Checklist	METHACHK_ADULT	*†
Methacholine Challenge Testing	METHA	*
Sputum Induction Checklist	SPUTUMCHK	*
Sputum Induction	SPUTUM	*
Microbiome Asthma Monitoring Log	P3_ASTHMA_LOG	
Microbiome Scheduled Medication	P3_MED	
Visit 5 Packet – Asthmatic: P3_VISIT5		
Asthma Control Questionnaire	ACQ	*
Microbiome Compliance Checklist	P3_COMPLY	
Microbiome Pulmonary Procedure Checklist	P3_PULMONARYCHK	

Spirometry Testing	SPIRO	*†
Post-Albuterol (4 puffs) Spirometry Testing	PALB4_SPIRO	*†
Microbiome Bronchoscopy Checklist Asthmatic	P3_BRONCHCHK_A	
Microbiome Asthma Monitoring Log	P3_ASTHMA_LOG	
Microbiome Participant Study Treatment Questionnaire	P3_PARTTXQX	
Microbiome Coordinator Study Treatment Questionnaire	P3_CTXQX	
Microbiome Bronchoscopy Procedure Documentation	P3_BPD	
Visits 6 Packet - Asthmatic: P3_VISIT6		
Asthma Control Questionnaire	ACQ	*
Adult Body Measurements	BODYMEAS_ADULT	*
Microbiome Pulmonary Procedure Checklist	P3_PULMONARYCHK	
Spirometry Testing	SPIRO	*†
Microbiome Asthma Monitoring Log	P3_ASTHMA_LOG	
Microbiome Termination of Study Participants Asthmatics	P3_TERM_A	

Form Name	Form Code	Refer to AsthmaNet General MOP (Section 10) for Instructions
Visit 0 Packet – Control: P3_VISIT0		
BioLINCC Consent Tracking Form	BIOLINCC	*
Microbiome Eligibility Checklist 0C	P3_ELIG0C	
Visit 1 Packet - Control: P3_VISIT1		
Sinonasal Questionnaire	SNQ	*
Microbiome Eligibility Checklist 1C	P3_ELIG1C	
Prior Conditions for All Participants	PRIOR_COND_ALL	*
Prior Conditions for Adult Participants	PRIOR_COND_ADULT	*
Adult Body Measurements	BODYMEAS_ADULT	*
Microbiome Pulmonary Procedure Checklist	P3_PULMONARYCHK	
Spirometry Testing	SPIRO	*†

Form Name	Form Code	Refer to AsthmaNet General MOP (Section 10) for Instructions
Adult Methacholine Challenge Testing Checklist	METHACHK_ADULT	*†
Methacholine Challenge Testing	METHA	*
Microbiome Eligibility Checklist 2C	P3_ELIG2C	
Sputum Induction Checklist	SPUTUMCHK	*
Sputum Induction	SPUTUM	*
Microbiome Laboratory Results	P3_LAB	
Genetic Analysis Blood Draw	GABLOOD	*†
Household Socio-Economic Information	HOUSEHOLD_SEI	*
Home Environment Questionnaire	HEQ	*
Microbial Exposure Questionnaire	P3_MEQ	
Visit 2 Packet – Control: P3_VISIT2C		
Microbiome Pulmonary Procedure Checklist	P3_PULMONARYCHK	
Spirometry Testing	SPIRO	*†
Post-Albuterol (4 puffs) Spirometry Testing	PALB4_SPIRO	*†
Microbiome Eligibility Checklist 3C	P3_ELIG3C	
Microbiome Bronchoscopy Checklist Control	P3_BRONCHCHK_C	
Microbiome Termination of Study Participants Control	P3_TERM_C	

† Additional protocol specific information concerning the standard form is included in this section of the MOP. More generalized information on the standard form can be found in Section 10 of the General AsthmaNet MOP.

4.2 Data Collection Forms

Packet data forms are found in visit-specific packets, and they are submitted to the DCC as packets. Individual data forms (single forms) are submitted on an as-needed basis. Concurrent forms (AECLIN, CMED) are completed at each study visit and can be updated periodically throughout the Microbiome study. All concurrent forms should be submitted when the participant concludes his or her participation in the Microbiome study. Some forms (i.e., Spirometry Testing form (SPIRO)) can be submitted as part of a visit packet or as a single form, depending on the specific circumstances. The following is a list of data forms alphabetized by form code and indicating the form type.

Form Name	Form Code	Packet	Single	Concurrent
Asthma Control Questionnaire	ACQ	*		
Clinical Adverse Events	AECLIN			*
Adult Asthma and Allergy History	ASTHMA_HX_ADULT	*		
BioLINCC Consent Tracking Form	BIOLINCC	*		
Adult Body Measurements	BODYMEAS_ADULT	*		
Concomitant Medications for Asthma/Allergy and Adverse Events	CMED			*
Cold History	COLD_HX	*		
Genetic Analysis Blood Draw	GABLOOD	*	*	
Home Environment Questionnaire	HEQ	*		
Household Socio-Economic Information	HOUSEHOLD_SEI	*		
Additional Treatment Post Methacholine Challenge Testing	METHA_ADD_TRT		*	
Methacholine Challenge Testing	METHA	*		
Adult Methacholine Challenge Testing Checklist	METHACHK_ADULT	*		
Microbiome Asthma Monitoring Log	P3_ASTHMA_LOG	*	*	

Form Name	Form Code	Packet	Single	Concurrent
Microbiome Bronchoscopy Procedure Documentation	P3_BPD	*		
Microbiome Bronchoscopy Checklist Asthmatic	P3_BRONCHCHK_A	*		
Microbiome Bronchoscopy Checklist Control	P3_BRONCHCHK_C	*		
Microbiome Compliance Checklist	P3_COMPLY	*	*	
Microbiome Coordinator Study Treatment Questionnaire	P3_CTXQX	*	*	
Microbiome Eligibility Checklist 0A	P3_ELIG0A	*		
Microbiome Eligibility Checklist 0C	P3_ELIG0C	*		
Microbiome Eligibility Checklist 1A	P3_ELIG1A	*		
Microbiome Eligibility Checklist 1C	P3_ELIG1C	*		
Microbiome Eligibility Checklist 2A	P3_ELIG2A	*		
Microbiome Eligibility Checklist 2C	P3_ELIG2C	*		
Microbiome Eligibility Checklist 3A	P3_ELIG3A	*		
Microbiome Eligibility Checklist 3C	P3_ELIG3C	*		
Microbiome Laboratory Results	P3_LAB	*	*	
Microbiome BAL Laboratory Results	P3_LAB_BAL		*	
Microbiome Scheduled Medication	P3_MED	*	*	
Microbial Exposure Questionnaire	P3_MEQ	*		
Microbiome Participant Study Treatment Questionnaire	P3_PARTTXQX	*	*	
Microbiome Pulmonary	P3_PULMONARYCHK	*	*	

Form Name	Form Code	Packet	Single	Concurrent
Procedure Checklist				
Microbiome Significant Asthma Exacerbation	P3_SIGEX		*	
Microbiome Termination of Study Participation Asthmatics	P3_TERM_A	*	*	
Microbiome Termination of Study Participation Control	P3_TERM_C	*	*	
Post-Albuterol (4 puffs) Spirometry Testing	PALB4_SPIRO	*	*	
Urine Pregnancy Test	PREG_TEST		*	
Prior Conditions for Adult Participants	PRIOR_COND_ADULT	*		
Prior Conditions for All Participants	PRIOR_COND_ALL	*		
Prior Asthma/Allergy Treatment	PRIOR_TRT	*		
Serious Adverse Event Reporting Form	SERIOUS		*	
Sinonasal Questionnaire	SNQ	*		
Spirometry Testing	SPIRO	*	*	
Sputum Induction Lab Values	SPUTLAB		*	
Additional Treatment Post Sputum Induction	SPUTUM_ADD_TRT		*	
Sputum Induction	SPUTUM	*		
Sputum Induction Checklist	SPUTUMCHK	*		

4.2.1 Clinical Adverse Events (AECLIN)

Purpose: To record the details and events that occur each time a participant experiences a clinical adverse event.

Who: An AsthmaNet coordinator completes the form.

When: Visits 0-6 Asthmatic, 0-2 Control

Note: This form should also be completed if the participant or participant's guardian contacts study personnel to report a clinical adverse event outside of scheduled visits. This form should also be updated if the participant reports having an asthma/allergy or adverse event between visits. Questions on other forms may also prompt a coordinator to complete this form.

Form Instructions:

Clinical adverse events that started in between visits but were reported by the participant at the following regular visit should be recorded on the current visit's AECLIN form. For example, events started in between Visit 3 and Visit 4 and reported at Visit 4 would get recorded on the Visit 4 AECLIN form.

If the participant contacts the clinic coordinator between visits, record the new event(s) on the AECLIN form completed at the last visit. This new event should be updated in the Participant Data module within the data management application.

For more information on recording clinical adverse events, see the Clinical Adverse Events discussion in Section 10 of the AsthmaNet General MOP.

4.2.2 Concomitant Medications for Asthma/Allergy and Adverse Events (CMED)

Purpose: To record any asthma/allergy and adverse event related concomitant medications that the participant uses during the study.

Who: An AsthmaNet coordinator completes the form.

When: Visits 0-6 Asthmatic, 0-2 Control

Note: This form should be completed if the participant contacts study personnel to report a concomitant medication used outside of scheduled visits. This form should also be updated if the participant reports taking an asthma/allergy or adverse event related concomitant medication between visits. Questions on other forms may also prompt a coordinator to complete this form.

Form Instructions:

Concurrent medications that were started in between visits but were reported by the participant at the following regular visit should be recorded on the current visit's CMED form. For example, medications started in between Visit 3 and Visit 4 and reported at Visit 4 would get recorded on the Visit 4 CMED form.

If the participant contacts the clinic coordinator between visits, record the new medication(s) on the CMED form completed at the last visit. This new medication should be updated in the Participant Data module within the data management application.

For more information on recording concomitant medications, see the Concomitant Medications discussion in Section 7 or Section 10 of the AsthmaNet General MOP.

4.2.3 Genetics Analysis Blood Draw (GABLOOD)

Purpose: To record information related to a participant's genetic analysis blood draw.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1 or at a later visit, if necessary

Form Instructions:

If the genetics blood draw is not done at Visit 1, but is instead deferred to a later visit in the Microbiome study, the Visit 1 packet Genetics Analysis Blood Draw (GABLOOD) form should be marked missing. The Genetics Analysis Blood Draw (GABLOOD) form should be completed and entered as a single form for the visit at which the blood draw takes place (Visit 2-6 for Asthmatics and Visit 2 for Controls).

If the participant terminates early from the study and never completes a blood draw at a subsequent visit, submit a data correction to have the Visit 1 Genetics Analysis Blood Draw (GABLOOD) form set to present. Q1000 and Q1010 should be completed, indicating that a blood sample was not obtained. When the data correction is submitted through the AsthmaNet application, first-enter the form and send the original copy of the Genetics Analysis Blood Draw (GABLOOD) form for Visit 1 to the DCC for second entry. A subsequent blood draw could also be missing because the participant is deemed ineligible or consent is withdrawn.

If the blood draw is attempted at Visit 1 but is unsuccessful, and the participant is unwilling to have another draw attempted at a future visit, then the Genetics Analysis Blood Draw (GABLOOD) form should be completed and data entered as part of the Visit 1 packet. In that case, Q1000 and Q1010 should be completed, indicating that a blood sample was not obtained, and the participant should provide source documentation.

See the Genetics Blood Draw discussion in Section 2 for more details on the genetics analysis blood draw and Appendix 4 of the AsthmaNet General MOP for more details on the Genetics Analysis Blood Draw (GABLOOD) form.

4.2.4 Methacholine Challenge Testing Checklist (METHACHK_ADULT)

Purpose: To determine if an adult participant is eligible to proceed with the diluent (solution #0) pulmonary function testing for methacholine challenge testing.

Who: A Pulmonary Function Technician completes the form.

When: Visits 1 and 4 Asthmatic Participants Only
Visit 1 Control Participants Only

Form Instructions:

Question 1050. Refer to the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form and the Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG) for records regarding systemic corticosteroid use for the treatment of an asthma exacerbation in the last 4 weeks.

For more information on the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT), see Section 10 of the AsthmaNet General MOP.

4.2.5 Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG)

Purpose: This form records the number of daily RESCUE Ventolin® inhaler puffs , as well as the participant's baseline rescue use value, non-study medication use, and medical problems.

Who: The participant completes the form.

When: Visits 2-6 Asthmatic Participants Only

Form Instructions:

Starting the day after Visit 1, the Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG) will be completed by the participant on a daily basis.

If the participant loses his or her Asthma Monitoring Log between visits, instruct the participant to return to the clinical center as soon as possible to receive new ones. The participant should only complete the remainder of the days until the next visit; he or she should not try to recall information from the lost days. Date and initial a note in the Comments section (Q6000) on the new Asthma Monitoring Log to indicate why days are missing.

If the participant returns to the clinic with an Asthma Monitoring Log that contains information that appears to be fabricated, instruct the participant on proper completion of the log. The P3_ASTHMA_LOG data should still be entered and submitted to the DCC as usual. Clinical staff should not edit the data on the logs, as it is reported by the participant.

If the participant is unable to complete a scheduled visit at the clinic, it is the coordinator's responsibility to ensure that the participant has enough logs until they come to the clinic for the next visit.

The first field of the Date column in the .pdf version of the Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG) is fillable. This form is available on the AsthmaNet secure website via the following path: Forms: Microbiome: Data Collection Forms.

Before printing out this form to give to the participant, the coordinator can prefill all date fields on the form by entering the current date in the first field of the Date column and then clicking elsewhere in the form. The rest of the fields will prefill with subsequent dates up to 6 weeks from the current date.

Question 1000. At Visit 2, the participant's baseline rescue use value will be calculated and entered in Q1000. To calculate baseline rescue use, find the sum of Q1010 for the days recorded on the baseline Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG) at Visit 2. Divide the sum of Q1010 by the number of days for which Q1010 was completed. This is the baseline rescue use value. This field should not be completed again at subsequent visits. During data entry, once you enter the date for the first day and the value for Q1000 for the first day, the Date (ddate) field and Q1000 will automatically populate for each additional day.

ddate. This field should be auto-populated by the coordinator using the fillable PDF posted on the AsthmaNet website prior to distribution to the participant. This allows the coordinator to prefill the header information, as well. For instructions on how to use the fillable form, refer to **the AsthmaNet website under Protocols: Microbiome:Training.** During entry in the Participant Data module, this field will also populate once the first 'ddate' is entered. This field is still editable after it has been populated.

Question 1010. The participant should record the total number of RESCUE Ventolin® inhaler puffs taken for each day, excluding preventive puffs. If the participant did not need any puffs for a day, then '00' should be recorded for Q1010.

The last page of the Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG) is designed to record the following information that occurred since the last study visit:

1. Any non-study medications the participant took since the last study visit

2. Details of any medical problems the participant experienced since the last study visit (adverse events)

Review the information found for items 1 and 2 above and update the AECLIN and CMED concurrent forms, if necessary.

At Visits 2 – 5, the coordinator should complete the RESCUE Ventolin® puff reference value in the "To the Participant" second paragraph before giving the Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG) to the participant. The value to be entered here is the high rescue use value, which is calculated by adding 8 to Q1000 (calculated at Visit 2, on the Asthma Monitoring Log completed between Visit 1 and Visit 2).

Collecting the Asthma Monitoring Log from the participant:

Make sure that the 'Return Visit' and 'Return Visit Date' match the current visit number and current date.

Review the logs for completeness and legibility. The logs must be legible and completed in black ink. If necessary, have the participant recopy the logs before they are sent to the DCC. (Store the originals in the participant's study folder.)

Puffs taken the day of a visit should be recorded on the subsequent visit's log. If the participant has documented rescue puffs taken the day of a visit (prior to the visit) on the log, instruct the participant to include those puffs in that day's evening assessment on the log he/she is given at the visit. Do not data enter these puffs in the database, as any puffs taken on the day of a visit should be recorded on the subsequent visit's log.

Distributing the Asthma Monitoring Log to the participant:

Access the fillable PDF P3_ASTHMA_LOG file on the AsthmaNet website. Complete the current date (date of the visit) in the first date field at the top of the form. All dates will be completed automatically throughout the rest of the form.

Complete the upper right-hand corner of each log. Write in the return visit number and the proposed visit date on which the logs will be returned.

Remind the participant to complete everything on the log. The log must be legible and completed in black ink.

When collating this form, all P3_ASTHMA_LOGs returned on the same date should be paper clipped together as one form. Complete the 'Form Page ___ of ___' fields at the bottom center of each page. For example, if three logs are returned, the first log should be numbered 'Form Page 1 of 3', the second as 'Form Page 2 of 3', and the third as 'Form Page 3 of 3'.

A photocopy of the logs should be made and filed in the participant's folder, while the original logs should be sent to the DCC.

4.2.6 Microbiome BAL Laboratory Results (P3_LAB_BAL)

Purpose: This form is completed after the local lab report with BAL white blood cell count levels is received for various samples taken during the study.

Who: An AsthmaNet coordinator completes the form.

When: Visits 2 and 5 Asthmatic Participants Only
Visit 2 Control Participants Only

Form Instructions:

Submit the original lab report with the participant's Visit 2 packet (and Visit 5 packet for asthmatics). The coordinator should record the participant's ID number in the upper right-hand corner of the report. All identifying information (name, medical record number, etc.) should be blackened-out prior to forwarding the report to the DCC with the packet. If the DCC receives a report for which the identifying information has not been blackened-out, a protocol violation may be assigned.

4.2.7 Microbiome Bronchoscopy Checklist Asthmatic (P3_BRONCHCHK_A)

Purpose: This form assists the coordinator in determining if the participant is eligible to proceed with bronchoscopy.

Who: The Bronchoscopist completes the form.

When: Visits 2 and 5 Asthmatic Participants Only

Form Instructions:

Question 1000. Do not ask the participant this question. Bronchoscopy can **only** be performed if the participant has signed an informed consent form for the bronchoscopy procedure. See the Informed Consent discussion in Section 2 for further details.

Question 1010. Refer to Q1040 on the participant's spirometry data collected on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form. If this value is greater than or equal to 70, the criterion is met. If the participant's postbronchodilator FEV_1 is not ≥ 70% predicted after 4 puffs of albuterol at Visit 5, the participant is ineligible to continue with the bronchoscopy procedure, however the participant should not be terminated. Visit 5 should be rescheduled and the ineligible visit packet should **not** be entered into the study database. Instead, the packet should be filed in the participant's study binder. If the participant's postbronchodilator FEV_1 is still not ≥ 70% predicted after 4 puffs of albuterol at the second attempt, the participant is ineligible and the visit should be stopped and the visit packet for the second attempt should be entered into the study database.

Question 1020. If the participant's pulse oximetry is demonstrating oxygen saturation of ≥ 90% on room air, the criterion is met. If the participant's pulse oximetry demonstrates oxygen saturation of < 90% on room air at Visit 5, the participant is ineligible to continue with the bronchoscopy procedure however the participant should not be terminated. Visit 5 should be rescheduled and the ineligible visit packet should **not** be entered into the study database. Instead, the packet should be filed in the participant's study binder. At the second attempt, if the participant's pulse oximetry is still demonstrating oxygen saturation of < 90% on room air, the participant is ineligible and the visit should be stopped. The visit packet for the second attempt should be entered into the study database. See the Bronchoscopy MOP for further details.

Questions 1040-1050. For more detail on the definition of an asthma exacerbation, see Section 2. Q1050 should only be completed at Visit 5.

Question 1070. To calculate the participant's average rescue inhaler use within the last 48 hours, review Q1010 on the P3_ASTHMA_LOG form for the last two full days recorded prior to the visit date. (Do **not** count the visit date). If Q1010 is not completed for either of the last two full log days prior to the visit, the coordinator should inquire as to how many puffs he/she took on the days with missing data. Q1010 on the P3_ASTHMA_LOG will remain blank for the day(s), and a comment should be added to the P3_BRONCHCHK_A form in Q6000 citing "the participant indicated he/she took…" to explain the answer to Q8 If either of these values (Q1010 on the last two full log days prior to the visit date) is greater than 8, the participant is ineligible to continue with the bronchoscopy procedure. If this occurs at Visit 2, the participant is ineligibile and the Microbiome Termination of Study Participation (P3_TERM_A) form should be completed.

Question 1080. Record the participant's average score from the ACQ_ SCORE form. The average score is the sum of all 6 values obtained from ACQ form divided by 6 (this is the value recorded in the last field on the ACQ_ SCORE form). If the score is less than or equal to 1.5, the criterion is met. If the value obtained has more than two decimal places, round it to one decimal place. For example, if the value is 1.35, round up to 1.4.

Question 1110. If any of the shaded boxes is completed, the participant is ineligible. The visit should be stopped. If Visit 2, enter and submit all collected data, along with the Microbiome Termination of Study Participation Asthmatic (P3_TERM_A) form. If Visit 5, and only Q1010 or Q1020 make the Asthmatic participant ineligible, reschedule the visit.

If the participant is eligible, continue with the bronchoscopy procedure.

General Instructions:

If an eligibility protocol exception was granted by the DCC, complete the question(s) that the exception was granted for accurately (i.e., complete the shaded box). Q1110 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

4.2.8 Microbiome Bronchoscopy Checklist Control (P3_BRONCHCHK_C)

Purpose: This form assists the coordinator in determining if the participant is eligible to proceed with bronchoscopy.

Who: The Bronchoscopist completes the form.

When: Visits 2 Control Participants Only

Form Instructions:

Question 1000. Do not ask the participant this question. Bronchoscopy can **only** be performed if the participant has signed an informed consent form for the bronchoscopy procedure. See the Informed Consent discussion in Section 2 for further details.

Question 1010. Refer to Q1040 on the participant's spirometry data collected on the Spirometry Testing (SPIRO) form. If this value is greater than or equal to 80, the criterion is met.

Question 1020. If the participant's pulse oximetry is demonstrating oxygen saturation of < 90% on room air, the participant is ineligible to continue with the bronchoscopy procedure and should be terminated.

Question 1110. If any of the shaded boxes is completed, the participant is ineligible. The visit should be stopped. Enter and submit all collected data, along with the Microbiome Termination of Study Participation Control (P3_TERM_C) form.

If the participant is eligible, continue with the bronchoscopy procedure.

General Instructions:

If an eligibility protocol exception was granted by the DCC, complete the question(s) that the exception was granted for accurately (i.e., complete the shaded box). Q1110 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

4.2.9 Microbiome Bronchoscopy Procedure Documentation (P3_BPD)

Purpose: This form assists the bronchoscopist in preparing, carrying out, and following up on the bronchoscopy procedure.

Who: A certified bronchoscopist and/or AsthmaNet technician/coordinator.

When: Visits 2 and 5: Asthmatic Participants prior to, during, and following bronchoscopy. Visit 2: Control Participants prior to, during, and following bronchoscopy.

Form Instructions:

Question 1000. Prior to performing the bronchoscopy procedure, record the participant's track designation (asthmatic or non-asthmatic).

Questions 1010-1060. The participant should sit quietly for five minutes before blood pressure measurements are recorded and maintain this position while all vital signs are taken.

Question 1070. Refer to Q1040 on the participant's spirometry data collected on the Spirometry Testing (SPIRO) form to answer Q1070.

Question 1080. Refer to Q1040 on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form to answer Q1080.

Question 1110. Any medications that the participant may be taking should be recorded on the Concurrent Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1130. If any of the questions from Question 1090 through Question 1130 are answered no, bronchoscopy should not be performed and the completion of the Microbiome Bronchoscopy Procedure Documentation (P3_BPD) should be stopped. The form should be set to missing in the database but should still be forwarded to the DCC with the rest of the visit packet.

Questions 1240-1360. If any of the bronchoscopy medications are used in Q1240-1360, answer yes and record the route and dose administered. The doses values should be rounded to the nearest demical place or whole number, where applicable.

Questions 1370-1410. The participant should sit quietly for five minutes before blood pressure measurements are recorded and maintain this position while all vital signs are taken.

Questions 1420 and 1430. Record the time using military time (24-hour clock).

Question 1490-1650. If any adverse events occured (including those listed in Q1500-1650), indicate yes in Q1490 and record the event on the Clinical Adverse Events (AECLIN) form. If 'Other' is selected, complete the description field provided.

Question 1660. If the bronchoscopy was not completed as intended or was started but not completed, indicate yes in Q1660 and describe what occurred in Q6000.

Questions 1670-1710. If any of the boxes are checked yes and are beyond what is normally expected with bronchoscopy, record the corresponding event on the Clinical Adverse Events (AECLIN) form.

Question 1730. The dose administered should match the route checked (nebulizations or puffs) in Q1735.

Questions 1740 and 1750. Record the time using military time (24-hour clock).

Question 1760. Refer to Q1030 on the participant's spirometry data collection on the Spirometry Testing (SPIRO) form for the prebronchodilator FEV_1.

Questions 1780-1860. If any of the boxes are checked yes, indicate the number of hours the symptom was experienced. If any of the post-procedure symptoms are beyond what is normally expected with bronchoscopy, record the corresponding event on the Clinical Adverse Events (AECLIN) form.

Questions 1880-1920. If any of the boxes are checked yes, record the medications the participant is taking on the Concurrent Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1930. If answered yes, review the Significant Asthma Exacerbation (asthmatics only) discussion in Section 2. This question will always be answered no for control participants.

Question 1930. The fever value should be recorded in degrees Fahrenheit.

This form is entered (or set to missing, if ineligible) during data entry and should be sent to the DCC with the Visit 2 and Visit 5 packets. The P3_BPD form will be reviewed by Dr. Boushey.

4.2.10 Microbiome Compliance Checklist (P3_COMPLY)

Purpose: The participant's compliance with dosing from the Diskus inhaler is recorded on this form.

Who: An AsthmaNet coordinator completes this form.

When: Visits 3-5 Asthmatic Participants Only

Form Instructions:

Question 1000. The number of scheduled puffs should include all doses the participant should have taken since leaving the last clinic visit.

Question 1010. The value for Q1010 is obtained from the participant's Diskus inhaler. If more than 1 Diskus was required between visits, record the remaining puffs on Diskus 1 + remaining puffs on Diskus 2 for Q1010. This will occur when the time between visits is greater than 30 days.

Question 1020. Calculate the number of puffs taken by subtracting the number of remaining puffs reflected on scheduled Diskus counter (Q1010) from 60*. If Visit 3 was rescheduled due to a percent compliance value less than 75%, Q1020 is calculated by subtracting the number of remaining puffs reflected on scheduled Diskus counter (Q1010) on the current P3_COMPLY form from the number of remaining puffs reflected on schedule Diskus counter (Q1010) on the previous Visit 3 P3_COMPLY form.

*If more than 1 Diskus was required between visits, Q1020 will be calculated by subtracting the remaining puffs (Q1010) from 120.

Question 1030. Calculate the percent compliance by dividing the number of puffs taken (Q1020) by the number of scheduled puffs since the last visit (Q1000) and multiplying by 100. Round to the nearest tenth of a percent and record the value in Q1030.

See the Dosing Compliance (asthmatics only) discussion in Section 2 for more details on the compliance calculations.

No compliance reports should be sent to the DCC

4.2.11 Microbiome Coordinator Study Treatment Questionnaire (P3_CTXQX)

Purpose: This form helps to determine whether the blind on the scheduled Diskus was effective from the coordinator's perspective.

Who: The AsthmaNet coordinator who was primarily responsible for the participant's Microbiome visits completes the form.

When: Visits 2-5 Asthmatic Participants Only

Form Instructions:

The Microbiome Coordinator Study Treatment Questionnaire (P3_CTXQX) form should be completed at Visit 5 or on the day of a randomized participant's last visit if he or she terminates prior to Visit 5.

If a randomized participant terminates:

- **during** a post-randomization visit, the Microbiome Coordinator Study Treatment Questionnaire (P3_CTXQX) form should be completed at the visit and entered as a single form at Visits 3-4, or as a packet form for Visit 5.

- **between** visits, the coordinator should complete the Microbiome Coordinator Study Treatment Questionnaire (P3_CTXQX) form and enter it as a single form with the number of the last visit completed in the upper right-hand corner. For instance, a participant could be terminated from the Microbiome study following Visit 3 due to loss to follow up. In this case, the Microbiome Coordinator Study Treatment Questionnaire (P3_CTXQX) should be entered as a single form with the last visit number completed in the upper right-hand corner of the form, or Visit 3.

The visit date recorded on the form should be the date the form was completed. If the coordinator who was primarily responsible for the participant's Microbiome study visits is not present during a visit when this form is to be completed, it should be completed upon his or her return and dated appropriately.

Question 1000. Q1000 should be answered with the option that most closely represents the coordinator's feelings about which type of Diskus the participant

received during the treatment period. If unsure of which type of study Diskus was received, Q1000 should be answered with option number 3, “I have no idea which type of Diskus the participant received, but my best guess would be:,” and choose either Placebo or Fluticasone for Q1010.

Question 1015D. Any comments with respect to any other observations the coordinator may have made that helped him or her make a choice in Q1 should be recorded in Q1015D and entered into the AsthmaNet database (up to 250 characters).

To verify that the information collected on this form is correct, the coordinator who completed the form should initial and date the form in the shaded source documentation box provided (Q1020-1030) at the bottom of the page.

4.2.12 Microbiome Eligibility Checklist 0 Asthmatic (P3_ELIG0A)

Purpose: This form is the first of four eligibility forms completed during Visits 0-2. It consists of basic interview questions that assist in determining if a participant is eligible to enroll in the Microbiome study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 0 Asthmatic Participants Only

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2.

Question 1000. Do not ask the participant this question. Data can **only** be collected if the participant has signed an informed consent form for the Microbiome study. See the Informed Consent discussion in Section 2 for further details.

Question 1010. The signature date should be the date the participant signed the consent document. If the consent was signed prior to the Visit 0, the consent should be reviewed by the participant on the day Visit 0 takes place. The date the consent form was signed should **not** be updated.

Question 1050. If the participant indicates historical evidence of a disease or medical condition, but has no current evidence, Q1050 should be answered 'No.' The participant must have current evidence of one of the medical conditions for Q1050 to be answered 'Yes.'

If a participant screened at Visit 0 has one of these exclusionary medical conditions and is being allowed to progress through the study per a DCC-approved protocol exception, then Q1050 should be answered 'Yes' and Q1350 should also be answered 'Yes.' Resulting errors should be marked unresolvable, and the participant's condition and physician approval to proceed should be documented in the comment provided.

Question 1080. An upper respiratory tract infection is defined as a cough, runny nose with or without fever, or sore throat that is not related to allergen exposure.

Question 1140. If the participant has taken one of the drugs that are listed as exclusionary within the specified time periods, but is allowed to progress through the study at the discretion of the study physician, Q1130 should be answered 'Yes' and Q1140 should also be answered 'No.' Resulting errors should be marked unresolvable, and the participant's Microbiome-exclusionary medication and physician approval to proceed should be documented in the comment provided. Such cases will be tracked as protocol exceptions.

Question 1190. Calculate pack-years by multiplying the number of packs smoked per day by the number of years smoked at that quantity. One pack equals 20 cigarettes. Pack-year history will be recorded on the Adult Asthma and Allergy History (ASTHMA_HX_ADULT) form at Visit 1.

Question 1230-1260. For more detail on the definition of an asthma exacerbation, see the Significant Asthma Exacerbation (asthmatics only) discussion in Section 2.

Question 1310. If there is any possibility that the participant is physically able to bear children, Q1310 should be answered 'Yes' (even if the participant indicates she is not currently engaging in heterosexual intercourse). If the participant is surgically sterile or post-menopausal for at least one year, Q1310 should be answered 'No.' If the participant is male, Q1310 should be answered 'N/A.'

Questions 1320 and 1330. Answer Q1320 and Q1330 only if the participant is able to bear children. If the participant is currently pregnant or lactating, she is ineligible to participate in the study at this time.

Question 1330. Show the participant the Birth Control Methods (BIRTH_CTRL) reference card found on the AsthmaNet secure website in the Standard Forms: Reference Cards folder and ask if she is using one of the listed birth control methods. A participant who is able to bear children **must** be using a birth control method listed on the reference card to be eligible for the study. If the participant is not engaging in heterosexual intercourse, abstinence applies as a legitimate birth control method.

Question 1340. Refer to the ACQ_SCORE form.

Question 1350. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped and the visit packet should **not** be entered into the AsthmaNet database nor sent to the DCC. File the visit packet in the participant's study folder at the clinic.

If the participant is eligible, continue with the rest of the Visit 0 visit procedures.

For more details pertaining to whether a coordinator should permit the participant to continue in the study, see the Eligibility Criteria discussion in Section 2.

General Instructions:

If an eligibility protocol exception was granted by the DCC, complete the question(s) that the exception was granted for accurately (i.e., complete the shaded box). Q1350 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

To verify that the information collected on this form is correct, have the participant initial and date the form in the shaded source documentation box provided (Q1360-1370) on the last page of the form.

4.2.13 Microbiome Eligibility Checklist 0 Control (P3_ELIG0C)

Purpose: This form is the first of four eligibility forms completed during Visits 0-2. It consists of basic interview questions that assist in determining if a participant is eligible to enroll in the Microbiome study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 0 Control Participants Only

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2.

Question 1000. Do not ask the participant this question. Data can **only** be collected if the participant has signed an informed consent form for the Microbiome study. See the Informed Consent discussion in Section 2 for further details.

Question 1010. The signature date should be the date the participant signed the consent document. If the consent was signed prior to the Visit 0, the consent should be reviewed by the participant on the day Visit 0 takes place. The date the consent form was signed should **not** be updated.

Question 1050. If the participant indicates historical evidence of a disease or medical condition, but has no current evidence, Q1050 should be answered 'No.' The participant must have current evidence of one of the medical conditions for Q1050 to be answered 'Yes.'

If a participant screened at Visit 0 has one of these exclusionary medical conditions and is being allowed to progress through the study per a DCC-approved protocol exception, then Q1050 should be answered 'Yes' and Q1350 should also be answered 'Yes.' Resulting errors should be marked unresolvable, and the participant's condition and physician approval to proceed should be documented in the comment provided.

Question 1080. An upper respiratory tract infection is defined as a cough, runny nose with or without fever, or sore throat that is not related to allergen exposure.

Question 1140. If the participant has taken one of the drugs that are listed as exclusionary within the specified time periods, but is allowed to progress through the study at the discretion of the study physician, Q1130 should be answered 'Yes' and Q1140 should also be answered 'No.' Resulting errors should be marked unresolvable, and the participant's Microbiome-exclusionary medication and physician approval to proceed should be documented in the comment provided. Such cases will be tracked as protocol exceptions.

Question 1190 and Q1300. Calculate pack-years by multiplying the number of packs smoked per day by the number of years smoked at that quantity. One pack equals 20 cigarettes. Record the values to the nearest hundredths of a decimal.

Question 1310. If there is any possibility that the participant is physically able to bear children, Q1310 should be answered 'Yes' (even if the participant indicates she is not currently engaging in heterosexual intercourse). If the participant is surgically sterile or post-menopausal for at least one year, Q1310 should be answered 'No.' If the participant is male, Q1310 should be answered 'N/A.'

Questions 1320. Answer Q1320 only if the participant is able to bear children. If the participant is currently pregnant or lactating, she is ineligible to participate in the study at this time.

Question 1350. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped and the visit packet should **not** be entered into the AsthmaNet database or sent to the DCC. File the visit packet in the participant's study folder at the clinic.

If the participant is eligible, continue with the rest of the Visit 0 visit procedures.

For more details pertaining to whether a coordinator should permit the participant to continue in the study, see the Eligibility Criteria discussion in Section 2.

General Instructions:

If an eligibility protocol exception was granted by the DCC, complete the question(s) that the exception was granted for accurately (i.e., complete the shaded box). Q1350 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field

(Q6000) provided on the last page of the form with additional information on the exception.

To verify that the information collected on this form is correct, have the participant initial and date the form in the shaded source documentation box provided (Q1360-1370) on the last page of the form.

4.2.14 Microbiome Eligibility Checklist 1 Asthmatic (P3_ELIG1A)

Purpose: This form is the second of four eligibility forms completed during Visits 0-2. It consists of questions that assist in determining if a participant is eligible to continue in the Microbiome study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1 Asthmatic Participants Only

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2.

Question 1000. Refer to the participant's Phadiatop results present on the Microbiome Participant Report on the AsthmaNet website. For more information on Phadiatop testing, see the Blood draw (Phadiatop and IgE) discussion in Section 2.

Question 1010. If answered yes, the corticosteroid medication should be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1020. If answered yes, the respiratory infection should be recorded on the Clinical Adverse Events (AECLIN) form.

Question 1030. If answered yes, the antibiotic medication should be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1040. If the participant has taken one of the drugs that are listed as exclusionary within the specified time periods, but is allowed to progress through the study at the discretion of the study physician, Q1040 should be answered 'Yes' and Q1070 should also be answered 'Yes.' Resulting errors should be marked unresolvable, and the participant's Microbiome-exclusionary medication and physician approval to proceed should be documented in the comment provided. Such cases will be tracked as protocol exceptions.

Question 1050. If the participant is currently taking prescription or OTC medications other than those listed on the Allowed Medications for Microbiome (P3_MEDALLOW) reference card, the coordinator should confirm through the DCC that the medication is allowed before continuing. If the medication is approved by the DCC, Q1050 should

be answered 'No.' If the medication is not approved by the DCC, the participant is ineligible to continue in the study and Q1050 should be answered 'Yes.'

Question 1060. If the participant has experienced a significant asthma exacerbation since Visit 0, the event should be recorded on the Clinical Adverse Events (AECLIN) form and a Microbiome Significant Asthma Exacerbation (P3_SIGEX) form should be completed and entered into the database.

Question 1070. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped. Enter and submit all collected data, along with the Microbiome Termination of Study Participation Asthmatic (P3_TERM_A) form.

If the participant is eligible, continue with the rest of the Visit 1 visit procedures.

General Instructions:

If an eligibility protocol exception was granted by the DCC, complete the question(s) that the exception was granted for accurately (i.e., complete the shaded box). Q1070 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

To verify that the information collected on this form is correct, have the participant initial and date the form in the shaded source documentation box provided (Q1080-1090) on the second page of the form.

4.2.15 Microbiome Eligibility Checklist 1 Control (P3_ELIG1C)

Purpose: This form is the second of four eligibility forms completed during Visits 0-2. It consists of questions that assist in determining if a participant is eligible to continue in the Microbiome study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1 Control Participants Only

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2.

Question 1000. Refer to the participant's Phadiatop results present on the Microbiome Participant Report on the AsthmaNet website. For more information on Phadiatop testing, see the Blood draw (Phadiatop and IgE) discussion in Section 2.

Questions 1001-1008. If the participant is not atopic according to the Phadiatop test, then Q1001-Q1008 should be completed. For eligibility as a non-allergic control in the Microbiome protocol, the participant must not have a history of allergic rhinitis, allergic conjunctivitis, eczema, anaphylaxis and/or hives/uticaria to food or stinging insects.

Question 1010. If answered yes, the corticosteroid medication should be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1020. If answered yes, the respiratory infection should be recorded on the Clinical Adverse Events (AECLIN) form.

Question 1030. If answered yes, the antibiotic medication should be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1040. If the participant has taken one of the drugs that are listed as exclusionary within the specified time periods, but is allowed to progress through the study at the discretion of the study physician, Q1040 should be answered 'Yes' and Q1070 should also be answered 'Yes.' Resulting errors should be marked unresolvable, and the participant's Microbiome-exclusionary medication and physician approval to proceed should be documented in the comment provided. Such cases will be tracked as protocol exceptions.

Question 1050. If the participant is currently taking prescription or OTC medications other than those listed on the Allowed Medications for Microbiome (P3_MEDALLOW) reference card, the coordinator should confirm through the DCC that the medication is allowed before continuing. If the medication is approved by the DCC, Q1050 should be answered 'No.' If the medication is not approved by the DCC, the participant is ineligible to continue in the study and Q1050 should be answered 'Yes.'

Question 1070. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped. Enter and submit all collected data, along with the Microbiome Termination of Study Participation Control (P3_TERM_C) form.

If the participant is eligible, continue with the rest of the Visit 1 visit procedures.

General Instructions:

If an eligibility protocol exception was granted by the DCC, complete the question(s) that the exception was granted for accurately (i.e., complete the shaded box). Q1070 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

To verify that the information collected on this form is correct, have the participant initial and date the form in the shaded source documentation box provided (Q1080-1090) on the second page of the form.

4.2.16 Microbiome Eligibility Checklist 2 Asthmatic (P3_ELIG2A)

Purpose: This form is the third of four eligibility forms completed during Visits 0-2. It consists of questions that assist in determining if a participant is eligible to continue in the Microbiome study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1 Asthmatic Participants Only

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2.

Question 1000. If a participant is found to have evidence of an exclusionary medical condition at Visit 1 based on the physical exam and medical history and is being allowed to progress through the study due to a DCC approved exception, then Q1000 should be answered 'Yes' and Q1080 should also be answered 'Yes.' Resulting errors should be marked unresolvable, and the participant's condition and physician approval to proceed should be documented in the comment section provided. Such cases will be tracked as protocol exceptions.

Question 1010. If the participant is over 45 years of age, Q1010 should be answered Yes or No, according to ECG results. In this case, Q1014 and Q1017 should be completed by the physician. If the participant is less than or equal to 45 years of age, Q1010 should be answered N/A and Q1014 and Q1017 should not be completed.

Question 1020. If answered no, therefore the asthmatic participant does not achieve a PC20 of less than or equal to 8 mg/ml, a continuation visit should be scheduled.

At the continuation visit, P3_PULMONARYCHK, SPIRO, PALB4_SPIRO and PALB4_RPT single forms will be completed first. The rest of the P3_ELIG2A form and Visit 1 forms should be completed as outlined by the visit procedure checklist, using the visit date of the continuation visit.

Question 1030. If the participant did not achieve a PC_{20} of less than or equal to 8 mg/ml, Q1030 should be completed. The percent difference in FEV_1 value should be calculated by hand from Q1030 on the Post-Albuterol (4 puffs) Spirometry Testing

(PALB4_SPIRO) form and Q1030 on the Spirometry Testing (SPIRO) form completed at the visit.

Question 1080. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped. Enter and submit all collected data, along with the Microbiome Termination of Study Participation Asthmatic (P3_TERM_A) form.

If the participant is eligible, continue with the rest of the Visit 1 visit procedures.

General Instructions:

If an eligibility protocol exception was granted by the DCC, complete the question(s) that the exception was granted for accurately (i.e., complete the shaded box). Q1080 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

To verify that the information collected on this form is correct, have the participant initial and date the form in the shaded source documentation box provided (Q1090-1100).

4.2.17 Microbiome Eligibility Checklist 2 Control (P3_ELIG2C)

Purpose: This form is the third of four eligibility forms completed during Visits 0-2. It consists of questions that assist in determining if a participant is eligible to continue in the Microbiome study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1 Control Participants Only

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2.

Question 1000. If a participant is found to have evidence of an exclusionary medical condition at Visit 1 based on the physical exam and medical history and is being allowed to progress through the study due to a DCC approved exception, then Q1000 should be answered 'Yes' and Q1080 should also be answered 'Yes.' Resulting errors should be marked unresolvable, and the participant's condition and physician approval to proceed should be documented in the comment section provided. Such cases will be tracked as protocol exceptions.

Question 1010. If the participant is over 45 years of age, Q1010 should be answered Yes or No, according to ECG results. In this case, Q1014 and Q1017 should be completed by the physician. If the participant is less than or equal to 45 years of age, Q1010 should be answered N/A and Q1014 and Q1017 should not be completed.

Question 1040. Refer to Q1030 on the participant's spirometry data collected on the Spirometry Testing (SPIRO) form. The baseline value is Q1030 on the Spirometry Testing (SPIRO) form at Visit 1.

Question 1050. Refer to the Highest FVC and FVC Predicted values present on the appropriate spirometry report from the current visit. Q1050 is calculated by the following equation: (Highest FVC/FVC Predicted)*100.

Question 1060. Refer to Q1050 on the participant's methacholine data collected on the Methacholine Challenge Testing (METHA) form.

Question 1080. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped. Enter and submit all collected data, along with the Microbiome Termination of Study Participation Control (P3_TERM_C) form.

If the participant is eligible, continue with the rest of the Visit 1 visit procedures.

General Instructions:

If an eligibility protocol exception was granted by the DCC, complete the question(s) that the exception was granted for accurately (i.e., complete the shaded box). Q1080 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

To verify that the information collected on this form is correct, have the participant initial and date the form in the shaded source documentation box provided (Q1090-1100).

4.2.18 Microbiome Eligibility Checklist 3 (P3_ELIG3A)

Purpose: This form is the fourth of four eligibility forms completed during Visits 0-2. It consists of questions that assist in determining if a participant is eligible to continue in the Microbiome study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 2 Asthmatic Participants Only

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2.

Question 1000. Results of the serum creatinine test are recorded on the Microbiome Laboratory Results (P3_LAB) form at Visit 1. Q1000 should be completed after local lab results are received. If the participant's serum creatinine value is greater than or equal to 1.30 mg/dL, the participant is ineligible to continue in the study. The Microbiome Laboratory Results (P3_LAB) form is completed at Visit 1 and submitted as part of the Visit 1 packet. The local lab report should be included in the packet. All identifying information on the lab report should be blackened-out prior to forwarding to the DCC.

Question 1010. If answered yes, the corticosteroid medication should be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1020. If answered yes, the respiratory infection should be recorded on the Clinical Adverse Events (AECLIN) form.

Question 1030. If answered yes, the antibiotic medication should be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1040. If the participant has received treatment with any drug that is considered exclusionary, but is allowed to progress through the study at the discretion of the study physician and per a DCC-approved protocol exception , Q1040 should be answered 'Yes' and Q1110 should also be answered 'Yes.' Resulting errors should be marked unresolvable, and the participant's Microbiome-exclusionary medication and physician approval to proceed should be documented in the comment provided.

Question 1050 and 1055. If the participant has experienced a significant asthma exacerbation since Visit 1, the event should be recorded on the Clinical Adverse Events (AECLIN) form and a Microbiome Significant Asthma Exacerbation (P3_SIGEX) form should be completed and entered into the database.

Question 1110. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped and a Microbiome Termination of Study Participation Asthmatic (P3_TERM_A) form completed.

If the participant is eligible, continue with the rest of the Visit 2 procedures.

General Instructions:

If an eligibility protocol exception was granted through the DCC, complete the question(s) that the exception was granted for accurately (i.e. complete the shaded box). Q1110 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

To verify that the information collected on this form is correct, have the participant initial and date the form in the shaded source documentation box provided (Q1120-1130).

4.2.19 Microbiome Eligibility Checklist 3 (P3_ELIG3C)

Purpose: This form is the fourth of four eligibility forms completed during Visits 0-2. It consists of questions that assist in determining if a participant is eligible to continue in the Microbiome study.

Who: An AsthmaNet coordinator completes the form.

When: Visit 2 Control Participants Only

Form Instructions:

For detailed information regarding eligibility criteria, see the Eligibility Criteria discussion in Section 2.

Question 1000. Results of the serum creatinine test are recorded on the Microbiome Laboratory Results (P3_LAB) form at Visit 1. Q1000 should be completed after local lab results are received. If the participant's serum creatinine value is greater than or equal to 1.30 mg/dL, the participant is ineligible to continue in the study. The Microbiome Laboratory Results (P3_LAB) form is completed at Visit 1 and submitted as part of the Visit 1 packet. The local lab report should be included in the packet. All identifying information on the lab report should be blackened-out prior to forwarding to the DCC.

Question 1010. If answered yes, the corticosteroid medication should be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1020. If answered yes, the respiratory infection should be recorded on the Clinical Adverse Events (AECLIN) form.

Question 1030. If answered yes, the antibiotic medication should be recorded on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Question 1040. If the participant has received treatment with any drug that is considered exclusionary, but is allowed to progress through the study at the discretion of the study physician and per a DCC-approved protocol exception , Q1040 should be answered 'Yes' and Q1110 should also be answered 'Yes.' Resulting errors should be marked unresolvable, and the participant's Microbiome-exclusionary medication and physician approval to proceed should be documented in the comment provided.

Question 1080. The percent difference in FEV_1 value should be calculated by hand from Q1030 on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form and Q1030 on the Spirometry Testing (SPIRO) form completed at the visit.

Question 1110. If any of the shaded boxes are completed, the participant is ineligible. The visit should be stopped and a Microbiome Termination of Study Participation Control (P3_TERM_C) form completed.

If the participant is eligible, continue with the rest of the Visit 2 procedures.

General Instructions:

If an eligibility protocol exception was granted through the DCC, complete the question(s) that the exception was granted for accurately (i.e. complete the shaded box). Q1110 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

To verify that the information collected on this form is correct, have the participant initial and date the form in the shaded source documentation box provided (Q1120-1130).

4.2.20 Microbiome Laboratory Results (P3_LAB)

Purpose: This form is completed after the local lab report with CBC and BUN/Creatinine levels is received for various samples taken at the beginning of the study.

Who: An AsthmaNet coordinator completes the form.

When: Visits 1 and 2

Form Instructions:

For more information regarding the samples collected on the Microbiome Laboratory Results (P3_LAB) form, see the Blood draw (CBC and BUN/Creatinine) discussion in Section 2.

Visit Date. Record the visit date as the date the blood was drawn.

Questions 1000-1090. Q1000-Q1090 are completed at Visits 1 and 2. Refer to the laboratory results printout generated at each clinical center's local laboratory to answer Q1000 – Q1090. Round each value to the nearest tenth, where applicable, and record the value on the form.

Submit the original lab report with the participant's Visit 1 or Visit 2 packet. The coordinator should record the participant's ID number in the upper right-hand corner of the report. All identifying information (name, medical record number, etc.) should be blackened-out prior to forwarding the report to the DCC with the packet. If the DCC receives a report for which the identifying information has not been blackened-out, a protocol violation may be assigned.

If the values recorded on the P3_LAB_RPT are censored, record the numeric value in the entry field and in Q6000 document how the value appears on the lab report. For example, if the creatinine is reported as < 5.2 on the report, 5.2 will be entered in field Q1020 and the comment 'Q1020 appears on the lab report as < 5.2' is entered into Q6000 on the P3_LAB form.

Questions 1100-1110. Q1100-Q1110 are completed at Visit 1 only.

Question 1110. The serum creatinine measurement is entered in Q1110. If the participant's serum creatinine value is greater than or equal to 1.30 mg/dL, the participant is ineligible to continue in the study.

4.2.21 Microbiome Microbial Exposure Questionnaire (P3_MEQ)

Purpose: To record information on exposures that may affect the participant's lung microbiome.

Who: The participant completes the form.

When: Visit 1 Asthmatic Participants Only
Visit 1 Control Participants Only

Form Instructions:

If a field only accepts whole numbers, round up to the nearest whole number for decimal values greater than or equal to 0.5 and round down to the nearest whole number for decimal values less than 0.5.

'House' is defined as the place where the participant lives most of the time.

Question 1000. Check only one box. If Q1000 is answered 'Other', Q1000D should be completed.

Question 1050. If Q1050 is answered 'Yes', complete Q1050D.

Question 1060. Check only one box. If Q1060 is answered 'Other', Q1060D should be completed.

Question 1080. Record the number of days per month the participant has used the wood burning fireplace/stove in his or her house during the past three months. For example, if the participant used the fireplace or stove for 10 full days each month out of the past 3 months, record 10 for Q1080. If unsure of the number of days per month, estimate to the nearest number of days.

Question 1130. Record the number of hours per week the participant spends in the yard. For example, if the participant responds that he/she spends 2 hours per day in the yard, record 14 for Q1130. If unsure of the number of hours per week, estimate to the nearest number of hours.

Question 1140. If Q1140 is answered 'No', skip to Q1200.

Questions 1150-1180. Record the number of hours per week the participant spends gardening duringe each of the four seasons. For example, if the participant responds that he/she spends 2 hours per day in the yard in the Spring, record 14 for Q1150. If unsure of the number of hours per week, estimate to the nearest number of hours.

Question 1190. Record the number of hours per week the participant spent gardening in the past month. For example, if the participant responds that he/she typically spends 1 hour per week gardening, record 1 for Q1190. If unsure of the number of hours per week, estimate to the nearest number of hours.

Question 1200. If Q1200 is answered 'No', skip to Q1230.

Question 1210. Record the number of children who spend time in the participant's houshold. For example, if the participant has two children and also babysits another child frequently, record 3 for Q1210.

Question 1220. Record the number of children who spend time in the participant's household that are not "potty-trained". Potty-trained is defined as able to use a toilet and no longer in need of diapers. For example, if the participant has a three month old infant, record 1 for Q1220.

Question 1240. If Q1240 is answered 'No', skip to Q1280.

Question 1250. Record the number of months per year the participant works on a farm. For example, if the participant responds that he/she only works on a farm during the months of June and July, record 2 for Q1250. If unsure of the number of months per year, estimate to the nearest number of months.

Question 1260. Record the number of hours per week the participant works on a farm during the months recorded in Q1250. For example, if the participant responds that he/she works on the farm 8 hours a day every day of the week, record 56 for Q1260. If unsure of the number of hours per week, estimate to the nearest number of hours.

Question 1270. Record the number of hours per week the participant worked on a farm in the past month. For example, if the participant responds that he/she worked on the farm 8 hours a day every day of the week, record 56 for Q1270. If unsure of the number of hours per week, estimate to the nearest number of hours.

Question 1300. If Q1300 is answered 'Yes', complete Q1310-Q1340.

Question 1340. If Q1340 is answered Yes, complete Q1340D.

Review the form prior to the participant leaving the clinic to ensure that the participant completed the form correctly.

The participant must complete the source documentation box (using 2 or 3 initials) on page 4 (Q1360 and Q1370). Enter the Date field in the database in the format mm/dd/yyyy.

4.2.22 Microbiome Participant Study Treatment Questionnaire (P3_PARTTXQX)

Purpose: Any observations the participant may have made during the Microbiome study that may have compromised the study blind on the Diskus are recorded on this form.

Who: The participant completes the form.

When: Visits 2-5 Asthmatic Participants Only

Form Instructions:

The Microbiome Participant Study Treatment Questionnaire (P3_PARTTXQX) should be completed at Visit 5 or on the day of a randomized participant's last visit if he or she terminates prior to Visit 5. If the participant completes the study and terminates at Visit 5, the form should be entered as part of the Visit 5 packet.

If the randomized participant terminates:

- **during** a post-randomization visit, the Microbiome Participant Study Treatment Questionnaire (P3_PARTTXQX) form should be completed at the visit and entered as a single form at Visits 3-4, or as a packet form for Visit 5.

- **between** visits, the coordinator should complete the Microbiome Participant Study Treatment Questionnaire (P3_PARTTXQX) form and enter it as a single form with the number of the last visit completed in the upper right-hand corner. For instance, a participant could be terminated from the Microbiome study following Visit 3but before Visit 4. In this case, the Microbiome Participant Study Treatment Questionnaire (P3_PARTTXQX) should be entered as a single form with the last visit number completed in the upper right-hand corner of the form, or Visit 3.

Question 1000. The participant should check the box that most closely represents his or her feelings about which type of study Diskus was used since randomization at Visit 2. If unsure of which type of study Diskus was received, Q1000 should be answered with option number 3, "I have no idea which type of Diskus I received, but my best guess would be:," and he or she should choose either Placebo or Fluticasone for Q1010.

Questions 1020-1040. The participant should check the boxes that most closely represent his or her feelings about the taste of (Q1020), smell of (Q1030), and physical sensations (Q1040) produced by the Diskus he or she received during the Microbiome study. If the participant chooses options 1 or 3 for any of the questions, he or she can comment on the taste of, smell of, or physical sensations produced by the Diskus.

Question 1050. If the participant answers Q1050 with option 2, he or she can comment further on observations made regarding the study Diskus.

Question 1050D. Any comments with respect to any other observations the participant may have made regarding his or her study Diskus should be recorded in Q1050D and entered into the AsthmaNet database (up to 250 characters).

To verify that the information collected on this form was provided by the participant, have the participant initial and date the form in the shaded source documentation box provided (Q1060-1070) on the second page.

4.2.23 Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK)

Purpose: This form assists the coordinator in determining if the participant is eligible to proceed with pulmonary function testing.

Who: The Pulmonary Function Technician or an AsthmaNet coordinator interviews the participant and completes the form. The coordinator **must** possess Microbiome protocol certification.

When: Visits 1-6 Asthmatic Participants Only
Visits 1-2 Control Participants Only

Form Instructions:

If any medications other than the study Flovent® or rescue Ventolin® medication were used, record the medications on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

Questions 1040-1047. If the participant is currently using any oral antihistamines (i.e., has an oral antihistamine listed on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form as ongoing or with a start date since the last visit) and has withheld the use of this medication for the past 48 hours prior to the visit, Q1040 should be answered 'No.' The most recent date and time the medication was taken since the last visit should be recorded in Q1043 and Q1047 for ongoing records.

Questions 1050-1057. If the participant is currently using any nasal antihistamines (i.e., has a nasal antihistamine listed on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form as ongoing or with a start date since the last visit) and has withheld the use of this medication for the past 48 hours prior to the visit, Q1050 should be answered 'No.' The most recent date and time the medication was taken since the last visit should be recorded in Q1053 and Q1057 for ongoing records.

Questions 1060-1067. If the participant is currently using any ophthalmic antihistamines (i.e., has an ophthalmic antihistamine listed on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form as ongoing or with a start date since the last visit) and has withheld the use of this medication for the past 48 hours prior to the visit, Q1060 should be answered 'No.' The most recent date and

time the medication was taken since the last visit should be recorded in Q1063 and Q1067 for ongoing records.

Questions 1070-1077. If the participant is currently using any oral decongestants or cold remedies (i.e., has an oral decongestant or cold remedy ingredient listed on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form as ongoing or with a start date since the last visit) and has withheld the use of this medication for the past 48 hours prior to the visit, Q1070 should be answered 'No.' The most recent date and time the medication was taken since the last visit should be recorded in Q1073 and Q1077 for ongoing records.

Questions 1080-1087. If the participant is currently using any nasal decongestants (i.e., has a nasal decongestant listed on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form as ongoing or with a start date since the last visit) and has withheld the use of this medication for the past 48 hours prior to the visit, Q1080 should be answered 'No.' The most recent date and time the medication was taken since the last visit should be recorded in Q1083 and Q1087 for ongoing records.

The antihistamine and/or decongestant records on the CMED form should be considered ongoing if taken daily or regularly. Regularly is defined as any medication that is taken at a consistent frequency.

Question 1110 and Q1120. A respiratory infection is defined as a cough, runny nose with or without fever, or sore throat that is not related to allergen exposure. At Visits 4 and 5, if the participant has had a respiratory infection within the past 4 weeks and it has not been 4 full weeks or more since the symptoms started, Q1120 should be answered No and the visit should be rescheduled for 4 weeks from the onset of symptoms. File any of the data collected at the first Visit 4/Visit 5 in the participant's folder but do not enter the data or forward it to the DCC. At the rescheduled visit, enter and forward all of the data collected then.

Question 1130. At Visits 4 and 5, if the participant has taken an antibiotic (except for topical) within the past 4 weeks, Q1130 should be answered 'Yes' and the visit should be rescheduled for 4 weeks from the last day of antibiotic use. File any of the data collected at the first Visit 4/Visit 5 in the participant's folder but do not enter the data or forward it to the DCC. At the rescheduled visit, enter and forward all of the data collected then.

Question 1160. The participant is ineligible to perform pulmonary function testing if any of the shaded boxes are completed.

If the participant is not eligible to proceed with spirometry and is willing to reschedule the visit, file the collected data in his or her study folder; do not enter the data or forward it to the DCC.

If a spirometry eligibility protocol exception was granted through the DCC, complete the question(s) for which the exception was granted accurately (i.e. complete the shaded box). Q1160 should be answered 'Yes' to indicate the participant is eligible to proceed and any entry errors that result from the exception should be marked unresolvable. In the unresolvable comment section, indicate that a protocol exception was granted, who granted it, and the justification for the exception. Also, complete the comment field (Q6000) provided on the last page of the form with additional information on the exception.

Question 1170. Only record an updated height (without shoes) for participants who have not yet had their 21st birthday at Visits 2-5. At Visits 1 and 6, refer to the height recorded on the Adult Body Measurements (BODYMEAS_ADULT) form and do not record the height on the Microbiome Pulmonary Procedure Checklist (P3_PULMONARYCHK) form.

4.2.24 Microbiome Scheduled Medication (P3_MED)

Purpose: The dispensation of post-randomization scheduled medication is recorded on this form.

Who: An AsthmaNet coordinator completes the form.

When: Visits 2-4 Asthmatic Participants Only

Note: This form must be completed every time scheduled medication is dispensed at regular visits (Visits 2-5) and in the event of backup dispensation for lost scheduled medication.

Form Instructions:

The Microbiome Scheduled Medication (P3_MED) form must be completed **every** time scheduled medication is dispensed.

Following the loss of medication, complete a new Microbiome Scheduled Medication (P3_MED) form with the current date and the visit number corresponding to the last visit completed in the upper right-hand corner. Indicate backup medication in Q1000.

Within 24 hours of distributing backup medication, immediately fax the Microbiome Scheduled Medication (P3_MED) form to the project coordinator at the DCC. In the comment field provided, describe the circumstances regarding the dispensation of backup medication. This comment field is not entered into the AsthmaNet database. For more information on backup drug procedures, see Section 5 of this MOP and the Study Medications (asthmatics only) discussion in Section 2.

If backup medication is dispensed, complete the Microbiome Scheduled Medication (P3_MED) form and enter it as a single form. For example, when scheduled medication is dispensed at Visit 4, complete the packet Visit 4 Microbiome Scheduled Medication (P3_MED) form. If the participant loses the Diskus dispensed at regular Visit 4, generate a backup study medication number and complete the Microbiome Scheduled Medication (P3_MED) form for the backup medication dispensation. Enter this form as a Visit 4 single form at the time of backup medication dispensation.

Question 1010 and Label. At Visits 2-4, cut the label from the empty diskus pouch and adhere to the P3_MED form in the box under Q1010 by completely overlaying the label with clear packaging tape.

Copy the vial number into field Q1010.

After affixing the label, the coordinator should sign and date the source documentation box provided (Q1020-1030).

4.2.25 Microbiome Significant Asthma Exacerbation (P3_SIGEX)

Purpose: This form outlines the significant asthma exacerbation criteria to determine if a participant experienced an event during the Microbiome study.

Who: An AsthmaNet coordinator completes the form.

When: Visits 0-6 Asthmatic Participants Only

Form Instructions:

The Microbiome Significant Asthma Exacerbation (P3_SIGEX) form is completed **only** if the participant experiences a significant asthma exacerbation as defined in the Significant Asthma Exacerbation (asthmatics only) discussion in Section 2.

The Microbiome Significant Asthma Exacerbation (P3_SIGEX) form is a single form that should be entered and forwarded to the DCC within one week of form completion. If this form is completed between visits, specify the number of the last visit completed and the current date in the upper right-hand corner.

Questions 1000 - 1040. These questions outline the criteria for diagnosis of a significant asthma exacerbation as defined for this study. If the participant does **NOT** meet at least one of these criteria, **do not complete this form**.

Question 1000. Refer to the participant's P3_ASTHMA_LOG to answer Q1000. If the participant used the value recorded in the "To the Participant" section (high rescue use value) on the P3_ASTHMA_LOG or more rescue albuterol inhalations a day for at least two days in a row, the criterion is met. If Q1010 on the P3_ASTHMA_LOG for the day corresponding to the sigex date is greater than the high rescue use value, and Q1010 for the day corresponding to the day before the sigex date is greater than the high rescue use value, answer Q1000 on the P3_SIGEX form as 'Yes'. Both of these diary days need to be greater than the high rescue use value for Q1000 to be answered 'Yes', otherwise answer Q1000 'No'.

If the P3_SIGEX form is completed at or prior to Visit 2, Q1000 should be answered 'N/A'.

Question 1010. To determine if the participant used his/her rescue inhaler greater than or equal to 16 puffs per 24 hours, refer to the day corresponding to the date of

the sigex. If Q1010 on the P3_ASTHMA_LOG is greater than or equal to 16 for that diary day, respond 'Yes' to Q1010 on the P3_SIGEX form. Otherwise, respond 'No'.

If the P3_SIGEX form is completed at or prior to Visit 1, Q1010 should be answered 'N/A'.

Question 1020. If the P3_SIGEX form is completed between visits, and spirometry testing was not done, Q1020 should be answered 'N/A'. Refer to Q1030 on the participant's spirometry data collected on the most recent Spirometry Testing (SPIRO) form. The baseline value is Q1030 on the Spirometry Testing (SPIRO) form at Visit 1. To calculate the participant's baseline FEV_1, multiply Q1030 on the baseline SPIRO form by .80. Compare the participant's baseline FEV_1 to the FEV_1 at the current visit. If the participant's FEV_1 at the current visit is less than 80% of the participant's baseline FEV_1, answer 'Yes', otherwise answer 'No'.

If the P3_SIGEX form is completed at Visits 0-1, Q1020 should be answered 'N/A'.

Question 1030. If the P3_SIGEX form is completed between visits, and spirometry testing was not done, or if P3_SIGEX form is completed at Visit 0 answer 'N/A'. Refer to Q1040 on the participant's spirometry data collected on the most recent Spirometry Testing (SPIRO) form. If this value is less than 50, the criterion is met.

Question 1050. If any of the shaded boxes is completed in Q1000-1040, the participant experienced a significant asthma exacerbation. If 'Yes', and the participant has not yet undergone bronchoscopy, the participant is ineligible for the study. Proceed with completion of the Microbiome Significant Asthma Exacerbation (P3_SIGEX) form and then complete the MIcrobiome Termination of Study Participation (P3_TERM_A) form. Complete the rest of the P3_SIGEX form and record the event on the Clinical Adverse Events (AECLIN) form using ICD-9 code 493.92. If non-study medication was taken for treatment of the significant asthma exacerbation, record the medication on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form and link the medication to the significant asthma exacerbation event. Do this by recording the event record ID in Q1020 on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form.

If the completed form indicates the participant did not experience a significant asthma exacerbation, do not complete the rest of the form and do **not** submit the form to the DCC.

If 'Yes' and the participant has undergone bronchoscopy, proceed with completion of the Microbiome Significant Asthma Exacerbation (P3_SIGEX) form. Asthmatic

participants experiencing an asthma exacerbation as a result of bronchoscopy at Visit 2 must not undergo the bronchoscopy procedure at Visit 5.

Question 1060. Record the date when the exacerbation criteria is met. If multiple criteria were met to indicate a significant asthma exacerbation, record the earliest date criterion was confirmed.

Question 1130. If the participant was hospitalized, complete the Serious Adverse Event Reporting (SERIOUS) form.

Question 1180-1230. If the participant required treatment with non-study inhaled corticosteroids, oral corticosteroids, IM or IV steroids, antibiotics, or other medications, record the details on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form. Link the medication to the significant asthma exacerbation event on the Clinical Adverse Events (AECLIN) form. Note that inhaled steroids refer to ICS prescribed in addition to, or in place of, the study dose of Flovent.

Question 1240. Q1240 should be completed at Visits 2, 3, 5, or 6. This question should be answered based on information from the participant and discussion between the site coordinator and the attending physician. Determine the likelihood, on a scale of 1 to 5, that the asthma exacerbation was related to the bronchoscopy procedure.

4.2.26 Microbiome Termination of Study Participation Asthmatics (P3_TERM_A)

Purpose: The date and the primary reason for a participant's termination of study participation are recorded on this form.

Who: An AsthmaNet coordinator completes the form.

When: Visits 0-6 Asthmatic Participants Only

Note: This form is completed at Visit 6 for those Asthmatic participants who complete the entire Microbiome study. It may be completed at regular study visits (Visits 1-5) when the participant withdraws consent, becomes pregnant, or is terminated by performance site staff.

Form Instructions:

Visit Date: This date should be the date the form was completed. It may not necessarily be the same as the most recent regular visit date. For example, if the participant is being terminated due to loss to follow up, the visit date would be the date the coordinator completed this form to document the termination, NOT the visit date of the last regular visit.

If a participant withdraws consent or is terminated from the Microbiome study during a visit, specify the number of the current visit and the current visit date in the upper right-hand corner. For example, if the participant terminates during Visit 5, then the visit number on the form should be '5.' This form is entered into the AsthmaNet database as a single form.

If a non-randomized participant withdraws between visits, submit the Microbiome Termination of Study Participant (P3_TERM_A) form with the number of the last visit completed in the upper right-hand corner. For instance, a participant could be terminated from the Microbiome study following Visit 1 due to moving out of the area. In this case, the Microbiome Termination of Study Participant (P3_TERM_A) form should be entered as a single form with the last visit number completed in the upper right-hand corner of the form, Visit 1.

Question 1020. If Q1010 is answered 'Participant,' complete Q1020 and Q1030D, if applicable, and skip to the signatures section of the form. Otherwise, skip to Q1040 and complete the rest of the form.

Question 1030D. An explanation should be provided for Q1030D if Q1020 was answered 1, 2, 6, 7, 8, or 10. If an explanation is provided, enter the full explanation (up to 100 characters) into the AsthmaNet database; otherwise, leave the field blank during data entry.

Question 1040. If the participant is male, Q1040 should be answered 'N/A.' If the participant is female and surgically sterile or postmenopausal, Q1040 should be answered 'No.' Q1040 should be answered 'Yes' if the participant becomes pregnant during the course of the Microbiome study.

Question 1160D. An explanation should be provided for Q1160D if Q1050, Q1080, Q1090, Q1100, Q1110, Q1120, Q1130, Q1140, and/or Q1150 was answered 'Yes.' If an explanation was provided, enter the full explanation (up to 100 characters) into the AsthmaNet database. Otherwise, leave the field blank during data entry.

Question 1170. At least one of the questions in Q1040-1150 must be answered 'Yes' if clinical staff terminated the participant. Of the questions Q1040-1150 marked 'Yes', indicate the letter associated with the **primary** reason for termination in Q1170.

This form requires the signatures of the coordinator and AsthmaNet investigator to verify that all data collected for this participant are correct to the best of their knowledge.

Questions 1180 and 1200. If a signature is not present, this field should be left missing during data entry.

Any AsthmaNet investigator (site director, Principal Investigator, or other) may sign field Q1200 to verify that all data collected for this participant are correct to the best of their knowledge.

4.2.27 Microbioime Termination of Study Participation Control (P3_TERM_C)

Purpose: The date and the primary reason for a participant's termination of study participation are recorded on this form.

Who: An AsthmaNet coordinator completes the form.

When: Visits 0-2 Control Participants Only

Note: This form is completed at Visit 2 for those Asthmatic participants who complete the entire Microbiome study. It may be completed at a regular study visit (Visit 1) when the participant withdraws consent, becomes pregnant, or is terminated by performance site staff.

Form Instructions:

Visit Date: This date should be the date the form was completed. This date may not necessarily be the same as the most recent regular visit date. For example, if the participant is being terminated due to loss to follow up, the visit date would be the date the coordinator completed this form to document the termination, NOT the visit date of the last regular visit.

Question 1020. If Q1010 is answered 'Participant,' complete Q1020 and Q1030D, if applicable, and skip to the signatures section of the form. Otherwise, skip to Q1040 and complete the rest of the form.

Question 1030D. An explanation should be provided for Q1030D if Q1020 was answered 1, 2, 6, or 10. If an explanation is provided, enter the full explanation (up to 100 characters) into the AsthmaNet database; otherwise, leave the field blank during data entry.

Question 1040. If the participant is male, Q1040 should be answered 'N/A.' If the participant is female and surgically sterile or postmenopausal, Q1040 should be answered 'No.' Q1040 should be answered 'Yes' if the participant becomes pregnant during the course of the Microbiome study.

Question 1160D. An explanation should be provided for Q1160D if Q1050, Q1100, Q1110, Q1130, and/or Q1150 was answered 'Yes.' If an explanation was provided,

enter the full explanation (up to 100 characters) into the AsthmaNet database. Otherwise, leave the field blank during data entry.

Question 1170. At least one of the questions in Q1040-1150 must be answered 'Yes' if clinical staff terminated the participant. Of the questions Q1040-1150 marked 'Yes', indicate the letter associated with the **primary** reason for termination in Q1170.

This form requires the signatures of the coordinator and AsthmaNet investigator to verify that all data collected for this participant are correct to the best of their knowledge.

Questions 1180 and 1200. If a signature is not present, this field should be left missing during data entry.

Any AsthmaNet investigator (site director, Principal Investigator, or other) may sign field Q1200 to verify that all data collected for this participant are correct to the best of their knowledge.

4.2.28 Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO)

Purpose: To record the outcome measurements from the participant's post-bronchodilator spirometry procedure.

Who: The Pulmonary Function Technician completes the form.

When: Visits 1, 2, 4, and 5 Asthmatic Participants Only
Visits 1 and 2 Control Participants Only

Form Instructions:

If post-albuterol (4 puffs) spirometry testing is completed at Visits 1, 2, 4, or 5, the Spirometry Testing Report (SPIRO_RPT) should be marked 'No' during entry in the Participant Data module. The Post-Albuterol (4 puffs) Spirometry Testing Report (PALB4_RPT) should be marked 'Yes' in this scenario. The spirometry session data is included on the Post-Albuterol (4 puffs) Spirometry Testing Report (PALB4_RPT) and a separate Spirometry Testing Report (SPIRO_RPT) does not need to be printed.

For more information on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form, see Section 10 of the AsthmaNet General MOP.

4.2.29 Serious Adverse Event Reporting Form (SERIOUS)

Purpose: To record the details of each serious adverse event.

Who: An AsthmaNet coordinator completes the form in collaboration with the Principal Investigator.

When: Visits 0-6 Asthmatic Participants Only
Visits 0-2 Control Participants Only

Form Instructions:

Question 1020. When answering this question, the study Flovent® Diskus and matching placebo, and open-label Flovent® given out as part of the study should be considered "study drug." However, Ventolin® and rescue prednisone should not be considered "study drug" when answering this question.

For more information on the Serious Adverse Event Reporting Form (SERIOUS), see Section 10 of the AsthmaNet General MOP.

4.2.30 Spirometry Testing (SPIRO)

Purpose: To record the outcome measurements from the participant's pre-bronchodilator spirometry procedure

Who: The Pulmonary Function Technician completes the form.

When: Visits 1-6 Asthmatic Participants Only
Visits 1-2 Control Participants Only

Form Instructions:

If methacholine challenge testing was completed at Visits 1 and 4, the Spirometry Testing Report (SPIRO_RPT) should be marked 'No' during entry in the Participant Data module. The Methacholine Challenge Testing Report (METHA_RPT) should be marked 'Yes' in the database. The spirometry session data is included on the Methacholine Challenge Testing Report (METHA_RPT) and a separate Spirometry Testing Report (SPIRO_RPT) does not need to be printed.

Similarly, if post-albuterol (4 puffs) spirometry testing is completed at Visits 1, 2, 4, or 5, the Spirometry Testing Report (SPIRO_RPT) should also be marked 'No.' The Post-Albuterol (4 puffs) Spirometry Testing Report (PALB4_RPT) should be marked 'Yes' in this scenario. The spirometry session data is included on the Post-Albuterol (4 puffs) Spirometry Testing Report (PALB4_RPT) and a separate Spirometry Testing Report (SPIRO_RPT) does not need to be printed.

For more information on the Spirometry Testing (SPIRO) form, see Section 10 of the AsthmaNet General MOP.

4.3 Administrative Forms

Administrative forms facilitate processing of the participant and visit flow by the performance sites and the DCC. They are not entered into the AsthmaNet database and they are not submitted to the DCC in most cases. The following is a list of all Microbiome study administrative forms and related instructions:

Administrative Form Name	Form Code
Microbiome Accountability Log 1% Lidocaine	P3_LIDOCAINE_1%
Microbiome Accountability Log 2% Lidocaine	P3_LIDOCAINE_2%
Microbiome Accountability Log 0.9% Sodium Chloride 250 mL Bag	P3_SALINE_BAG
Microbiome Accountability Log 0.9% Sodium Chloride 10 mLSyringe	P3_SALINE_SYRINGE
Microbiome Allergen-specific IgE Serum Sample Log	P3_SIGE_SAMP_LOG
Microbiome Biomarker Serum Sample Log	P3_BIOM_SAMP_LOG
Microbiome Drug Dispensing Log: Flovent® Inhaler	P3_DRG_FLO
Microbiome Drug Accountability Log: Post-Randomization Study Medications	P3_DRG_SCH
Microbiome Drug Accountability Log: Post-Randomization Study Medications	P3_DRG_SCH_ALL
Microbiome Drug Dispensing Log: Rescue Prednisone Tablets	P3_DRG_PRED
Microbiome Drug Dispensing Log: Ventolin® (RESCUE) Inhaler	P3_DRG_RESC
Microbiome Participant Assignment Log	P3_LOG
Microbiome Phadiatop and IgE Serum Sample Log	P3_PHAD_SAMP_LOG
Microbiome Phone Contact Form	P3_PHONE_CONTACT
Microbiome Stool Sample Log	P3_STOOL_SAMP_LOG
Microbiome Visit Procedure Checklists	P3_VISIT0, P3_VISIT1, P3_VISIT2A, P3_VISIT2C, P3_VISIT3, P3_VISIT3A, P3_VISIT4, P3_VISIT5, P3_VISIT6

4.3.1 Microbiome Accountability Log 1% Lidocaine (P3_LIDOCAINE_1%)

Purpose: This form must be completed for each participant every time the bronchoscopy procedure is performed.

Who: An AsthmaNet coordinator completes the form.

When: Visits 2 and 5 Asthmatic Participants Only
Visit 2 Control Participants Only

Form Instructions:

For the 1% lidocaine that will be used for the bronchoscopy procedure, complete an entry on the Microbiome Accountability Log 1% Lidocaine (P3_LIDOCAINE_1%) by recording the visit number, visit date, participant ID, participant intials, lot number, expiration date, number dispensed, current balance, and dispenser's intials.

For further information on 1% lidocaine, see the Bronchoscopy MOP.

This log will be reviewed during AsthmaNet site visits.

4.3.2 Microbiome Accountability Log 2% Lidocaine (P3_LIDOCAINE_2%)

Purpose: This form must be completed for each participant every time the bronchoscopy procedure is performed.

Who: An AsthmaNet coordinator completes the form.

When: Visits 2 and 5 Asthmatic Participants Only
Visit 2 Control Participants Only

Form Instructions:

For the 2% lidocaine that will be used for the bronchoscopy procedure, complete an entry on the Microbiome Accountability Log 2% Lidocaine (P3_LIDOCAINE_2%) by recording the visit number, visit date, participant ID, participant intials, lot number, expiration date, number dispensed, current balance, and dispenser's intials.

For further information on 2% lidocaine, see the Bronchoscopy MOP.

This log will be reviewed during AsthmaNet site visits.

4.3.3 Microbiome Accountability Log 0.9% Sodium Chloride 250 mL Bag (P3_SALINE_BAG)

Purpose: This form must be completed for each participant every time the bronchoscopy procedure is performed.

Who: An AsthmaNet coordinator completes the form.

When: Visits 2 and 5 Asthmatic Participants Only
Visit 2 Control Participants Only

Form Instructions:

For the 0.9% Sodiume Chloride 250 mL Bag that will be used for the bronchoscopy procedure, complete an entry on the Microbiome Accountability Log 0.9% Sodium Chloride 250 mL Bag (P3_SALINE_BAG) by recording the visit number, visit date, participant ID, participant intials, lot number, expiration date, number dispensed, current balance, and dispenser's intials.

For further information on 0.9% Sodium Chloride 250 mL Bag, see the Bronchoscopy MOP.

This log will be reviewed during AsthmaNet site visits.

4.3.4 Microbiome Accountability Log 0.9% Sodium Chloride 10 mL Syringe (P3_SALINE_SYRINGE)

Purpose: This form must be completed for each participant every time the bronchoscopy procedure is performed.

Who: An AsthmaNet coordinator completes the form.

When: Visits 2 and 5 Asthmatic Participants Only
Visit 2 Control Participants Only

Form Instructions:

For the 0.9% Sodiume Chloride 10 mL Syringe that will be used for the bronchoscopy procedure, complete an entry on the Microbiome Accountability Log 0.9% Sodium Chloride 10 mL Syringe (P3_SALINE_SYRINGE) by recording the visit number, visit date, participant ID, participant intials, lot number, expiration date, number dispensed, current balance, and dispenser's intials.

For further information on 0.9% Sodium Chloride 10 mL Syringe, see the Bronchoscopy MOP.

This log will be reviewed during AsthmaNet site visits.

4.3.5 Microbiome Allergen-specific IgE Serum Sample Log (P3_SIGE_SAMP_LOG)

Purpose: This form must be completed each time blood is drawn and processed at the site for allergen-specific IgE.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1 Asthmatic Participants Only
Visit 1 Control Participants Only

Form Instructions:

After collecting one 10 ml red-top vacutainer with the participant's blood, complete an entry for the blood draw on the Microbiome Allergen-specific Serum Sample Log (P3_SIGE_SAMP_LOG) by recording the participant's Microbiome ID number, visit number, and collection date/time.

After the blood sample has been allowed to clot at room temperature between 20 minutes and 1 hour, complete the time spinning is initiated on the Microbiome Allergen-specific Serum Sample Log (P3_SIGE_SAMP_LOG).

After labeling the serum vial with a barcode label generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system, complete the microtube barcode number and sample volume on the Microbiome Allergen-specific Serum Sample Log (P3_SIGE_SAMP_LOG).

After storing the serum sample at -80 degrees Celsius, record the date/time the sample is placed in the freezer and the current freezer temperature on the Microbiome Allergen-specific Serum Sample Log (P3_SIGE_SAMP_LOG).

This log will be reviewed during AsthmaNet site visits.

4.3.6 Microbiome Biomarker Serum Sample Log (P3_BIOM_SAMP_LOG)

Purpose: This form must be completed each time blood is drawn and processed for Microbiome Biomarker analysis.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1 Asthmatic Participants Only
Visit 1 Control Participants Only

Form Instructions:

After collecting one 10 ml red-top vacutainer with the participant's blood, complete an entry for the blood draw on the Microbiome Biomarker Serum Sample Log (P3_BIOM_SAMP_LOG) by recording the participant's Microbiome ID number, visit number, and collection date/time.

After the blood sample has been allowed to clot at room temperature between 20 minutes and 1 hour, complete the time spinning is initiated on the Microbiome Biomarker Serum Sample Log (P3_BIOM_SAMP_LOG).

After labeling the serum vial with a barcode label generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system, complete the microtube barcode number and sample volume on the Microbiome Biomarker Serum Sample Log (P3_BIOM_SAMP_LOG).

After storing the serum sample at -80 degrees Celsius, record the date/time the sample is placed in the freezer and the current freezer temperature on the Microbiome Biomarker Serum Sample Log (P3_BIOM_SAMP_LOG).

This log will be reviewed during AsthmaNet site visits.

4.3.7 Microbiome Drug Dispensing Log: Flovent® Inhaler (P3_DRG_FLO)

Purpose: This is a log for recording all Flovent® inhalers dispensed and returned during the Microbiome study.

Who: An AsthmaNet coordinator completes the log.

When: This log is completed at Visit 5 Asthmatic Participants Only, or every time a Flovent® inhaler is dispensed or returned.

Form Instructions:

When a Flovent® inhaler is dispensed or returned, complete the appropriate part of the Microbiome Drug Dispensing Log: Flovent® Inhaler (P3_DRG_FLO).

When a Flovent® inhaler is dispensed, record the visit number, visit date, the participant's ID, the participant's initials and the dispenser's initials in the next available row on the log. The balance entered for the new row should be 1 less than the balance in the prior row. The recorded balance in the last completed row is the total number of remaining, unissued Flovent® inhalers at the performance site. When the balance gets low (<**4** inhalers left), contact the DCC for replenishments.

When a Flovent® inhaler is returned, record the date returned and the collector's initials. If a Flovent® inhaler is lost or not returned, note this in the Date Returned column. Indicate the reason the Flovent® inhaler was not returned, if known.

See the Drug Logging and Dispensation discussions in Section 5 for more information.

This log will be reviewed during AsthmaNet site visits.

4.3.8 Microbiome Drug Accountability Log: Post-Randomization Study Medications (P3_DRG_SCH)

Purpose: This is a log for recording all Flovent® Diskus or matching placebo (study medications) inhalers dispensed and returned during the Microbiome study for an individual participant.

Who: An AsthmaNet coordinator completes the log.

When: Visits 2-4 Asthmatic Participant Only

Form Instructions:

When Diskus inhalers are dispensed, record the date dispensed, the dispenser's initials, and the scheduled Diskus inhaler's three-digit ID.

When medications are returned, record the date returned, the collector's initials, and whether the scheduled Diskus inhaler was returned (indicated by checking in the box).

See the Drug Logging and Dispensation discussions in Section 5 for more information.

This log will be reviewed during AsthmaNet site visits.

4.3.9 Microbiome Drug Accountability Log: Post-Randomization Study Medications (P3_DRG_SCH_ALL)

Purpose: This is a log for recording all Flovent® Diskus or matching placebo (study medications) inhalers dispensed and returned during the Microbiome study for all participants at a site.

Who: An AsthmaNet coordinator completes the log.

When: Visits 2-4 Asthmatic Participants Only

Form Instructions:

When Diskus inhalers are dispensed, record the date dispensed, the participant ID, the participant initials, the lot number, the expiration date, the number dispensed, current balance, and dispenser's intials. The balance entered for the new row should be 1 less than the balance in the prior row. The recorded balance in the last completed row is the total number of remaining, unissued Flovent® Diskus or matching placebo inhalers at the performance site.

When medications are returned, record the number returned, the date returned, and the collector's initials.

See the Drug Logging and Dispensation discussions in Section 5 for more information.

This log will be reviewed during AsthmaNet site visits.

4.3.10 Microbiome Drug Dispensing Log: Rescue Prednisone Tablets (P3_DRG_PRED)

Purpose: This is a log for recording all rescue prednisone tablets dispensed and returned during the Microbiome study.

Who: An AsthmaNet coordinator completes the log.

When: Visit 2 Asthmatic Participants Only

Form Instructions:

When rescue prednisone tablets are dispensed or returned, complete the appropriate part of the Microbiome Drug Dispensing Log: Rescue Prednisone Tablets (P3_DRG_PRED).

When rescue prednisone tablets are dispensed, record the date dispensed, the participant's ID, the participant's initials, the dispenser's initials and the number of tablets dispensed in the next available row on the log.

When rescue prednisone tablets are returned, record the date returned and the collector's initials. If rescue prednisone tablets are lost or not returned, note this in the "If Not Returned" column. Indicate the reason the rescue prednisone tablets were not returned, if known.

See the Drug Logging and Dispensation discussions in Section 5 for more information.

This log will be reviewed during AsthmaNet site visits.

4.3.11 Microbiome Drug Dispensing Log: Ventolin® (RESCUE) Inhaler (P3_DRG_RESC)

Purpose: This is a log for recording all Ventolin® (RESCUE) inhalers dispensed and returned during the Microbiome study.

Who: An AsthmaNet coordinator completes the log.

When: This log is completed at Visits 1-5 Asthmatic Participants Only, or every time a Ventolin® inhaler is dispensed or returned.

Form Instructions:

When a Ventolin® inhaler is dispensed or returned, complete the appropriate part of the Microbiome Drug Dispensing Log: Ventolin® (RESCUE) Inhaler (P3_DRG_RESC).

When a Ventolin® inhaler is dispensed, record the visit number, visit date, the participant's ID, the participant's initials and the dispenser's initials in the next available row on the log. The balance entered for the new row should be 1 less than the balance in the prior row. The recorded balance in the last completed row is the total number of remaining, unissued Ventolin® inhalers at the performance site. When the balance gets low (<**10** inhalers left), contact the DCC for replenishments.

When a Ventolin® inhaler is returned, record the date returned and the collector's initials. If a Ventolin® inhaler is lost or not returned, note this in the Date Returned column. Indicate the reason the Ventolin® inhaler was not returned, if known.

See the Drug Logging and Dispensation discussions in Section 5 for more information.

This log will be reviewed during AsthmaNet site visits.

4.3.12 Microbiome Participant Assignment Log (P3_LOG)

Purpose: This form is a log of all participants enrolled in the Microbiome study.

Who: An AsthmaNet coordinator completes the log.

When: Visits 0, 2-4 Asthmatic Participants Only
Visit 0 Control Participants Only

Form Instructions:

The Microbiome Participant Assignment Log (P3_LOG) must be used each time a **new** participant ID number is assigned. A new participant ID number is assigned by completing the next available blank entry on the log at Visit 0. The protocol ID, site ID, and participant ID will be pre-filled on the assignment log printed from Forms: Microbiome: Admin Forms section on the AsthmaNet secure website.

Participant initials must have three letters. The letter "X" should be used for an initial only if a participant does not have a middle initial. The participant's initials must be the same initials entered in the AsthmaNet Registry module.

The participant's name should be written last name first, followed by first name on the Microbiome Participant Assignment Log (P3_LOG).

At Visits 2-4, record the assigned Diskus numbers at each visit on the log.

This log will be reviewed during AsthmaNet site visits.

4.3.13 Microbiome Phadiatop and IgE Serum Sample Log (P3_PHAD_SAMP_LOG)

Purpose: This form must be completed each time blood is drawn and processed for Microbiome Biomarker analysis.

Who: An AsthmaNet coordinator completes the form.

When: Visit 1 Asthmatic Participants Only
Visit 1 Control Participants Only

Form Instructions:

After collecting one 5 ml red-top vacutainer with the participant's blood, complete an entry for the blood draw on the Microbiome Phadiatop and IgE Serum Sample Log (P3_PHAD_SAMP_LOG) by recording the participant's Microbiome ID number, visit number and collection date/time.

After the blood sample has been allowed to clot at room temperature between 20 minutes and 1 hour, complete the time spinning is initiated on the Microbiome Phadiatop and IgE Serum Sample Log (P3_PHAD_SAMP_LOG).

After labeling the serum vial with a barcode label generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system, complete the microtube barcode number and sample volume on the Microbiome Phadiatop and IgE Serum Sample Log (P3_PHAD_SAMP_LOG).

After storing the serum sample at 4 degrees Celsius (refrigerated), record the date/time the sample is placed in the refrigerator and the current refrigerator temperature on the Microbiome Phadiatop and IgE Serum Sample Log (P3_PHAD_SAMP_LOG).

This log will be reviewed during AsthmaNet site visits.

4.3.14 Microbiome Phone Contact Form (P3_PHONE_CONTACT)

Purpose: This form guides the coordinator in completing a scheduled phone contact with the participant. The questions assist in checking the participant's asthma control, scheduled medication usage, and medical care.

Who: An AsthmaNet coordinator interviews the participant while completing this form.

When: Visit 3A Asthmatic Participants Only

Form Instructions:

Complete the gray box with coordinator ID, date, time, and if contact occurred for each attempt made to contact the participant. Record any comments regarding the contact attempt in the Contact Occurred column.

When contact is made with the participant, ask him or her to refer to the Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG) as the coordinator will be asking the participant questions regarding RESCUE Ventolin® use, compliance, and treatment failure assessment.

Question 5. Before calling the participant, the coordinator should record the participants high rescue use from the Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG) in the question text. If the participant indicates that he or she has used the same amount or more than his or her High Rescue Use value (recorded on the Microbiome Asthma Monitoring Log (P3_ASTHMA_LOG) of RESCUE Ventolin® on any day since the last visit, complete Q5a for assessment of significant asthma exacerbation.

Question 8. If the participant indicates that he or she experienced a medical problem since the last visit, record the event on the Clinical Adverse Events (AECLIN) form at the next visit.

Question 10. If the participant indicates that he or she took any new medications other than those given as part of the study since the last visit, record the medication on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form at the next visit.

Question 11. If the participant indicates that he or she had any changes to non-study medications since the last visit, record the medication change on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form at the next visit. Depending on the type of medication that was changed, the Concomitant Medications for Non-Asthma Drugs (CMED_NON) form may instead need to be updated.

If, based on the results of the phone contact, the coordinator feels the participant has had a significant asthma exacerbation, the coordinator should review the proper procedure for treating an exacerbation (If Your Asthma Gets Worse (P3_ASWORSE) handout, Participant ID (P3_ID) card), and should proceed as outlined in the Significant Asthma Exacerbation (asthmatics only) discussion in Section 2.

This form will be reviewed during AsthmaNet site visits.

For use only at the performance site – DO NOT forward to the DCC.

4.3.15 Microbiome Stool Sample Log (P3_STOOL_SAMP_LOG)

Purpose: This form must be completed each time stool is collected.

Who: An AsthmaNet coordinator completes the form.

When: Visit 2 Asthmatic Participants Only
Visit 2 Control Participants Only

Form Instructions:

After collecting the stool collection tube, complete an entry for the stool sample on the Microbiome Stool Sample Log (P3_STOOL_SAMP_LOG) by recording the participant's Microbiome ID number, visit number, collection date and date of sample receipt at the clinic.

After labeling the stool collection tube with a barcode label generated through the Biological Sample Tracking (BST) module of the AsthmaNet database management system, complete the tube barcode number on the Microbiome Stool Sample Log (P3_STOOL_SAMP_LOG).

After storing the stool sample at -80 degrees Celsius, record the date/time the sample is placed in the freezer and the current storage temperature on the Microbiome Stool Sample Log (P3_STOOL_SAMP_LOG).

This log will be reviewed during AsthmaNet site visits.

4.3.16 Microbiome Visit Procedure Checklists (P3_VISIT0, P3_VISIT1, P3_VISIT2A, P3_VISIT2C, P3_VISIT3, P3_VISIT3A, P3_VISIT4, P3_VISIT5, P3_VISIT6)

Purpose: To provide the coordinator with a checklist of all procedures and forms completed during a visit.

Who: An AsthmaNet coordinator completes the form.

When: At the specified visit

Form Instructions:

These checklists serves as guides for the coordinator and should be sent to the DCC, in front of the visit packet, with the other forms in the packet.

For all procedures and forms, indicate whether or not the procedure or form was completed. If it was not completed, indicate the reason in the comment field.

At Visits 3, 3A, 4, and 5: If a visit is missed, complete the checklist indicating the missed visit and document if any other actions were completed (i.e., dispensation of additional study medications, etc.). The completed checklist should be filed at the performance site and does not need to be sent to the DCC.

Procedures should be followed in the order they are presented on the visit checklist for applicable visits. If certain procedures, such as pulmonary function testing and questionnaire completion, are performed out of order, a protocol deviation will be assigned.

This form is not entered during data entry.

MICROBIOME BRONCHOSCOPY

MANUAL OF OPERATIONS

I. AsthmaNet Bronchoscopy

I.1. Purpose

To provide guidelines for the use of fiberoptic bronchoscopy, tissue handling and analyses for the AsthmaNet Microbiome Protocol. These guidelines are based on National Institutes of Health, American College of Chest Physicians, and American Thoracic Society recommendations for investigative studies [1, 2]. and are generally concordant with the MOP's for bronchoscopy for the ACRN, SARP and SPIROMICS networks.

The procedures outlined herein are meant to serve as guidelines, with room for institutional flexibility for issues including sedation and topical anesthesia methods, and participant monitoring.

The overall purpose of the procedures described here is to obtain samples of the bronchial epithelium and of bronchoalveolar lining fluid with minimal contamination from oropharyngeal secretions. Because avoiding all such contamination is not possible, procedures for cleansing the mouth, and for sampling oral secretions as well as the contents of the bronchoscope's suction channel immediately before passing the bronchoscope through the glottis are described. At least a subset of these will be analyzed for their microbial content, to compare to the microbial content of the bronchial brushings, in addition to subsets of sputum and bronchoalveolar lavage. The procedures described here differ slightly from those of the ACRN MOP for the "Macrolides in Asthma" trial (MIA) Add-on Study, in which bronchial brushings were also obtained for microbial analysis. To reduce contamination of the bronchial mucosa by oral secretions aspirated during bronchoscopy, tongue scraping and mouth wash are now to be performed before topical anesthesia is applied to the oropharynx and larynx. Additionally, the bronchoscope is now to be removed after lidocaine is applied to the vocal cords, the suction channel flushed with sterile saline to be saved for microbial analysis, and the bronchoscope reinserted and passed through the cords. To the same end, this MOP also emphasizes the importance of aspirating oral secretions by Yankauer suction during the bronchoscopy and of minimizing suctioning fluid back through the bronchoscope channel before the bronchial brushings are obtained.

1 Busse, et al. Investigative Bronchoprovocation and Bronchoscopy in Airway Diseases. Am J Respir Crit Care Med 2005; 172: 807-816.

2 Jarjour, et al. Investigative Use of Bronchoscopy in Asthma. Am J Respir Crit Care Med 1998; 157: 692-697.

Inclusion/Exclusion Criteria

Inclusion:

- Participants enrolled in the Asthma Microbiome Proof of Concept study who have consented to research bronchoscopy and in whom appropriate laboratory tests have been obtained.

Exclusions:

- Age > 60
- History of cardiac disease or other comorbid condition severe enough to significantly increase risks based on investigator discretion (eg., platelet count and BUN/Cr)
- Post-BD FEV1 < 70% predicted
- Use of anticoagulation (patients on warfarin or clopidogrel will be excluded, patients on aspirin or other NSAID can be studied if none has been taken for > 7 days)

I.2. Supplies and Equipment for sample collection, processing, and storage

Note: Listed items below are needed in bronchoscope suite or readily nearby for sample processing immediately after collection. See also specific "**Sample Processing**" instructions in Section I.9 for further instructions and ensure necessary equipment/supplies are available for additional processing prior to shipment.

For blood sampling (immunophenotyping):

1. Two 5 mL Streck lavender/black striped top Cyto-Chex BCT blood collection tubes (Samples to be kept at room temperature until shipped same day by Priority Overnight Monday – Wednesday and First Overnight on Thursday to Ansel Lab at UCSF).
2. One 4 mL lavender-top tube for submission to local clinical lab for CBC with differential cell count.

For tongue scraping and oral rinse (microbiome analysis)

1. One 10 mL pre-filled sterile saline syringe (provided by DCC)
2. One 60 mL sterile collection cup containing 20 mL of RNALater, labeled "ORAL WASH" (will include both tongue scraping and oral rinse)
3. Three clean disposable tongue scrapers (Breath Rx tongue scraper; http://www.smilox.com/breathrx-tonguecleaner.cfm)
4. One 50 mL conical polypropylene tube (Corning)

For Saline Flush of Bronchoscope Suction Channel (before and after anesthetizing upper airways – microbiome analysis)

1. 250 mL sterile saline bag (to be used for BAL)
2. Two 10 mL sterile syringes (for drawing up 10 mL of saline for scope channel flushes from 250 mL saline bag provided by DCC)
3. Two sterile 30-60 mL cups (for introduction of sterile saline to be suctioned back through bronchoscope suction channel)
4. Two sterile collection traps (for collecting saline suctioned back through scope suction channel)
5. Two 50 mL sterile conical polypropylene tubes, each containing 20 mL RNALater, one labeled as “SCOPE FLUSH 1” and one labeled as “SCOPE FLUSH 2”

For Epithelial Protected Brushings (microbiome analysis)

1. Five BARD Disposable Microbiology Brushes (ConMed, Catalog # 130, phone # (800) 448-6506)
2. Four 2.0 mL microtubes pre-filled with 1.0 mL of RNALater™ (provided by UCSF)
3. One 2.0 mL microtube pre-filled with 1.0 mL 35% glycerol (provided by UCSF)
4. Wire Cutters
5. Individually packaged alcohol preps

For Bronchoalveolar Lavage (will be separated into aliquots for immunophenotyping, microbiome analyses, and cytokine measurements)

1. >200 mL of warmed (37° C) sterile normal saline (0.9%) from 250 mL saline bag (provided by DCC)
2. Two 60 mL Luer-Lok sterile syringes
3. Three sterile specimen traps (different from above trap used to collect saline flush of bronchoscope)
4. Sterile beaker for mixing/pooling lavage fluid
5. One sterile 50 mL conical polypropylene centrifuge tube containing 20 mL RNAlater (labeled “BAL MICROBIOME”)
6. Two sterile 50 mL conical polypropylene centrifuge tubes
7. Two 15 mL conical polypropylene tubes (for remaining supernatant, labeled “BAL Supernatant”)
8. Twelve 2 mL screw cap vials (labeled “BAL Supernatant”)
9. Conical tube for 10 mL BAL (for cell count)

10. Materials for BAL cell count including PBS, Turks solution, hemocytometer, slides, Kwik-Diff stain
11. Immunophenotyping vial with Streck Cell Preservative (provided by UCSF)
12. Basin containing crushed ice for cooling samples to 4° C

For Nasal Brushing

1. Lidocaine 1% (used for topical anesthesia of lower airways)
2. DeVilbiss Atomizer model 15-RD
3. Disposable nasal speculum (Bionix)
4. Head light
5. Cytology brush (CytoSoft, Medical Packing Corporation, Cat no: CYB-1, individually wrapped – provided by DCC)
6. Two 2.0 mL screw cap vials with 1 mL of RNA later
7. Scissors
8. Alcohol swabs

Preparation of RNALater for collection tubes described above (Note: each site will need to aliquot RNALater into collection tubes other than those for bronchial brushes, which are provided by UCSF)**:**

RNALater (Ambion) should be pre-aliquoted in a certified biohazard hood PRIOR to bronchoscopy and sample collection. Use a Pipet aid and sterile 10 mL pipet tips. Record date the RNALater was opened on bottle. It can be used for up to 1 year and longer if no white precipitate forms. If a precipitate does form, heat it to 37° C and agitate to re-dissolve it. Please store RNALater at room temperature (25° C) or at 4° C. RNALater is used to preserve the integrity of RNA and DNA in cells for microbiome analysis. Mix sample with RNALater immediately after collection and keep on ice. Samples in RNALater should be kept at 4° C for at least 24 hours. Thereafter they can remain at 4° C for up to 4 weeks, or transferred to -80° C for indefinite storage. RNALater can be safely discarded down the sink and flushed with water. Note: it is known to react with hypochlorite solution (bleach).

a. For ORAL sample: Fill 60 mL sterile collection cup with 20 mL of RNALater
b. For Saline flush of bronchoscope: Fill two 50 mL sterile conical polypropylene tubes with 20 mL of RNALater
c. For BAL (aliquot for microbiome analysis): Fill one 50 mL sterile conical polypropylene tube with 20 mL RNALater

d. For Microbiological brushes: set aside four 2.0 mL microtubes containing 1.0 mL of RNALater™ and one 2.0 mL microtube containing 1.0 mL 35% glycerol sitting on ice (provided by UCSF)

e. For Nasal brushing: Fill two 2.0 mL microtubes with 1.0 mL of RNALater™

I.3. General Preparation

I.3.1. Universal Precautions

- Wear protective glasses and a mask.
- Wear gloves and use good hand washing technique. Avoid latex if latex allergic.
- Wear a protective gown.

I.3.2. Pre-Procedure Setup

1. Have gloves, safety glasses, masks and gowns available for bronchoscopy team
2. Check wall and/or portable wall suction for:
   a. Adequate vacuum
   b. Canister with Isosorb (liquid medical weight solidifier)
   c. Tubing and Yankauer tip
3. Check wall and/or portable O2 supply and regulator
4. Check wall and/or portable medical air supply and regulator
5. Check for all necessary stock and instruments
6. For tongue scraping and oral rinse: see above
7. For Participant preparation:
   a. 1% or 2% lidocaine (in atomizer or nebulizer) marked in 5 cc increments; all lidocaine will be purchased from **the same source** and stored at room temperature (provided by DCC)
   b. 1% lidocaine in specimen cup with 5 cc syringes for 2 cc aliquots
   c. Connect tubing to oxygen or compressed air to power atomizer
   d. 2% viscous lidocaine (for lubricating the scope and the bite block)
   e. Tongue blade, 6” Dacron tips and 4x4s
   f. Box of tissues and emesis basin
   g. Nasal cannula, nebulizer (with 2.5mg albuterol/3ml saline), and oxygen mask

8. For Insertion of intravenous access and blood draw for sample for immunophenotyping
    a. Intravenous catheter, tubing, three way stop-cock, 500 mL sterile D5W or 0.5N saline for “tko” infusion during procedure, per local bronchoscopy procedure custom
    b. Syringes to clear line of saline and for withdrawal of 20 mL blood sample for immunophenotyping, and for later infusion of drugs for conscious sedation (e.g., midazolam and fentanyl) and antagonists (flumazenil and naloxone)
    c. Two 5 mL Streck lavender/black striped top Cyto-Chex BCT blood collection tube, sitting at room temperature (will be shipped same day overnight to Ansel lab at UCSF)
    d. One 4 mL lavender top tube for submission to local clinical lab for CBC with differential cell count (at Visit 2 only)
9. For Bronchial brushings and Bronchoalveolar lavage – *Check for items listed above under Section I.3 which should include*:
    a. Five BARD Disposable Microbiology Brushes
    b. Four 2.0 mL microtubes pre-filled with 1.0 mL of RNALater and labeled with sample (“BRUSHING R”) sitting on ice (see above)
    c. One 2.0 mL microtube pre-filled with 1.0 mL 35% glycerol and labeled with sample (“BRUSHING G”) sitting on ice (see above)
    d. Individually packaged alcohol preps
    e. Wire cutters
    f. Sterile normal saline (0.9%) warmed to 37° C in water bath (>200 mL)
    g. Two 60 mL Luer-Lok sterile syringes
    h. Three sterile specimen traps
    i. One sterile 50 mL conical polypropylene tube containing 20 mL RNALater (labeled “BAL Microbiome”)
    j. Two sterile 50 mL conical tubes
    k. Conical tube for 10 mL BAL (for cell count)
    l. Materials for BAL cell count including PBS, Turks solution, hemocytometer, slides, Kwik-Diff stain
    m. Sterile beaker
    n. Bucket with crushed ice
10. Equipment and supplies for any special needs per physician order
11. Ensure access to up-to-date stat (“emergency, Crash” etc.) cart with:

- Allocated albuterol MDI and albuterol nebulizer
- Sedation reversal medications: naloxone and flumazenil

12. Monitoring system for recording pulse oximetry, blood pressure, and ECG
13. Leak test bronchoscope

I.4. Participant Preparation

1. Ensure informed consent has been obtained at bronchoscopy visit.
2. Confirm that participant has no history of bleeding disorder and has not taken aspirin or other NSAID within past 7 days.
3. Confirm that participant history and physical exam has been done by physician prior to procedure.
4. For patients over 45 years old, ensure that EKG has been obtained and reviewed within previous 6 weeks.
5. NPO 8 hours prior to procedure (no eating or drinking 8 hours prior).
6. Confirm that the participant has no allergies to medication or latex.
7. Medications per physician's order.
8. Participant should understand written instructions for "Post Bronchoscopy Instructions."
9. Collect baseline vitals signs (heart rate, blood pressure, respiratory rate, SpO_2 and temperature).
10. Pre bronchoscopy spirometry:

    Perform baseline followed by 4 puffs of albuterol MDI using standard AsthmaNet protocol. Repeat spirometry 15 minutes after albuterol. The post-albuterol FEV1 must be $\geq$ 70% of predicted. If not, physician must be notified and procedure cancelled

11. Establish IV, withdraw blood sample into collection tubes per below

    Protocol for blood sampling for immunophenotyping: Blood drawn during the bronchoscopy visit (at the time of the IV placement) will be collected for immunophenotyping as well as a CBC with differential (for normalization of immunophenotyping data). The CBC will be conducted at the local, clinical lab at Visit 2 only. The preferred protocol will be to draw the blood from the IV itself at the time of placement (after first using a separate syringe to pull back through the IV to clear blood diluted with saline introduced at the time the IV catheter was inserted). If that is not possible, a separate phlebotomy will be acceptable.

    - Fill a 4 mL lavender-top tube for submission to local laboratory for CBC with differential cell count.

- Fill each of the two 5 mL Streck lavender/black striped top Cyto-Chex BCT blood collection tubes labeled with participant and center identifiers. Invert gently 8 times, and package for overnight shipping at room temperature to the Ansel lab at UCSF (see "Shipping Instructions" in Section I.10 below).

12. Perform tongue scraping and oral rinse per below:

Protocol for Tongue Scraping and Oral Rinse: The purpose of the Tongue Scraping and Oral Rinse is to remove cellular and salivary debris that may contaminate lower airway samples and to collect samples of the bacterial communities in the mouth and pharynx. The combined sample of tongue scraping and oral rinse will be frozen and stored for all participants, so that its microbial content can be analyzed and compared to the microbial content of the protected brushings and BAL fluid in at least a subset of participants, if not all participants.

Step 1: Tongue Scraping

1) Have the participant extend his/her tongue, and hold tip gently with sterile gauze pad.
2) Using moderate pressure, rapidly scrape the tongue from back to front 6 times to accumulate a buildup of debris on the tongue scraper.
3) Swirl the tongue scraper in the ORAL specimen container containing 20 mL of RNALater to remove tongue debris. Discard tongue scraper. If necessary to facilitate transfer of material from the scraper, use a sterile syringe to aspirate fluid from the container and flush over the scraper.
4) Repeat steps 1, 2 and 3, using a new tongue scraper, 2 more times.
5) Please keep lid of specimen container closed as much as possible to reduce environmental contamination.

Step 2: Oral Rinse

1) Have participant clear mouth and spit secretions into the ORAL specimen container and then ACTIVELY swish and gargle 10 cc of sterile saline for 20 seconds. Use a stopwatch to time the swish/gargle. Do not include time the patient pauses to rest.
2) At end of swish/gargle, instruct patient to gently spit oral rinse into the ORAL specimen container.
3) Cap and gently invert the oral rinse specimen container 10 times to thoroughly mix. Place on ice.
4) Under a biosafety hood, transfer 30 mL of oral rinse into a 50 mL conical polypropylene tube. Do not exceed 30 mL in tube. (see also Section I.9.2)
5) Store at 4° C for 24 hours or at least overnight.
6) Move sample to -80° C freezer until ready to ship.
IMPORTANT: Tube should be stored vertically using an open slot rack,

such that air will surround the whole tube. Tubes should NOT be stored on a Styrofoam rack for freezing as this can lead to asymmetric volume expansion and tube cracking.

****PLEASE NOTE****:** If there is an unforeseen delay of >60 min from the time of ORAL collection and the application of topical anesthesia for bronchoscopy, please tongue scrape patients 1x and swish with sterile saline solution 1x again prior to bronchoscopy. If this second oral rinse is completed do not save the saline or tongue scraping from this rinse. Write a note on the collection sheet noting that a second oral rinse was completed.

13. Prepare participant for Bronchoscopy
    1) Complete Microbiome Bronchoscopy Checklist (P3_BRONCHCHK_A or P3_BRONCHCHK_C) form
    2) Attach monitoring system (heart rate, blood pressure, pulse oximetry, 3-lead ECG) to participant.
    3) Administer topical anesthesia with topical spray of 2% lidocaine. (Total lidocaine limit will be the lesser of 600 mg or 9 mg/kg. Additional details are below).
        a. While anesthetizing, but after patient has expectorated oral secretions, ask participant to swallow. The participant should state that it is difficult to swallow.
        b. Check the gag reflex suppression with Dacron Q-tip. The participant may feel deep pressure but not touch.
        c. Instill 5 ml 1% lidocaine directly onto larynx via curved blunt-tipped 4" needle on end of 10 ml syringe, passed over tongue to posterior pharynx.
    4) Provide supplemental oxygen
        a. Use a single or double lumen nasal cannula.
        b. Be sure tubing is connected to oxygen port, not compressed air.
        c. Flow at 4 L/min or per physician's request.
        d. Observe and record pulse oximetry, blood pressure every 5 minutes, and ECG throughout.
    5) Administer 0.6 mg atropine sulfate through IV line; and administer medications for conscious sedation (see below). Confirm presence of sedation and availability of reversal medications.
    6) Place participant in a slight Trendelenburg position (supine, with feet 5-10^0 elevated above head level) and place bite block between participant's teeth. Advise participant of gesture he or she should make to indicate sense of accumulation of secretions in oropharynx (e.g., pointing to submandibular area); remove secretions by Yankauer tube suction as needed.

7) Just prior to insertion of scope into patient:
   a. Using sterile syringe, draw 10 mL from 250 mL sterile saline bag and place into sterile 30-60 mL cup. Insert tip of scope into this cup and suction saline through scope into sterile collection trap (labeled SCOPE FLUSH #1). This will ensure patency of channel and adequate function of suction apparatus, and will provide sample of bronchoscope channel contents for microbiome analysis. (See Saline Flush of Bronchoscope Suction Channel in Section I.2. See also Step 3 in Section I.6 below.) Remove the collection trap, take one 10 mL aliquot (under hood if possible, but should be done as soon as possible after collection) and add into the designated 50 mL conical tube containing 20 mL of RNALater (labeled "SCOPE FLUSH #**1**"). Total volume in tube should not surpass 30 mL. Store on ice.
   b. Lubricate distal 3-5 inches of scope with thin film of 2% viscous lidocaine.
8) When injecting fluids (i.e. 1% lidocaine, 0.9% saline flushes) through scope:
   a. Ask physician exactly what volume and what concentration of each fluid.
   b. Insert syringe firmly to suction port.
   c. Inject slowly (may need to pinch off suction tubing depending on what brand of bronchoscope) unless otherwise directed.

I.5. Procedure Monitoring

Note: The procedure described below may vary according to individual institutional practice or policy. These are guidelines only.

1. The patient is lying supine or semi-supine and the monitors (blood pressure cuff, electrocardiogram leads and pulse oximeter) have been activated and are measuring blood pressure, heart rate, heart rhythm and oxygen saturation. Document vital signs (blood pressure, heart rate and oxygen saturation) prior to beginning procedure. These vital signs should be measured every 5 minutes during the procedure using a monitoring system.
2. Potential complications to assess for during bronchoscopy:
   a. Respiratory Depression and/or Hypotension
      i. Benzodiazepines such as midazolam (Versed) and narcotics such as fentanyl (Sublimaze) may cause respiratory depression with decreased oxygen saturation and/or hypotension
      ii. Falling SaO_2
      iii. Decreasing blood pressure (< 90/60)

b. Dyspnea

c. Cardiac arrhythmias (i.e. HR > 120 or <50, etc.)

d. Severe bronchospasm secondary to airway irritation from bronchoscope

e. Laryngospasm

f. Cardiac/respiratory arrest

g. Lidocaine toxicity

h. Anaphylaxis

I.6. Bronchoscopy Procedure

Note: The procedure described below may vary according to individual physician style. These are guidelines only.

1. Conscious Sedation

   a. Administer 0.5-2 mg midazolam (Versed) intravenously over 5-10 seconds, if needed for sedation; flush the IV with 2 ml of normal saline. Normal saline infusion at a minimum of 30 ml/hour can also be instituted in place of the saline flush. During bronchoscopy, additional midazolam can be administered in 0.5-2 mg increments. Intravenous fentanyl (25 mcg increments) can also be administered prior to bronchoscopy for cough suppression and/or during the bronchoscopy if additional sedation and/or cough suppression is required. The maximum doses of midazolam and fentanyl should adhere to the individual institution's conscious sedation guidelines.

   b. When the patient is sedated, proceed with the bronchoscopy.

2. Topical Anesthesia

   a. The spirit of this guideline is to minimize the lidocaine dose delivered to the participant, though the dose given should be sufficient to minimize participant discomfort, at the discretion of the physician performing bronchoscopy. For purposes of calculation, all lidocaine administered is to be recorded, whether delivery is via gargle, aerosol, spray, or instillation. For participant safety, the following limit on lidocaine dose is suggested, based on a publication (Langmack EL, et al. Chest 2000; 117: 1055-60) which demonstrates levels after administration of up to 9 mg/kg of lidocaine:

      Total lidocaine limit will be the less than 600 mg or 9 mg/kg

   b. The amount of lidocaine used should be recorded in the procedure record sheet.

3. The bronchoscope channel should have been flushed prior to introduction into mouth, and the sample set aside for storage in RNALater. (See item 13.7a in Section I.4 above.) A new/un-used sterile collection trap (labeled SCOPE FLUSH #2) should be in place once the mouth and pharynx are anesthetized and the bronchoscope is introduced through the mouth for application of lidocaine to the vocal cords. Use 1% lidocaine in 2 ml increments separated by at least 20 seconds until cords are anesthetized.

4. After this application of the lidocaine to the vocal cords, the bronchoscope should be removed to collect a 10 mL sample of sterile saline, drawn from the 250 mL saline bag and placed into a sterile 30-60 mL cup. Insert tip of scope into this cup and suction saline through scope into sterile collection trap (labeled scope flush #2). This will provide a post oral introduction sample of the bronchoscope channel contents for microbiome analysis. Remove the collection trap, take one 10 mL aliquot (under hood if possible, but should be done as soon as possible after collection) and add into the designated 50 mL conical tube containing 20 mL of RNALater and labeled "SCOPE FLUSH #**2**". Total volume in tube should not surpass 30 mL. Store on ice.

   This sample will be used to comparatively analyze for bacterial contamination from passage of the bronchoscope through the mouth. **Once re-introduced, touching of the bronchoscope to the oropharyngeal mucosa should be avoided if possible,** as should suctioning back through the bronchoscope until brushings and BAL are performed. It is understandable if additional lidocaine needs to be administered to the vocal cords before the bronchoscope is passed into the trachea.

5. When the bronchoscope has entered the trachea, instill 2 ml of 1% lidocaine in the trachea, 2 ml in the mainstem bronchus and 2 ml in the segments from which the brushings are to be taken. If needed, additional 1% lidocaine is available for the more sensitive participants. Be sure to record all lidocaine used for the entire bronchoscopy as you use it. **Note that if possible, NO SUCTIONING through the bronchoscope should be performed until the scope is through the vocal cords and wedged into position for bronchoalveolar lavage following the bronchial brushings. If oropharyngeal suctioning is required, please use a trochar.**

6. Bronchial brushings should be performed first in one lung (right or left lower lobe, with the site determined by coin toss; this site should be recorded in the bronchoscopy record, for the opposite side will be sampled in the asthmatic participants after 6 weeks of treatment with inhaled fluticasone or placebo, per the study protocol). Once the bronchial brushings have been performed, bronchoalveolar lavage should be done in the contralateral lung. Advance the BARD Disposable Microbiology Brush into the segment and "drop" the plug. Advance the brush and gently brush the bronchial mucosa (about 0.5 to 1 inch) while rotating the brush 360°. After about 10 seconds of brushing, retract the brush completely into the inner catheter. Then retract the inner catheter into the outer catheter by pulling the blue and white section apart. Withdraw the

microbiology brush assembly from the bronchoscope. See description below on how to remove the brush. Collect 4 additional microbiology brush samples for a total of 5 brushing samples.

7. Remove the brush sample by first wiping the outer catheter approximately 5 mm distal to the inner catheter with an alcohol prep (proposed cut site), and then cut the outer catheter at the alcohol cleansed site, and discard. Then, completely advance the inner catheter. Wipe the inner catheter about 5 mm distal to the brush tip (proposed cut site) with another alcohol prep, and then cut the inner catheter at the alcohol cleansed site. Advance the brush directly and completely into a 2.0 mL microtube containing 1.0 mL RNALater™ (4 tubes) or 2.0 mL microtube containing 1.0 mL 35% glycerol (1 tube). **Note: The first four brushings should be placed in tubes with RNALater; the last brushing should be placed in tube with glycerol.** Cut the wire level with the top of the tube, so that brush is kept fully immersed in the RNALater™. Screw the cap on tightly and place on ice.
8. Bronchial lavage (BAL) (200 mL total) is to be performed after endobronchial brushings, in the opposite (contralateral) lung. Without suctioning secretions (if possible) the bronchoscope should be moved to the opposite mainstem bronchus, and passed to the lingula or right middle lobe. Lidocaine may be used to decrease cough, but if possible secretions should not to be suctioned. **Prior to performing lavage, ensure that a new sterile collection trap is in place.** The bronchoscope is then wedged into the medial or lateral segment of the RML, or the superior or inferior segment of the lingula. 50 ml of warm, sterile saline from a syringe is placed through the port and fluid returned via gentle suction. Instillation and suction of three additional 50 ml aliquots of warmed saline is performed, with contents collected into each syringe with gentle suction. **The BAL fluid collected in the traps should be mixed in the sterile beaker and then divided under a biosafety hood (to minimize environmental contamination for microbiome analysis), as follows:**

    - 10 mL for total cell count with differential (conical tube).
    - 10 mL aliquot (for bacterial microbiome analysis) added to 50 mL conical polypropylene tube containing 20 mL RNALater pre-aliquoted according to Section I.3. Store at 4° C.
    - Remaining BAL volume should be divided equally into two 50-mL sterile conical collecting tubes for further processing for immunophenotyping and cytokine measurements (See additional instructions under “Sample Processing and Storage” in Section I.9.5)

I.7. Nasal Brushing

Nasal brushing will be performed immediately following bronchoscopy procedure.

1. Remind participant of the details of the procedure.

2. Have subject blow his/her nose.
3. Using speculum and head light visualize where lumen between lower turbinate and floor of the nose is. Observe if any septum spur and other anatomical variations are in the way.
4. Using atomizer, apply 2 sprays of lidocaine 1% aiming at the lumen between lower turbinate and floor of the nose. Anesthetize both nasal cavities. (Note: If atomizer not used at site, an adapter that directs nebulizer output into the nose for 5 seconds or so is an acceptable alternative.)
5. Have subject blow his/her nose again.
6. Unwrap a brush; insert and remove the brush while rotating it, gently. Make sure you insert far enough so that all brittles are inserted under the lower turbinate. This means inserting the brush about 1.5 to 2 inches into the nose. Make a note whether there was any bleeding. (Note: The training video states the brush was inserted 4-5 inches inside the nose. The 4-5 inches was from the back of the nasal speculum. The first 2 inches make the length of the nasal speculum; beyond 2 inches goes inside the nasal cavity, so that all bristles go between the lower turbinate and floor of the nose.)
7. Insert the brush into the 2 mL screw cap vial with 1 mL of RNA later, cut the brush. Screw the cap on tightly and place on ice.
8. Repeat 6-7 on the other nasal cavity.

I.8. Post-Procedure Monitoring

Post-procedure monitoring and decision regarding discharge are expected to vary according to institutional policy and investigator preference. These guidelines for monitoring and discharge are general in nature and may be modified at any time by study personnel as dictated by the clinical scenario.

1. A physician (usually the bronchoscopist) should examine the patient immediately after the procedure for state of alertness, cardiac function, and pulmonary function, noting especially signs of airway obstruction or pneumothorax.

   Follow the individual institutional policy on Conscious Sedation and requirements for recovery and discharge. Vital signs (blood pressure, heart rate, telemetry monitor and oxygen saturation) should be checked and recorded every 15 minutes for the first 60 minutes. Vital signs should be recorded every 30 minutes thereafter.
2. Administer either albuterol MDI (2-4 puffs) or nebulizer (2.5mg premix solution) to participants with dyspnea, wheeze, chest tightness, or hypoxia. May be repeated as necessary at the supervising physician's discretion.

3. Participants may request to drink liquids; be sure to check gag reflex prior to ingestion, and ask them to take small sips of liquids prior to solid food. Participants should wait at least 30 minutes to 1 hour after the procedure to drink or eat.

4. Prior to discharge, a series of checks should be performed:

   Note: The procedure described below may vary according to individual institutional practice or policy. These are guidelines only.

   a. Spirometry:

      If the FEV_1 is < 90% of the baseline value (prior to pre-bronchoscopy administration of albuterol), albuterol should be administered by MDI (2-4 puffs) or by nebulizer (2.5 mg in 2.5-5.0 ml normal saline), and spirometry repeated 15 minutes later. If the FEV1 is still < 90% of the baseline value, a physician should be notified to make a final determination regarding discharge.

      If using the AsthmaNet spirometry system for post-bronchoscopy spirometry testing, the participant's AsthmaNet ID number (AB-CDE-FGH) should be constructed as follows:

      • AB = 98

      • CDE= site number

      • FGH=participant's number in the study

      This modified participant ID should be used for post-bronchoscopy spirometry monitoring so that the AsthmaNet over-reader does not grade those tests.

   b. Vital signs should be close to baseline values and recorded.

   c. Gag reflex should be present.

   d. Mobility status: walk with patient in immediate area and observe for loss of balance.

   e. Participant should understand <u>written</u> Post Bronchoscopy Instructions

5. If these checks are performed and the results are satisfactory and/or approved by a study physician, remove participant's IV and discharge participant from the laboratory. Be sure he/she receives written Post Bronchoscopy Instructions. Plan to call the participant every day for three days following bronchoscopy date to check for adverse events.

6. The following forms should be completed:

   a. Microbiome Bronchoscopy Procedure Documentation (P3_BPD) form (not entered but mailed to DCC with visit packet).

   b. Procedure Note for participant's hospital chart (physician).

   c. Copy of the procedure notes for participant's AsthmaNet study folder.

   d. Vital Signs/medication sheet (technician/nurse with physician's signature).

e. Print the vital sign record from the monitoring system and staple to "Procedure Monitoring form" and/or record individually.

f. Post procedure checklist (technician).

g. 72-hour Follow-up checklist.

I.9. Sample Processing and storage

I.9.1. Blood for immunophenotyping.

**TO BE SHIPPED AT ROOM TEMP ON SAME DAY AS COLLECTION **

Remember that blood drawn at the time of the IV placement for bronchoscopy will be used for immunophenotyping, as well as a CBC with differential (for normalization of immunophenotyping data). The CBC will be conducted at the local, clinical lab at Visit 2 only.

1. Prior to bronchoscopy, blood was drawn into two 5 mL lavender/black striped-top Cyto-Chex BCT tubes for immunophenotyping.
2. Label each 5 mL lavender/black striped-top Cyto-Chex BCT tube with barcode label (Tough-Tag or Cryo-Tag 1.50 x 0.75") for the Cyto-Chex BCT tubes (barcode starting with '3IMMUN') generated through the Biological Sample Tracking (BST) module. Place label vertically on each tube so that the barcode can be scanned. On each label, participant ID needs to be added.
3. Enter the participant's immunophenotyping sample information into the Biological Sample Tracking module. The participant's immunophenotyping sample information should be entered into the Biological Sample Tracking module on the day the bronchoscopy is performed and label with the barcode is placed on the tubes.
4. Store at room temperature until ready to be shipped
5. Ship blood samples at room temperature TODAY to Immunophenotyping Core (Ansel Lab UCSF) following shipping procedures described in next Section I.10. (Combine shipment with BAL sample for immunophenotyping after latter is processed per instructions under Section I.9.5)

I.9.2. Oral Wash (includes tongue scraping plus oral rinse)

1. Prior to bronchoscopy, oral wash was allocated into a 50 mL conical polypropylene tube.
2. Label tube with barcode label (Cryo-Tags 1.50 x 0.75") for the oral wash (barcode starting with '3WASH') generated through the Biological Sample Tracking (BST) module. Place label vertically on tube so that the barcode can be

scanned. On label, use an alcohol-proof permanent marker to complete the following information: participant ID and visit number.

3. Enter the participant's oral wash sample information into the Biological Sample Tracking module. The participant's oral wash sample information should be entered into the Biological Sample Tracking module on the day the bronchoscopy is performed and label with the barcode is placed on the tube.
4. Using an alcohol-proof permanent marker, write the last three digits of the participant's ID and visit number (i.e. V2 or V5) on the caps of the tube.
5. Store oral wash sample at 4° C at least overnight to 24 hours. Then move to storage at -80° C until shipped (see Section I.10).
   IMPORTANT: Tube should be stored in an open slot rack such that air surrounds the whole tube. Tubes should NOT be stored on a Styrofoam rack for freezing as this can lead to asymmetric volume expansion and tube cracking.

I.9.3. Saline Flush of Bronchoscope

BEFORE Bronchoscope placement and application of lidocaine to upper airway:

1. Prior to bronchoscope placement and anesthesia, a saline flush of the bronchoscope was performed, and one 10 mL aliquot was added to a sterile 50 mL conical tube containing 20 mL RNALater.
2. Label 50 mL conical tube with barcode label (Cryo-Tags 1.50 x 0.75") for the saline flush (barcode starting with '3FLSH**1**') generated through the Biological Sample Tracking (BST) module. Place label vertically on tube so that the barcode can be scanned. On label, use an alcohol proof permanent marker to complete the following information: participant ID and visit number.
3. Enter the participant's saline flush sample information into the Biological Sample Tracking module. The participant's saline flush sample information should be entered into the Biological Sample Tracking module on the day the bronchoscopy is performed and label with the barcode is placed on the tube.
4. Using an alcohol proof permanent marker, write the last three digits of the participant's ID and visit number (i.e. V2 or V5) on the caps of the tube.
5. Store saline flush sample at 4° C at least overnight to 24 hours. Then move to storage at -80° C until shipped (see Section I.10).
   IMPORTANT: Tube should be stored in an open slot rack such that air surrounds the whole tube. Tubes should NOT be stored on a Styrofoam rack for freezing as this can lead to asymmetric volume expansion and tube cracking.

AFTER application of lidocaine to upper airway:

1. After application of lidocaine to upper airway, the bronchoscope was removed, a saline flush of the bronchoscope was performed, and one 10 mL aliquot was added to a sterile 50 mL conical tube containing 20 mL RNALater.

2. Label 50 mL conical tube with barcode label (Cryo-Tags 1.50 x 0.75") for the saline flush (barcode starting with '3FLSH**2**') generated through the Biological Sample Tracking (BST) module. Place label vertically on tube so that the barcode can be scanned. On label, use an alcohol-proof permanent marker to complete the following information: participant ID and visit number.

   *(**Note:** #5 – #7 same as for scope wash before application of lidocaine)*

3. Enter the participant's saline flush sample information into the Biological Sample Tracking module. The participant's saline flush sample information should be entered into the Biological Sample Tracking module on the day the bronchoscopy is performed and label with the barcode is placed on the tube.
4. Using an alcohol-proof permanent marker, write the last three digits of the participant's ID and visit number (i.e. V2 or V5) on the caps of the tube.
5. Store saline flush sample at 4° C at least overnight to 24 hours. Then move to storage at -80° C until shipped (see Section I.10).
   IMPORTANT: Tube should be stored in an open slot rack such that air surrounds the whole tube. Tubes should NOT be stored on a Styrofoam rack for freezing as this can lead to asymmetric volume expansion and tube cracking.

I.9.4. Protected specimen brushes

Tubes in RNALater:

1. During the bronchoscopy, four microbiology brushes were placed directly into a 2.0 mL microcentrifuge tube containing 1 mL of RNALater™ and labeled "BRUSHING **R**".
2. Label each microtube with barcode label (Cryo-Tags 1.50 x 0.75") for the bronchoscopy brushes in RNALater (barcode starting with '3BRSHR') generated through the Biological Sample Tracking (BST) module. Place label vertically on each tube so that the barcode can be scanned. On each label, use an alcohol-proof permanent marker to complete the following information: participant ID and visit number.
3. Enter the participant's brushing collection information into the Biological Sample Tracking module. The participant's brushing collection information should be entered into the Biological Sample Tracking module on the day the bronchoscopy is performed and label with the barcode is placed on the microtubes.
4. Using an alcohol-proof permanent marker, write the last three digits of the participant's ID and visit number (i.e. V2 or V5) on the caps of the tubes.
5. Wrap parafilm around caps of each tube to prevent leakage. These four BARD Disposable Microbiology brush samples immersed in RNALater should be stored at 4° C at least overnight to 24 hours. Then move to storage at -80° C until shipped on dry ice (See Section I.10).

Tube in Glycerol:

1. During the bronchoscopy, one microbiology brush was placed directly into a 2.0 mL microcentrifuge tube containing 1 mL of 35% glycerol and labeled "BRUSHING **G**".
2. Label the microtube with barcode label (Cryo-Tags 1.50 x 0.75") for the bronchoscopy brushes in glycerol (barcode starting with '3BRSHG') generated through the Biological Sample Tracking (BST) module. Place label vertically on tube so that the barcode can be scanned. On label use an alcohol-proof permanent marker to complete the following information: participant ID and visit number.

   *(**Note:** #3 and #4 same as for tubes in RNALater)*
3. Enter the participant's brushing collection information into the Biological Sample Tracking module. The participant's brushing collection information should be entered into the Biological Sample Tracking module on the day the bronchoscopy is performed and label with the barcode is placed on the microtubes.
4. Using an alcohol-proof permanent marker, write the last three digits of the participant's ID and visit number (i.e. V2 or V5) on the caps of the tube.
5. Wrap parafilm around cap of the tube to prevent leakage. This BARD Disposable Microbiology brush sample immersed in 35% glycerol should be stored at -80° C until shipped on dry ice (see Section I.10).

I.9.5. BAL processing

1. **For cell count**: place 10 mL BAL fluid in conical tube for total cell count with differential, and centrifuge at 450 x g for 10 minutes at 4^{o}C. Refer to Microbiome BAL Processing worksheet (P3_BAL_PROCESS_WKS) as a guide in completing calculations in #2-4 below.

   Resuspend the cell pellet in 2 mL PBS and keep on ice. Cell counts are performed on the resuspension, and 4 cytocentrifuge slides are prepared as follows:

   1) Ten microliters of the resuspended cells (tube shaken immediately before sampling) are taken and added to ten microliters of Turks solution (Sigma, cat# C0775) to perform the cell count in a hemocytometer. (If more than 50 cells/ counted square, prepare an appropriately diluted sample [record dilution] and repeat the ½ dilution with Turks solution as above).

   2) All cells in 4 corner squares are counted and the average of the 4 squares calculated. This value is multiplied by 2 (dilution with Turks) x 10,000 (x any

additional dilution, for example, x 4 for a 4-fold dilution of resuspended cells made before mixing with Turks blue). The result is recorded as cells/mL in the resuspended PBS. Multiply the cells/mL by 2 to obtain the total cell count of the cells resuspended in PBS (this TCC is also equal to the total cell count from the 10mL BAL aliquot).

3) The total cell/mL is used to calculate the volume of cell suspension needed to deposit 20,000 – 25,000 cells per slide (e.g. for 25,000 cells/slide: (25,000/TCC of resuspension) x 2 = volume to use in mL per slide. Four slides are prepared by cytocentrifugation at 500rpm for 5 minutes.

4) Summary of cell count formula:
(# cells counted in 4 squares/4) x 2 x 10,000 x additional Dilution Factor (if any) = cells/mL of resuspension. Cells/mL x 2 = TCC of the resuspension = TCC of the 10mL BAL aliquot. To express this as number of cells per mL in the ten mL aliquot of BAL fluid, divide this by 10 (cells per mL). To express this as cells per mm3, divide this number by 1000. Note that the number of cells per mm3 is the same as the number of thousands of cells per mL. So, for example, entering the number "250" for Q1000 on the Microbiome BAL Laboratory Results (P3_LAB_BAL) form, correctly refers to "250 cells per mm3", but reading it as "250 thousand cells/mL" would be perfectly accurate.

5) Cytospin slides should be air dried and stained with either the HEMA 3 stain set (Cat#122-911) or Kwik-Diff. stain for standard differential count on a minimum of 400 cells. BAL slides should be labeled with participant ID and visit number.

Results of BAL cell count should be recorded on Microbiome BAL Laboratory Results (P3_LAB_BAL) form.

Slides should be saved and stored at the site. At study completion, should BAL cell count data be in question, UCSF will request the slide for data in question for over-reading.

2. **For bacterial microbiome analysis:**

1) Under a biosafety hood, take one 10 mL aliquot of BAL and add to the prepared 50 mL sterile conical polypropylene tube containing 20 mL of RNALater labeled “BAL MICROBIOME”. Total volume in tube should not surpass 30 mL. Mix and invert.

2) Label tube with barcode label (Cryo-Tags 1.50 x 0.75”) for the BAL microbiome sample (barcode starting with ‘3BAL’) generated through the Biological Sample Tracking (BST) module. Place label vertically on tube so that the barcode can be scanned. On label, using an alcohol-proof

permanent marker, complete the following information: participant ID and visit number.

3) Enter the participant's BAL sample information into the Biological Sample Tracking module. The participant's information should be entered into the Biological Sample Tracking module on the day the bronchoscopy is performed and label with the barcode is placed on the tube.

4) Using an alcohol-proof permanent marker, write the last three digits of the participant's ID and visit number (i.e. V2 or V5) on the caps of the tube.

5) Store BAL microbiome sample at 4° C at least overnight to 24 hours. Then move to storage at -80° C until shipped (see Section I.10).
IMPORTANT: Tube should be stored in an open slot rack such that air surrounds the whole tube. Tubes should NOT be stored on a Styrofoam rack for freezing as this can lead to asymmetric volume expansion and tube cracking.

3. For immunophenotyping and cytokine measurements: ****Further sample processing is required prior to shipment.****

Materials needed:

- Two additional 50 mL conical collection tubes
- Centrifuge for 50 mL tubes with biocontainment lids
- 1 mL aliquot of provided FACS Buffer (Store at 4° C. Consists of PBS + 2mM EDTA + 0.1% bovine serum albumin + 0.1% sodium azide)
- One BAL immunophenotyping vial (consists of screwcap vial containing 1 mL Streck Cell Preservative (SCP). Store at room temperature.)
- Two 15 mL conical tubes to store supernatant
- Twelve 2 mL screwcap tubes to store supernatant

Processing:

1) Pour remaining BAL volume (after aliquots taken for cell count and microbiome analysis) into two 50 mL conical tubes. Fill to equal volumes (up to 50 mL/tube). Two tubes (up to 100 mL of BAL) will be used for immunophenotyping.

2) Centrifuge the two conical tubes for immunophenotyping at 300xg (~1200rpm in most table top centrifuges) for 10 minutes.

3) Remove the supernatant from the two, 50 mL conical tubes carefully to avoid disturbing the cell pellet. Do not pour/decant. Using a Pipet-Aid, transfer all but 1 to 5 mL of the supernatant into the fresh 50 mL conical collecting tube(s) and place on ice to be aliquoted later.

4) Remove and dispose of the final 1-5 mL with a pipetman, taking care not to disturb the pellet, while leaving less than 100 µL residual fluid in each tube

(enough to cover the cells), so as not allow the cell pellet to dry.

5) Gently resuspend one of the cell pellets in the 50 mL conical tubes using up to 0.8 mL of FACS buffer (provided). If large residual BAL fluid volumes were unavoidable, reduce the amount of buffer to keep the total final volume under 1 mL.

6) After resuspending the pellet in one tube, pipet the entire volume including cells into the second tube and resuspend the second cell pellet with the transferred volume.

7) Use the FACS buffer to adjust the volume of resuspended cells until it reaches ~1 mL (± 0.1 mL). Adjust the volume of resuspended cells to ~1 mL with FACS buffer (± 0.1 mL; measure with pipet while transferring).

8) Transfer the resuspended cells into BAL immunophenotyping collection vial, which contain 1 mL Streck Cell Preservative (provided). If volume is >1.1 mL, do not transfer more than 1.1 mL of resuspended cells into the BAL immunophenotyping vial. Dispose of excess cells if necessary.

9) Cap BAL immunophenotyping vial. Invert 2-3 times to mix.

10) Label vial with barcode label (Tough-Tag or Cryo-Tag 1.50 x 0.75") for the BAL immunophenotyping sample (barcode starting with '3BALCP') generated through the Biological Sample Tracking (BST) module. Place label vertically on tube so that the barcode can be scanned. On label, using an alcohol-proof permanent marker, complete the following information: participant ID and visit number.

11) Enter the participant's BAL immunophenotyping sample information into the Biological Sample Tracking module. The participant's sample information should be entered into the Biological Sample Tracking module on the day the bronchoscopy is performed and label with the barcode is placed on the tubes.

12) Hold at room temperature until shipping (see Section I.10).

13) Ship sample at room temperature TODAY to Immunophenotyping Core using procedures described below in Section I.10. These should be shipped along with Blood sample for immunophenotyping.

14) Next aliquot the BAL supernatant into twelve 2 mL tubes (12 x 1 mL aliquots).

15) Aliquot remaining supernatant in 5 mL aliquots in 15 mL conical tubes, UP TO 5 tubes. Any remaining BAL supernatant should be discarded.

16) Label BAL supernatant tubes with barcode label (Cryo-Tags 1.50 x 0.75") for the BAL supernatant samples (barcode starting with '3BALST') generated through the Biological Sample Tracking (BST) module. Place label vertically on each tube so that the barcode can be scanned. On each label, using an alcohol-proof permanent marker, complete the following information: participant ID and visit number.

17) Enter the participant's BAL supernatant sample information into the Biological Sample Tracking module. The participant's sample information should be entered into the Biological Sample Tracking module on the day the bronchoscopy is performed and label with the barcode is placed on the tubes.

18) Using an alcohol-proof permanent marker, write the last three digits of the participant's ID and visit number (i.e. V2 or V5) on the caps of the tubes.

19) Freeze and store BAL supernatants by placing at -80° C. Ship in batches using procedures described in Section I.10.

I.9.6. Nasal brushings

1. Immediately following bronchoscopy, two cytology brushes were placed directly into 2.0 mL microcentrifuge tubes containing 1 mL of RNALater™ and labeled "NASAL".
2. Label each microtube with barcode label (Cryo-Tags 1.50 x 0.75") for the nasal brushes in RNALater (barcode starting with '3NASAL') generated through the Biological Sample Tracking (BST) module. Place label vertically on each tube so that the barcode can be scanned. On each label, use an alcohol-proof permanent marker to complete the following information: participant ID and visit number.
3. Enter the participant's nasal brushing collection information into the Biological Sample Tracking module. The participant's brushing collection information should be entered into the Biological Sample Tracking module on the day the bronchoscopy is performed and label with the barcode is placed on the microtubes.
4. Using an alcohol-proof permanent marker, write the last three digits of the participant's ID and visit number (i.e. V2 or V5) on the caps of the tubes.
5. Wrap parafilm around caps of each tube to prevent leakage. These two cytology brush samples immersed in RNALater should be stored at 4° C at least overnight to 24 hours. Then move to storage at -80° C until shipped on dry ice (See Section I.10)

I.10. Shipping Instructions

I.10.1. General information

Personnel shipping samples must adhere to IATA and Dept of Transportation shipping regulations. Please check with your institution for guidelines. Failure to comply may result in fines.

Note that shipment of samples for immunophenotyping differ from shipment of all other samples. See specific instructions below. In general:

- **Specimens for immunophenotyping (blood and BAL) must be sent on the same day as each bronchoscopy procedure to the Ansel lab at UCSF. To avoid Thursday shipment issues (which has resulted in Saturday and Monday deliveries), it is preferred for bronchoscopies to be scheduled for Monday – Wednesday. Samples shipped Monday – Wednesday should be shipped by Priority Overnight to the Ansel Lab at UCSF. For Thursday bronchoscopies, samples should be shipped by First Overnight to the Ansel Lab at UCSF. Should there be concerns about a shipment (due to existing or impending extreme weather, holiday, etc.), ship First Overnight.**
- Specimens for microbiome analysis and cytokine measurements should be stored at -80° C after appropriate processing. (Note: Samples for microbiome analysis must be stored at 4° C overnight to 24 hours after collection, before being moved to -80° C.) These samples should be shipped in batches, for every 3 participants (asthmatic and/or non-asthmatic) who have completed the study. **Samples must be shipped on Monday - Thursdays only, on dry ice, by Priority Overnight to the Boushey Lab at UCSF.**
- On the day of shipment, clinical personnel should access the AsthmaNet Biological Sample Tracking module to mark the samples as shipped and to generate a tracking log that will be shipped with the bronchoscopy samples to the appropriate lab in San Francisco (Ansel or Boushey lab). Include a shipment comment detailing the contents of the shipment (i.e., blood, BAL cell pellet, oral wash, bronchoscope flush, bronchoscopy brushes, BAL fluid, BAL supernatants, and nasal brushes). Because this log is generated directly from the tracking database, it is imperative that all bronchoscopy samples are entered into the system before generating the tracking log.

  Refer to the AsthmaNet Data Management System User Manual in Section 7 of the AsthmaNet General Manual of Operations for instructions on marking the sample shipped, excluding missing samples and generating the shipment inventory.

  A shipment inventory will need to be generated for each sample type.

  The shipment inventory that is generated can be saved on the computer for electronic filing. If the shipping tracking # is included during generation of the log it will be included on the log automatically. If the tracking # is not available when the sample log is generated, handwrite the shipment tracking number on the printed copy of the shipment inventory that will be sent with the shipment.
- If the shipment inventory will not be saved electronically, make a copy for clinic records. Place the original inventory in a waterproof Ziploc bag to include it at the top of the shipping box. Shipment inventories for all samples can be included in the same Ziploc bag.

I.10.2. Instructions for shipping immunophenotyping samples (at room temperature, overnight priority) to Ansel Lab UCSF

The immunophenotyping samples include the 2 Cyto-Chex BCT tubes containing blood collected at bronchoscopy visit, and BAL immunophenotyping screwcap vial containing BAL cells resuspended in FACS buffer and mixed with Streck Cell Preservative.

Shipment materials include: cardboard shipper box, bubble wrap, parafilm, absorbant pad, Ziploc bag, "Biohazard" label, and "Exempt Human Specimen" label. See Section I.11 for additional information.

Shipping Instructions:

1. **Ship immunophenotyping specimens Monday - Thursday only. Samples shipped Monday - Wednesday should be shipped Priority Overnight. Samples shipped on Thursday should be shipped First Overnight. Should there be concerns about a shipment (due to existing or impending extreme weather, holiday, etc.), ship First Overnight.**
2. Laura Christian (laura.christian@ucsf.edu) should have been notified of the bronchoscopy visit at least one week ahead of the visit, as indicated on the Visit 1 (P3_VISIT1) and Visit 4 (P3_VISIT4) Checklists.
3. Email the Immunophenotyping Core (Ansel Lab) at least 48 hours in advance at the emails listed below to insure they are ready for your shipment.
4. Immunophenotyping samples must be shipped the day they are collected!
5. Wrap the cap of the BAL immunophenotyping vial with parafilm to insure against leakage.
6. Place BAL immunophenotyping vial, blood collection tubes, and absorbant pads into Ziploc bag. Seal bag.
7. Wrap the sealed bag in bubble wrap. Use tape to secure the bag inside the bubble wrap.
8. Place in box, using additional packing material generously to prevent breakage.
9. Place the shipment inventory in a waterproof Ziploc bag at the top of the shipping box. Seal box.
10. Attach one large "Biohazard" label and one "Exempt Human Specimen" label to the shipping box.

11. Ship by Fedex to Ansel Lab (Immunophenotyping Core).

    Ansel Lab (c/o Laura Christian)
    University of California San Francisco
    513 Parnassus Avenue, HSE-201
    San Francisco, CA 94143-0414
    (Phone: 1-415-476-5373)

12. Use Fedex website or manually send an e-mail message containing the tracking number and date of shipment to all of the addresses below:

    Laura Christian (laura.christian@ucsf.edu)
    Sana Patel (sana.patel@ucsf.edu)
    Mark Ansel (mark.ansel@ucsf.edu)

I.10.3. Instructions for shipping all other sample types (dry ice, priority overnight) to the UCSF Microbiome Core (Boushey Lab)

The labeled samples should include:

- ORAL WASH in one 50 mL conical tube
- SCOPE FLUSH 1 (before insertion into mouth) in one 50 mL conical tube
- SCOPE FLUSH 2 (after upper airway anesthesia achieved) in one 50 mL conical tube
- BRUSHINGS, four tubes containing microbiological brushes in RNALater
- BRUSHING, one tube containing microbiological brush in Glycerol
- BAL MICROBIOME in one 50 mL conical tube
- BAL supernatant aliquots in up to five 15 mL conical tubes, and twelve 2 mL screw cap microtubes
- NASAL brushings, two tubes containing cytology brushes in RNALater

Sputum supernatant, pellets, and SS samples (with and without glycerol) will be shipped with bronchoscopy samples to UCSF. Sputum pellets should be packaged in a separate Ziploc bag and sputum supernatant and SS samples should be packaged together in a separate box.

Samples should be shipped in batches, after every 3 subjects have completed the study at the site. These samples must be sent on Monday – Thursday only on dry ice. See Section I.11 for additional information on shipping supplies.

Since samples from multiple participants are included in each shipment, please ensure participant identifiers on samples are clear (to avoid any mix-up).

Shipping Instructions:

1. **Ship specimens Monday - Thursday only by Priority Overnight.**
2. Email the Boushey Lab at least 48 hours in advance at the emails listed below to ensure they are ready for your shipment. Include Sputum Core (Zesemayat.Mekonnen@ucsf.edu) on this e-mail as well.
3. Place the 2 ml screwcap vials containing the bronchoscopy brushes, aliquots of BAL supernatant, and nasal brushes in a cardboard freezer storage box (USA Scientific # 9023-8100, Deep-lid, Premium cardboard box without grid/divider; Insert/use 100-well cardboard dividers which can be obtained from Fisher, e.g. item # TF4000013 Thermo Scientific). If using only 1 box, place the bronchoscopy brush tubes in top rows of the box, BAL supernatant tubes in subsequent rows, followed by nasal brushes in bottom rows so that the three specimen types are distinguished. On the cover of each storage box indicate that they contain “BRONCH BRUSHES” or “BAL SUPERNATANT” or “NASAL BRUSHES” and the name of your “SITE”.
4. Place the specimen storage box(es) in Ziploc bag(s), and securely tape the bag(s) closed with packaging tape.
5. Place the samples frozen in 50 ml conical tubes in large size Ziploc bags (one bag for each sample type), and securely tape the bag closed with packaging tape. Be sure that participant identifiers and specimen labels are in place and secure on the tubes. Place Ziploc bags in cardboard storage box(es), and stuff box(es) with crumpled paper towel balls to secure tubes and prevent them from moving and hitting each other during shipping. Tape box closed.
6. Place the BAL supernatant samples frozen in 15 ml conical tubes in a large size Ziploc bag, and securely tape the bag closed with packaging tape. Be sure that participant identifiers and specimen labels are in place and secure on the tubes. Place Ziploc bag in cardboard storage box(es), and stuff box(es) with crumpled paper towel balls to secure tubes and prevent them from moving and hitting each other during shipping. Tape box closed.
7. Place the sputum pellets in another Ziploc bag, and securely tape the bag closed with packaging tape. Be sure that participant identifiers and specimen labels are in place and secure on the tubes.
8. Place the storage box containing sputum supernatant and SS samples in a Ziploc bag, and securely tape the bag closed with packaging tape.
9. Place the cardboard freezer box(es) containing 2 ml screwcap vials, the cardboard freezer box(es) containing 15 ml conical tubes, the cardboard freezer box(es) containing 50 ml conical tubes, the Ziploc bag containing sputum pellets, and the freezer box containing sputum supernatant and SS samples into a Thermosafe shipping container (Fisher Sci 03-530-003, Tegrant E327UPS, 23x23x24; *or* Tegrant E89UPS, 3-530-001, 21x14x17). Size of Thermosafe box used is at your discretion, but should hold all samples to be shipped with

sufficient dry ice.

10. Fill the Thermosafe shipping container with enough dry ice to cover the specimens.
11. Place the shipment inventory in a waterproof Ziploc bag at the top of the shipping container. Seal container.
12. Attach one “Exempt Human Specimen” sticker to the container.
13. Attach dry ice label “DRY ICE – UN 1845” to the container. Mark the approximate weight of dry ice in kg for each shipment.
14. Ship samples overnight to:

    Attention: Snehal Nariya
    UCSF
    Health Sciences East, Room 1355B
    513 Parnassus Avenue
    San Francisco, CA 94143
    (415) 476-5985

15. Use FedEx website or manually send an e-mail message containing the tracking number and date of shipment to all of the addresses below:

    Snehal Nariya (snehal.nariya@ucsf.edu, Boushey lab manager)
    Homer Boushey (homer.boushey@ucsf.edu)
    Yvonne Huang (yvonne.huang@ucsf.edu)
    Kelsey Wollen (kelsey.wollen@ucsf.edu)

Complete instructions for accessing and interacting with the Biological Sample Tracking module in the AsthmaNet Database Application can be found in Section 7, “Computing and Networking Environment”, of the AsthmaNet General Manual of Operations, specifically Section 7.5.8.

I.11. Bronchoscopy Supplies

Supplies Needed for Bronchoscopy Sample Collection, Processing, Storage

Item	Vendor/ Manufacturer	Catalog #	# Per Collection
RNALater	Life Technologies	AM7021M (500 mL)	20 mL each for oral sample, bronchoscope flush and BAL (total of 60 mL)
Lidocaine (1% and 2%)	Supplied by DCC		
Bucket with Ice	Per study site		1
Barcode labels for frozen samples (Laser Cryo-Tags 1.50 x 0.75")	Diversified Biotech	LCRY-1200	22
Barcode labels for immunophenotyping blood and BAL cell pellet (Laser Tough-Tags 1.50 x 0.75")	Diversified Biotech	TTGP-1050	3
Immunophenotyping			
5 mL Streck lavender/black striped top Cyto-Chex BCT	Supplied by UCSF		2
4 mL lavender-top vacutainer	Fisher Sci	02-683-99C (BD Medical)	1
Oral rinse and tongue scraping			
BD POSIFLUSH™ NORMAL SALINE SYRINGES (pre-filled 10 mL)	Supplied by DCC		1
60 mL sterile collection cup	Starplex Scientific	B60210	1
Breath Rx tongue scrapers (3 scrapers per pack) http://www.smilox.com/breathrx-tonguecleaner.cfm	www.smilox.com		1
50 mL conical polypropylene tube	Fisher Sci (Corning)	05-526-B	1
Bronchoscope saline flush			
10 mL sterile syringe			2
30-60 mL sterile collection cup			2
Sterile specimen trap	Fisher/Busse	406 (Busse)	2
50 mL conical polypropylene tube	Fisher Sci (Corning)	05-526-B	2

Brushings			
BARD Disposable Microbiology brushes	ConMed (800) 448-6506	130	5
2 mL microtube containing RNALater	Supplied by UCSF		4
2 mL microtube containing 35% Glycerol	Supplied by UCSF		1
Alcohol preps			
Wire cutter (any simple wire cutter will do)	Fisher Sci	S43981	1
Bronchoalveolar Lavage			
200 mL warmed sterile saline (250 mL bag)	Supplied by DCC		1
Conical tube			1
50 mL conical polypropylene centrifuge tubes	Fisher Sci	05-526-B	3
2 mL screw cap tubes	Fisher Sci (USA Scientific)	1420-9700	12
15 mL conical tubes	Fisher Sci (Corning)	05-538-59A	2
Immunophenotyping vial with SCP	Supplied by UCSF		1
Luer-Lok 60 mL syringe	VWR	BD - 309653	2
Sterile specimen trap	Fisher/Busse	406 (Busse)	3
FACS buffer	Supplied by UCSF		
Sterile beaker			1
Pipet-Aid			1
Sterile 10 mL Pipet-Tips			
Nasal Brushings			
DeVilbiss Atomizer model 15-RD			
Head light			
Disposable nasal speculum	www.bionixmed.com	9877	1
2 mL screw cap tubes	Fisher Sci (USA Scientific)	1420-9700	2
Cytology brush	Supplied by DCC		2
Scissors			
Alcohol swabs			

Supplies Needed for Bronchoscopy Shipping

Item	Vendor/ Manufacturer	Catalog #	# Per Shipment
Cardboard freezer storage box (Deep-lid, premium cardboard box without grid/divider)	USA Scientific	9023-8100	1-2
100-well cardboard dividers	Fisher Sci	TF4000013	1-2
Thermosafe shipping container (Tegrant E-327UPS, 23x23x24, or E89UPS, 21x14x17).	Fisher Sci	03-530-003 or 03-530-001	1
Ziploc bags			
Packaging tape			
Exempt Human Specimen labels Therapak 2.5”x2”	Fisher Sci.	22-130-070	1
Dry Ice labels (DRY ICE – UN 1845)	Air Sea Containers http://www.airseacontainers.com 1-866-272-9880	Dry Ice UN 1845 Label, Roll of 500 (No product number)	1

Supplies Needed for Immunophenotyping Shipping

Item	Vendor	Catalog #	# Per Shipment
Cardboard Shipper Box			1
Parafilm	Fisher Sci.	13-374-10	
Absorbant pad	Fisher Sci.	19-075-383C	
Ziploc bag			2
Bubble wrap			
Packaging tape	Staples	380107	
Exempt Human Specimen labels Therapak 2.5”x2”	Fisher Sci.	22-130-070	1
Biohazard labels (large) 3”x3”	Fisher Sci.	18-999-936	1
Shipment inventory from BST			1