ADVERSE EVENTS

4. ADVERSE EVENT

4.1. Reporting Procedures for AsthmaNet

This section outlines the identification, handling, and reporting of adverse events (AEs). Adverse events experienced by study participants must be documented appropriately in order to:

Provide the accurate reporting of all adverse events, both serious and non-serious (CFR 312.64(b). These reports will be submitted to the DSMB who will determine if one or more of the treatment groups place individuals at undue risk by participating in the study.

Permit the use of adverse events as outcome variables to allow comparisons of participant safety between treatment groups.

4.2. Definitions

4.2.1. Laboratory Adverse Event

A laboratory adverse event is any clinically important worsening in a laboratory variable that occurs during the course of the study, whether or not considered to be drug-related.

4.2.2. Clinical Adverse Event

A clinical adverse event is any unintended worsening in the structure or function of the body, whether or not it is considered to be drug-related or study-related. This includes any side effect, injury, sensitivity reaction, or any other illness or condition occurring while a participant is in the study. Worsening of variables monitored by an ECG would be considered a clinical adverse event.

4.2.3. Serious Adverse Event

A serious adverse event (SAE) is any experience that suggests a significant hazard, contraindication, side effect, or precaution. With respect to human clinical experience, a serious adverse event includes any experience that is fatal or life threatening, is permanently disabling, requires or prolongs an existing hospitalization, or is a congenital anomaly, cancer, or overdose.

Note that any hospitalization, even for elective surgery, constitutes a serious adverse event and should be documented as such. This includes a hospitalization for an asthma exacerbation. If the elective surgery required hospitalization afterwards, then it should be considered a serious adverse event. If the elective surgery took place in an ambulatory surgery area and the participant went home the same day, then it would not be considered a serious adverse event.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an ER or at home, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or abuse.

4.2.4. Unanticipated Problem

An unanticipated problem (UP) is any incident, experience, or outcome that meets all of the following criteria: 1) unexpected; 2) related or possibly related to participation in the research; and 3) suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized. An unanticipated problem may not necessarily be an adverse event, which is the case when the problem does not cause actual harm to participant(s). For example, if a laptop computer with sensitive, identifiable study data is stolen, this theft places the participants at greater risk of psychological or social harm; this is an unanticipated problem that is not an adverse event.

4.2.5. Unexpected Adverse Event

An unexpected adverse event is any adverse event that is not consistent with the currently approved research protocol, investigator brochure, or informed consent form, or one that is not part of the normal disease progression.

4.3. Adverse Event Reported to the Clinic Coordinator

Study personnel may learn of the occurrence of an adverse event in a variety of ways:

> Study visit: The participant (or his/her parent/guardian) reports the adverse event at a scheduled study visit. Participants are instructed to note any medical problems and non-study medications which are reviewed at each visit in order to aid in the identification and reporting of recent medical

problems. In addition, at each study visit, participants are asked about the occurrence of any medical problems since the last visit.
Telephone contact: The participant reports the adverse event during a telephone contact.
Unscheduled reporting: The participant or treating physician calls or visits the clinic to report an adverse event.

In each case, the incident must be brought to the attention of the clinic coordinator so that appropriate documentation can be completed.

4.3.1. Was there a Laboratory Adverse Event?

Laboratory tests are done if required by a protocol at a given visit or if a participant exhibits related symptoms. If the results of any of these tests are abnormal, the participant is experiencing a laboratory adverse event and the Clinical Adverse Events form (AECLIN) should be completed. The definition of abnormal for relevant tests is determined by the particular protocol. Abnormal may be defined as being out of range as a percent of normal (e.g., >125% of normal, where normal may be a participant's baseline value). Normal ranges for tests from each clinical lab will be maintained at the DCC along with copies of the lab certifications from the College of American Pathologists.

Participants may exhibit symptoms that call for additional tests that have not been specified by the protocol. Results that are not within the normal range, as specified by the clinical lab, will also be reported as laboratory adverse events.

4.3.2. Was there a Clinical Adverse Event?

If there was a clinical adverse event, then the Clinical Adverse Events form (AECLIN) should be completed. This form documents all clinical adverse events experienced by a participant for the duration of a study. The DCC will perform interim reporting to the DSMB of adverse events for all studies.

Only conditions that first appear or become worse after study entry will be recorded. It is essential, therefore, that pre-existing medical conditions be accurately identified, characterized, and documented at the time of trial entry to prevent a medical event from being interpreted as an adverse event, when it is simply a reoccurrence of an existing medical problem. For example, a migraine attack may occur during the course of the study in a participant with a long-standing history of such headaches. Such an attack should be recorded as an adverse experience only if the event is different than before (e.g., of worse severity, duration, or frequency). If the pre-existing condition requires a medication change, where the new medication is equivalent to the old medication (i.e. a

change from one antihistamine to another), it does not require reporting as an adverse event.

Any unfavorable sign, symptom, disease, injury or syndrome that occurs during the study period should be recorded. This includes: any intercurrent illness, lab abnormality, symptoms significant enough to warrant therapy (including over-the-counter medications), any symptom significant to the participant, or any worsening of baseline conditions. Minor traumas such as bumps, bruises, hangnails, etc. that are part of normal childhood should not be recorded, unless they are clinically significant or significantly increased from baseline (i.e. sudden, diffuse bruising that can not be explained).

Participants who experience minor illnesses while in the study (intercurrent illnesses) may continue study participation. Examples of minor intercurrent illnesses include acute rhinitis, sinusitis, urinary tract infections, and gastroenteritis. Medications allowed for treatment of these conditions are described in the study-specific protocol and are in accordance with the judgment of the responsible physician. Illnesses constitute adverse events and require documentation ***only*** if they are appearing for the first time or if they represent a worsening of a prior condition. Adverse events are identified by the clinic coordinators in collaboration with the Clinical Center Investigators.

4.3.3. Was the Event Serious?

If the adverse event is deemed to be serious, appropriate procedures must be followed. This applies to significant asthma exacerbations as well as laboratory and clinical adverse events. The definition of a serious adverse event is described in the MOP and specific procedures for reporting are outlined. Investigators at the clinical centers will be responsible for assessing severity of adverse events and prescribing treatment.

4.3.4. Adverse Event Follow-Up

Adverse events are followed until the condition is resolved or stabilized. The medical judgment of the investigators must be used to determine what events must be followed after study termination. Events that are thought to be related to the study drug should be followed to resolution or stability of the event.

Adverse events due to either illnesses or injury may be grounds for withdrawal if the condition is considered to be significant by the investigator or if the participant is no longer able to effectively participate in the study.

4.3.5. Enter data and Submit Forms to DCC

The Clinical Centers are responsible for the timely documentation of adverse events and the submission of documentation to the DCC. A Serious Adverse Event Reporting (SERIOUS) form should be completed and faxed to the DCC at (717) 531-3922 within 72 hours of notification of a serious adverse event. Adverse Event forms should receive priority entry to maintain up-to-date safety information. It is the responsibility of the DCC to confirm that each adverse event is sufficiently documented.

Descriptions of adverse events should be clear and concise and accompanied by an ICD9 code. Note that codes should indicate the underlying condition causing an adverse event; procedure codes should not be submitted. Diseases or syndromes should be documented, not the individual symptoms (i.e. URI is documented, not the individual symptoms associated with the URI). A reference ICD9 code list (ICD9) which contains a list of common adverse events can be used for ease of reference.

For AsthmaNet purposes, reported ICD-9 codes should describe the underlying condition causing an adverse event; procedure codes should not be submitted. For example, if a participant undergoes a hysterectomy for treatment of uterine fibroids and related dysmenorrhea, codes for dysmenorrhea and uterine fibroids should be reported on the AECLIN form; no code for the hysterectomy procedure is necessary.

When assigning an ICD-9 code to an adverse event, the AsthmaNet Adverse Events ICD-9 Code Excel spreadsheet should be consulted first. This spreadsheet contains a comprehensive list of ICD-9 codes for common symptoms and conditions. It serves as the only acceptable reference for assigning ICD-9 codes for participants in any AsthmaNet protocols. As new conditions arise, the spreadsheet will be supplemented with additional codes, as needed. The spreadsheet is located on the AsthmaNet secure website in folder Application: ICD9 Codes. Alternatively, there is a search tool available during data entry of the AECLIN form.

It is essential that the following adverse event information be documented: date of onset, date stopped, type (intermittent, ongoing), maximum intensity (mild, moderate, severe), seriousness, likelihood of relationship of the adverse experience to study drugs, associated change in the drug dosage, outcome and treatment required. An explanation of all the data collected and copies of the forms are provided in the protocol-specific MOP.

The primary characteristics used to classify adverse events are:

1. the maximum intensity of the event, and;
2. the likelihood of its relationship to the study drug.

4.4. Maximum Intensity of the Event

Adverse events will be graded according to maximum intensity on a 3-point scale:

Maximum intensity	Event characteristics
Mild	No interruption in normal activities, study medications, or procedures. Symptoms noticeable but easily tolerated.
Moderate	Some interruption of normal activities (e.g., loss of school day or parent's work day due to illness or doctor's visit) or brief interruption of study medications or procedures.
Severe	Significant interruption of activities and unlikely to be able to effectively continue participation in the study; an incapacitating event.

The term "severe" is a measure of intensity. A severe reaction is not necessarily serious. For example, complete alopecia (hair loss) may be considered severe, but not serious. Conversely, a serious reaction may not necessarily be severe, e.g., a stroke that results in only a limited disability may be considered mild, but it would be a serious adverse event.

While **cancer and overdose** are not included in the ICH E2A definition of "serious" which has been adopted by worldwide regulators, the AsthmaNet will record these events and report them to the DSMB.

4.5. Relationship to Study Drug

The causal relationship between an adverse event and a protocol-prescribed medication will be assessed and categorized:

Relationship to study drug	Event characteristics
None - not related	Event for which there is another explanation, i.e., gunshot wound.

Unlikely - remote	Event relationship to drug is doubtful, but it cannot be completely ruled out.
Possible	Event may have been caused by clinical state or other therapy.
Probable	Condition recedes when the study drug is withdrawn and cannot be explained by participant's clinical state.

4.6. Reporting Adverse Events

Routine adverse event reporting is an important component of the interim and final analyses presented to the DSMB. Adverse event reports will include the frequency, severity and likelihood of relationship to study drug for documented events for each treatment group. Investigators must submit a written summary of the DSMB periodic review to their IRB (per the 7/1/99 NIH policy entitled, "Guidance on Reporting Adverse Events to Institutional Review Boards for NIH-Supported Multicenter Clinical Trials").

4.7. Reporting Serious Adverse Events and Unanticipated Problems

Studies involving human subjects research must include procedures for identifying, monitoring, and reporting adverse events and unanticipated problems. For clinical trials and studies with greater than minimal risk, these procedures should be described in the study's Institutional Review Board-approved data and safety monitoring plan which is sent to the NHLBI.

4.7.1 Expedited Reporting

Expedited reporting to the NHLBI Program Official or Project Officer is required for unanticipated problems or unexpected serious adverse events that may be related to the study protocol as follows:

Any event or problem that is:

1. Unexpected

AND

2. Possibly, probably, or definitely related to study participation

AND one of the following:

3a. Is fatal, life-treatening or serious (SAE + UP) – **Report within 7 calendar days**

OR

3b. Suggests greater risk of harm to study participant(s) than was previously known or recognized (UP) – **Report within 30 calendar days**

4.7.2 Review of Serious Adverse Event Report with M.D. at Clinical Center

1. *Complete Serious Adverse Event Reporting form (SERIOUS).* Fax all completed forms to the DCC within 72 hours of initial report to Clinical Center. The completed forms and supporting documentation includes the associated Clinical Adverse Event (AECLIN) form, the Concomitant Medication (CMED) form and the appropriate protocol-specific Change in Medication (PX_CHANGE_MEDS) form (if applicable). Any Emergency Department records, clinical notes, and other clinical documentation should be included as well, with all identifying information blacked out and replaced with participant's study ID number and initials.
2. In addition, the PI should send a final resolution report to the DCC, with relevant documents attached (including ER report, discharge summary, relevant clinical notes, pathology reports, ECG reports, laboratory reports, etc.). Participant name and other clear identifiers should **NOT** appear on these records; only the participant ID and initials should be used.
3. The P.I. or Co-Investigator should also provide the DCC with a brief summary on the disposition of a participant who experienced a serious adverse event. These follow-up plans should include the participant's status in the study, if they will be continuing on study medication, if they will be dropped from the study, when the next visit is scheduled and what communication occurred with the treating physician and participant's parent.

4.7.3 Review of Serious Adverse Event Report by PI or Co-PI at DCC

Does the event require immediate reporting? The PI or Co-PI at the DCC will review all documentation. Based on the seriousness of the event and the likelihood of its relationship to the study drug, urgency of reporting will be determined. Members of the AsthmaNet Steering Committee will be consulted as needed. If AsthmaNet holds an investigator IND and the SAE is thought to be related to the study drug, a report will be filed with the FDA in accordance with FDA guidelines (21 CFR 312.32) detailed in item 4.7.4.

- DCC reports to DSMB, NIH and AsthmaNet Steering Committee; pharmaceutical supplier and FDA (when appropriate; 4.7.4). These individuals will be informed of the event, within 24 hours of receipt at the DCC and assessment of urgency.
- DCC and PI's at Clinical Centers report to Institutional Review Boards. The DCC and PI's at the Clinical Centers are responsible for informing their local Institutional Review Boards.
- DSMB members must review the SAE and complete a comment ballot within one week of receipt. The comment ballot allows the DSMB members to make comments, request a conference call to discuss the SAE, or request other action. The comment ballots are sent directly to the AsthmaNet project scientist at NIH.
- A report recognizing the reporting of the SAE to the IRB at the clinical centers should be forwarded to the DCC and NHLBI. This will ensure that appropriate local reporting has been accomplished.

4.7.4 Reporting of Serious Adverse Events by DCC PI or Co-PI – FDA

If an SAE occurs in a protocol under FDA Investigational New Drug (IND) oversight, follow reporting requirements under 21 CFR 312.32.

Written IND Safety Reports:

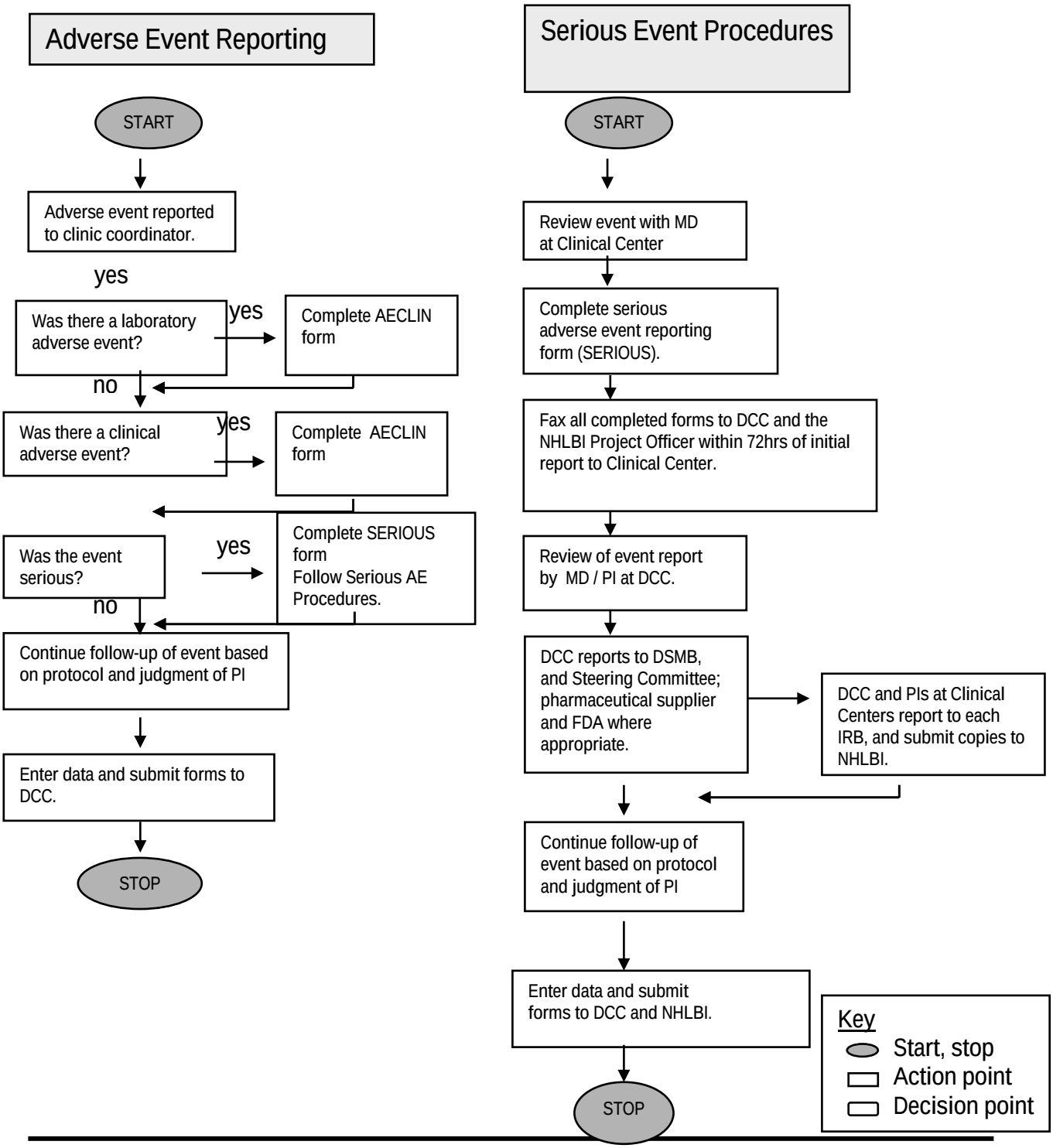
- The DCC PI or Co-PI will submit a written IND Safety Report (i.e., completed FDA Form 3500A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be a *serious and unexpected, suspected adverse reaction*. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating investigators (if applicable) and sub-investigators.
- Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the DCC PI or Co-PI's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.
- For each written IND Safety Report, the DCC PI or Co-PI will identify all previously submitted IND Safety Reports that addressed a similar suspected adverse reaction experience and will provide an analysis of the significance of newly reported, suspected adverse reaction in light of the previous, similar report(s) or any other relevant information.

- Relevant follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the information is available and will be identified as such (i.e., “Follow-up IND Safety Report”).
- If the results of the DCC PI or Co-PI’s follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the DCC PI or Co-PI will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

Telephoned IND Safety Reports – Fatal or Life-threatening Suspected Adverse Reactions:

- In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the DCC PI or Co-PI will notify the responsible review division of the FDA by telephone or facsimile transmission of any *unexpected, fatal or life-threatening suspected adverse reaction.*
- The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the DCC PI or Co-PI’s receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

Flow Chart for Handling of Adverse Events



4.8. Rescue algorithm for asthma research participants

In order to identify and possibly prevent serious adverse events, the following guidelines must be followed by Clinical Center coordinators unless they conflict with institutional policies. Institutional policies override the instructions in this section.

4.8.1. Prevention of Exacerbations

1. Never leave a participant unattended.
2. Do not perform procedures with the potential for worsening asthma (e.g., methacholine testing, skin testing, sputum induction) without ready access to a physician.
3. Do not perform procedures with the potential for worsening asthma (e.g., methacholine testing, skin testing, sputum induction) during times of unstable asthma.
4. Perform appropriate clinical assessments and vital signs including peak flows should asthma worsening occur. Document this information in the participant's clinic notes.

4.8.2. Treatment of Exacerbations

1. If a participant is in impending or actual respiratory or cardiac arrest, activate Code _____, call 911, or STAT call AsthmaNet MD or designate:
   _____
   _____
   _____

   Evaluate for CPR, administer 6L/min O_2, and consider epinephrine (0.01 ml/kg, max 0.3 ml IM) in outer surface of upper arm.
2. If a participant is in severe respiratory distress based on one or more of the following symptoms:
   - Cyanotic (blue nail beds and lips)
   - Inability to complete full sentences
   - Accessory muscle use

- O_2 saturation < 90%
- Respiratory rate > 30/minute
- Heart rate > 130/min (>160/min for participants < 18 years)
- Diaphoretic (sweating)
- Asthma score (see page 16 for determination) $\geq$ 5
- Confused
- PF below 25% predicted
- FEV_1 below 25% predicted

Immediately call the AsthmaNet MD or designate to the participant's room.

_____
_____
_____

Assess participant and obtain vital signs (B/P, HR, RR, and oxygen saturation) and administer:

- Oxygen at 4L/min to maintain O_2 saturations > 90%
- Albuterol (4-6 puffs MDI or nebulized albuterol solution (0.5 ml of 0.5% multidose solution and 2.0 ml saline or unit dose of 0.083% albuterol)

and if not responding

- Epinephrine (0.01 ml/kg, max 0.3 ml IM) OR SQ.

3. If the participant is not severe enough to declare an emergency, administer albuterol by MDI 2-4 puffs or, then perform PEF 15 minutes later and evaluate asthma score (see page 16), then contact AsthmaNet MD:

_____
_____
_____

4. If the participant's PEF is less than 25% predicted (40% for participants < 18 years) or asthma score is ≥ 4 (see page 16 for determination), nebulized albuterol solution (0.5 ml of 0.5% multi-dose solution and 2.0 ml saline or unit dose of 0.083% albuterol). If needed, repeat nebulized treatment at the same dose. MD will completely assess the participant's clinical status.
5. If the participant's PEF is equal to or greater than 60% predicted or the asthma score is ≤ 1 (See page 16 for determination), the participant should be instructed to

use the "PRN beta-agonist MDI" for home treatment according to their established protocol guidelines.

6. If the participant's FEV_1 is greater than 25% predicted (40% for participants < 18 years) but less than 60% predicted or the asthma score is 2 but less than 4 (see page 16 for determination) repeat albuterol 2-4 puffs or nebulized albuterol solution every 20 minutes up to an hour. Continue to assess clinical status and pulmonary function and follow NAEPP guidelines for acute asthma treatment.
7. By physician discretion add prednisone 80 mg po or Methylprednisolone 60 mg IV. Follow the prednisone course per AsthmaNet schedule. (For participants > 18 years: By physician discretion add prelone (15 mg/ml) or prednisone at a dose of 2 mg/kg (maximum 60 mg or 20 ml) p.o. if determined necessary. Follow the prelone course (2 mg/kg per day for 2 days, 1 mg/kg per day for 2 days))
8. If clinical status does not improve or PEF or FEV_1 remains below 50% predicted despite aggressive therapy consider hospitalization or transfer to ER.
9. If appropriate, contact the participant's primary care physician to inform him/her of the exacerbation.
10. Follow-up in 1 week to ensure stability of asthma.

Asthma Score

Sum the scores for the individual parameters to determine the total score. If the score is greater than or equal to 5, the participant is in severe respiratory distress.

Asthma Score	0	1	2
	On Room Air	On Room Air	On 40% Oxygen
O_2 saturation or Cyanosis	≧ 93% No	< 93% Yes	< 93% Yes
Breath sounds	Equal	Unequal	Absent
Wheezing	None	Moderate	Marked
Accessory muscles	None	Moderate	Marked
Consciousness	Normal	Agitated or depressed	Coma

4.9. Rescue Algorithm for Systemic Allergic Reactions to Skin Testing in Research Participants

4.10. Activation of CODE BLUE

The following guidelines need to be followed by Clinical Center coordinators unless they conflict with institutional policies.

To call CODE BLUE, dial _____, listen for tone and an operator will come on to assist you.

Comments:

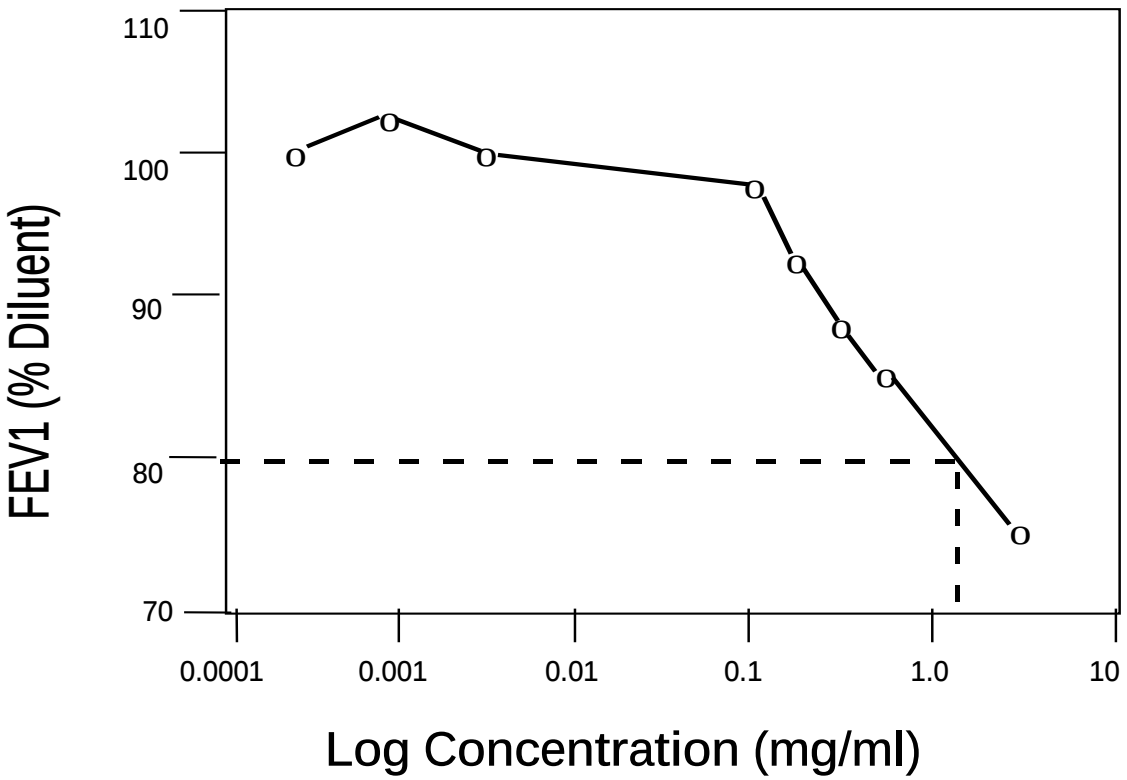
_____

_____

4.11. Emergency Equipment Locations

Emergency Equipment	Clinic Location (Room #, etc.)
Crash Cart	
EKG Machine	
Pulse Oximeter	
Bag and Mask (O_2 Setup)	
Suction Setup	
Nebulizer Treatment Setup	
IV Equipment (Catheters, Fluids, etc.)	
1:1,000 Epinephrine	

AsthmaNet



METHACHOLINE
MANUAL OF OPERATIONS

Introduction

Dilution Sequence Protocol

- These instructions are for the preparation of Provocholine and prepared according to the protocol provided by AsthmaNet
- The following are step-by-step instructions to dilute two 100 mg vials of Provocholine® into the 10 (ten) requisite concentrations for use in a bronchoprovocation challenge test
- **Please refer to the Package Insert for full instructions and safety precautions**
- Accurate sterile mixing is essential for the accuracy of the test results and to maintain patient safety
- Only trained individuals should mix and label methacholine solutions
- The following protocol is used to prepare two 100 mg vials of Provocholine® for testing a single patient only

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Important Notes

- Do not inhale powder during preparation of dilutions
- Do not handle Provocholine® if you have asthma or hay fever
- Provocholine® dilutions should be mixed by a pharmacist or other well-trained individual using sterile technique
- All vials should be labeled with the appropriate Lot Number, concentration, diluent used (in this case 0.9% Saline), preparation, initials of person preparation, and expiration dates.
- To reduce back pressure, vent vials with an extra needle as needed
- All dilutions should be made with:
  - 0.9% Saline
  - Sterile, empty USP Type I borosilicate glass vials
- When transferring solution from each vial (at least 2 mL) to a nebulizer use a sterile bacterial-retentive filter (porosity 0.22 µm)
- Practitioners should consult the Provocholine package insert for complete product safety information.

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Supplies Required

Suggested supplies for the dilutions of Provocholine® 100 mg

Quantity	Description
2	100 mg/vials of Provocholine®
1	100 mL 0.9% Saline
8	10 mL Sterile empty USP Type I borosilicate glass vials
1	10 mL Syringe* ** (labeled for diluent)
1	3 mL Syringe* ** (labeled for methacholine)
1	1 mL Syringe* ** (labeled for diluent)
3	20 Gauge, 1" Syringe Needles*
1	Millex GV 0.22 µm filter (Millipore)*
11	Alcohol Preparation Swabs*
1 set	Labels for vials
X	Set of Directions
X	Provocholine® Dilution Sequence Check Sheet and Control Record
X	Package Insert for Provocholine®

* Quantity of supplies subject to facility protocol

** Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

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Overview of the Dilution Process

TAKE Provocholine® (Methacholine Chloride U.S.P. Powder for Inhalation)	**ADD** 0.9% Saline (SHAKE WELL!)	**OBTAIN DILUTION**	**VIAL NAME**
Provocholine® 100 mg	3.125 mL	32 mg/mL	VIAL A - 32 mg/mL
Provocholine® 100 mg	6.25 mL	16 mg/mL	VIAL B - 16 mg/mL
3 mL from viaL B	3 mL	8 mg/mL	VIAL C- 8 mg/mL
3 mL from viaL C	3 mL	4 mg/mL	VIAL D - 4 mg/mL
3 mL from viaL D	3 mL	2 mg/mL	VIAL E - 2 mg/mL
3 mL from viaL E	3 mL	1 mg/mL	VIAL F - 1 mg/mL
3 mL from viaL F	3 mL	0.5 mg/mL	VIAL G - 0.5 mg/mL
3 mL from viaL G	3 mL	0.25 mg/mL	VIAL H - 0.25 mg/mL
3 mL from viaL H	3 mL	0.125 mg/mL	VIAL I - 0.125 mg/mL
3 mL from viaL I	3 mL	0.0625 mg/mL	VIAL J - 0.0625 mg/mL

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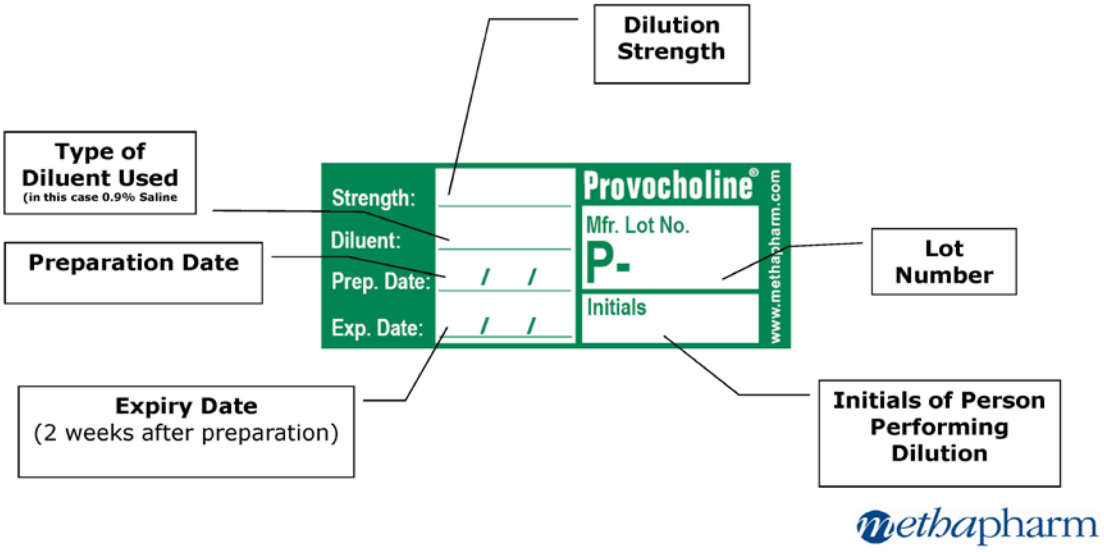
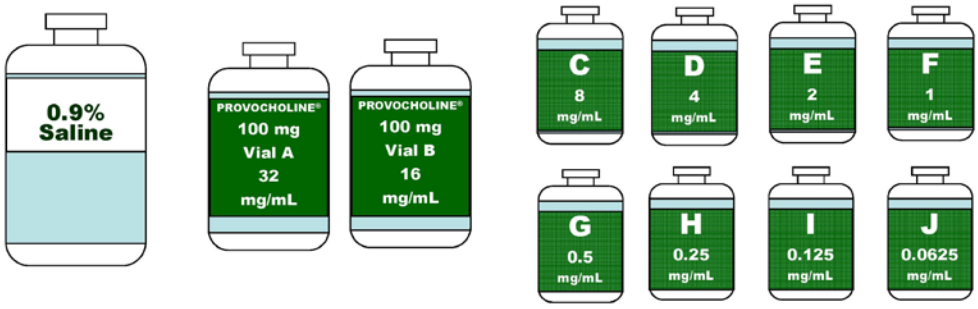
Getting Started –Labels

- Fill in all information on **labels**
- Include **14 day expiration** date on vials A - J

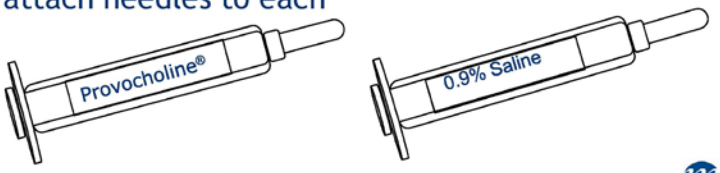


Getting Started

1. Attach labels to sterile empty vials
2. Wipe down the tops of the Sterile Empty vials, Provocholine®, and Saline vials with alcohol swabs:



3. Label syringes, (one for Provocholine® and one for 0.9% Saline) and attach needles to each*



*Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

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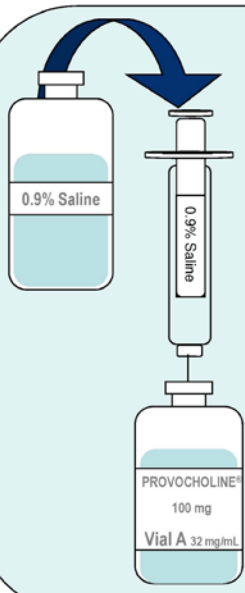
Step I: Preparing Vial A - Provocholine® **32 mg/mL** Solution



1. Using the 0.9% Saline needle and syringe*:
   - Draw 3.125 mL of 0.9% Saline
   - Transfer the 3.125 mL of 0.9% Saline to the Provocholine® vial
2. Shake Well

After Completing Step I:

Vial A contains 3.125 mL of Provocholine® Solution @ 32 mg/mL

* Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

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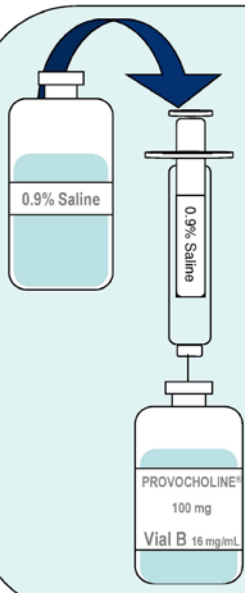
Step II: Preparing Vial B - Provocholine® **16 mg/mL** Solution



1. Using the 0.9% Saline needle and syringe*:
   - Draw 6.25 mL of 0.9% Saline
   - Transfer the 6.25 mL of 0.9% Saline to the Provocholine® vial
2. Shake Well

After Completing Step II:

Vial B contains 6.25 mL of Provocholine® Solution @ 16 mg/mL

* Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

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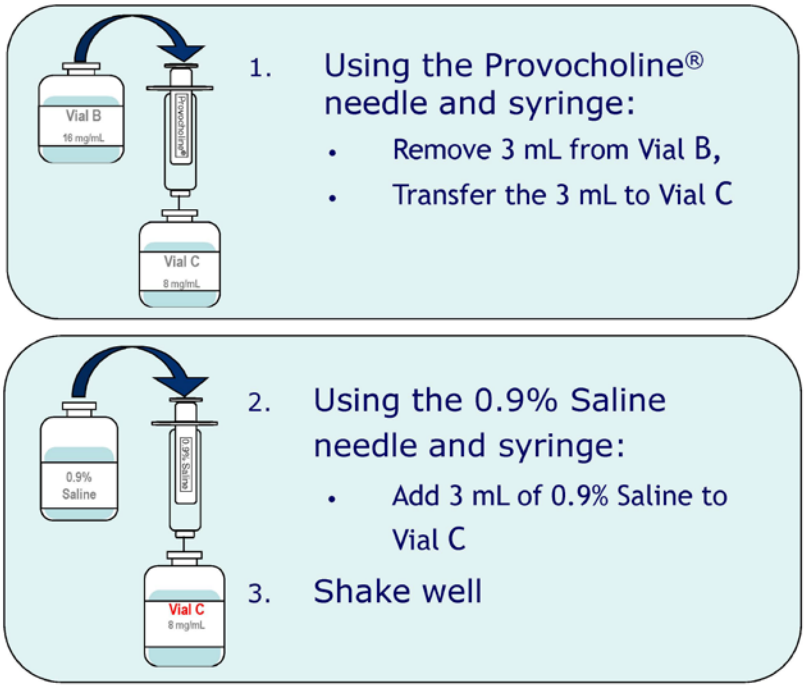
Step III: Preparing Vial C - Provocholine® **8 mg/mL** solution



After Completing Step III:

Vial B contains 3.25mL of Provocholine® solution @ 16 mg/mL

-and-

Vial C contains 6 mL of Provocholine® solution @ 8 mg/mL

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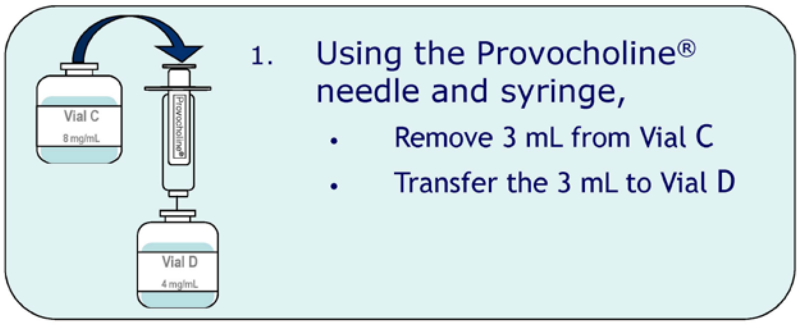
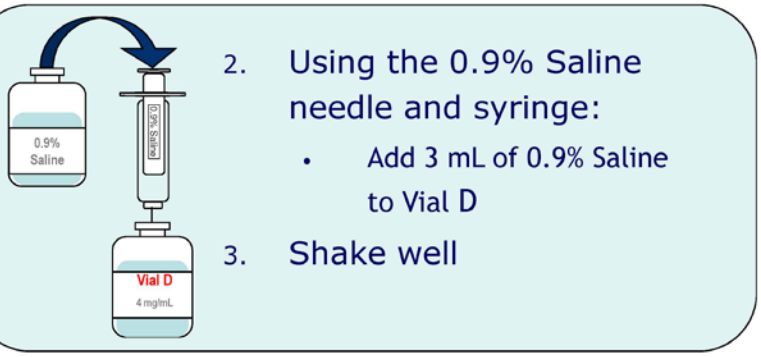
Step IV: Preparing Vial D - Provocholine® **4 mg/mL** solution



1. Using the Provocholine® needle and syringe,
   - Remove 3 mL from Vial C
   - Transfer the 3 mL to Vial D



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial D
3. Shake well

After Completing Step IV:
Vial C contains 3 mL of Provocholine® solution @ 8 mg/mL

-and-

Vial D contains 6 mL of Provocholine® solution @ 4 mg/mL

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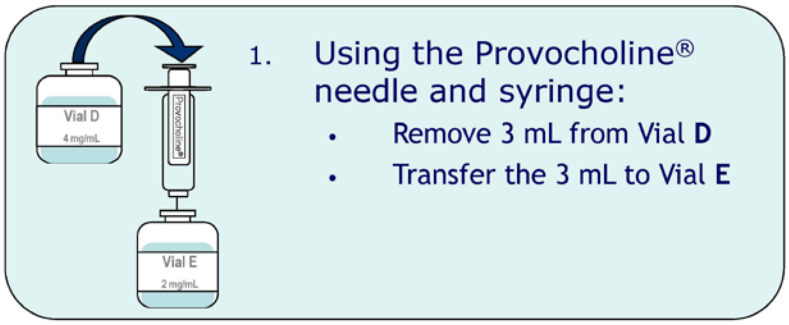
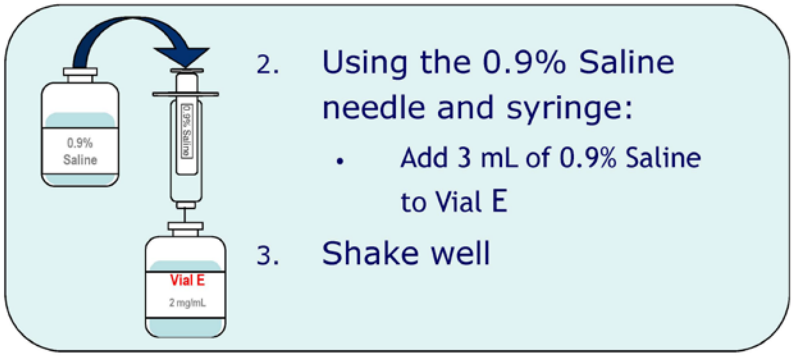
Step V: Preparing Vial E - Provocholine® **2 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **D**
   - Transfer the 3 mL to Vial **E**



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial E
3. Shake well

After Completing Step V:

Vial D contains 3 mL of Provocholine® solution @ 4 mg/mL

- and-

Vial E contains 6 mL of Provocholine® solution @ 2 mg/mL

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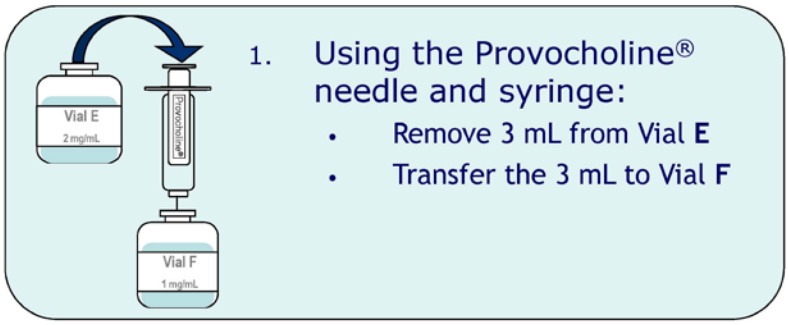
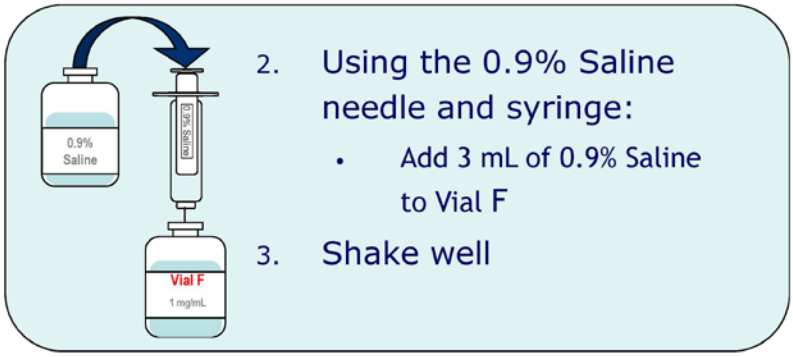
Step VI: Preparing Vial F - Provocholine® **1 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **E**
   - Transfer the 3 mL to Vial **F**



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial F
3. Shake well

After Completing Step VI:

Vial E contains 3 mL of Provocholine® solution @ 2 mg/mL

-and-

Vial F contains 6 mL of Provocholine® solution @ 1 mg/mL

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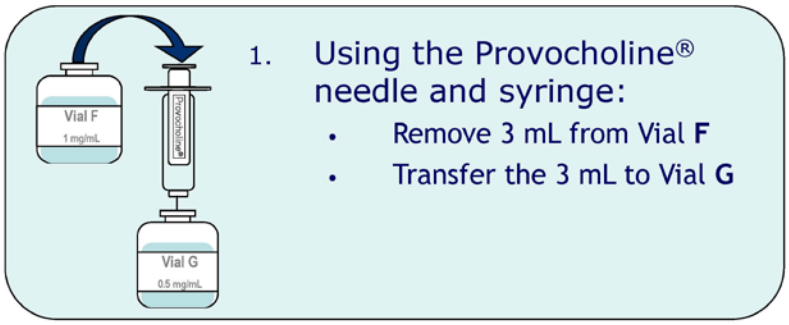
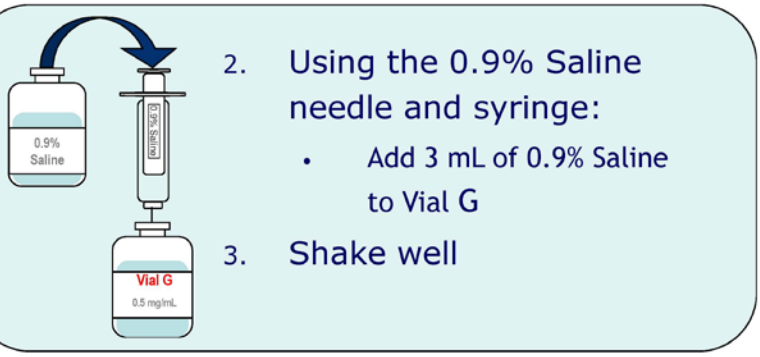
Step VII: Preparing Vial G - Provocholine® **0.5 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **F**
   - Transfer the 3 mL to Vial **G**



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial G
3. Shake well

After Completing Step VII:

Vial F contains 3 mL of Provocholine® solution @ 1 mg/mL

-and-

Vial G contains 6 mL of Provocholine® solution @ 0.5 mg/mL

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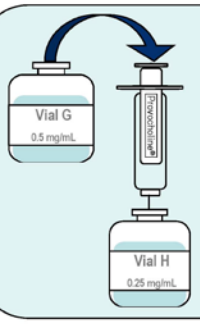
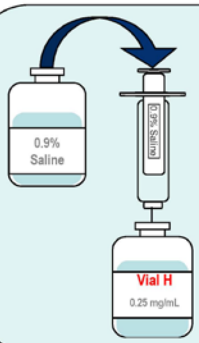
Step VIII: Preparing Vial H - Provocholine® **0.25 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **G**
   - Transfer the 3 mL to Vial **H**



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial H
3. Shake well

After Completing Step VIII:

Vial G contains 3 mL of Provocholine® solution @ 0.5 mg/mL

-and-

Vial H contains 6 mL of Provocholine® solution @ 0.25 mg/mL

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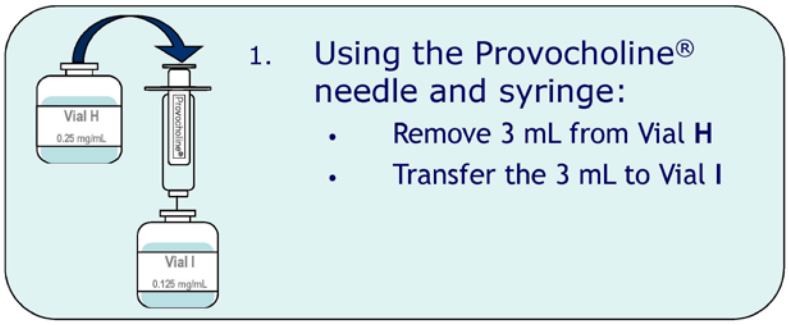
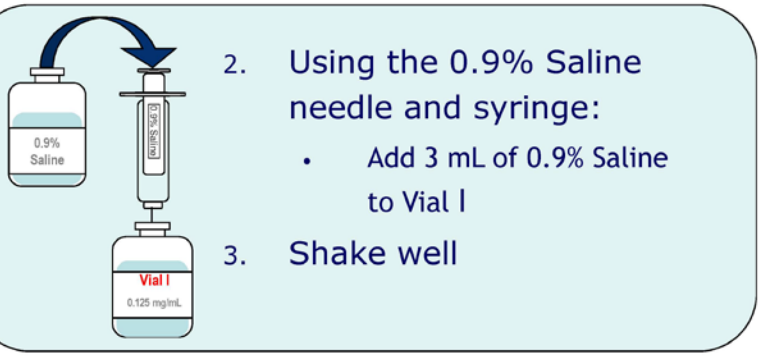
Step IX: Preparing Vial I - Provocholine® **0.125 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **H**
   - Transfer the 3 mL to Vial **I**



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial I
3. Shake well

After Completing Step IX:

Vial H contains 3 mL of Provocholine® solution @ 0. 25 mg/mL

-and-

Vial I contains 6 mL of Provocholine® solution @ 0.125 mg/mL

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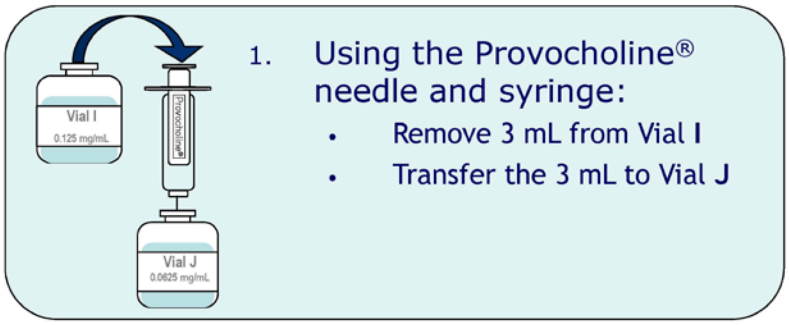
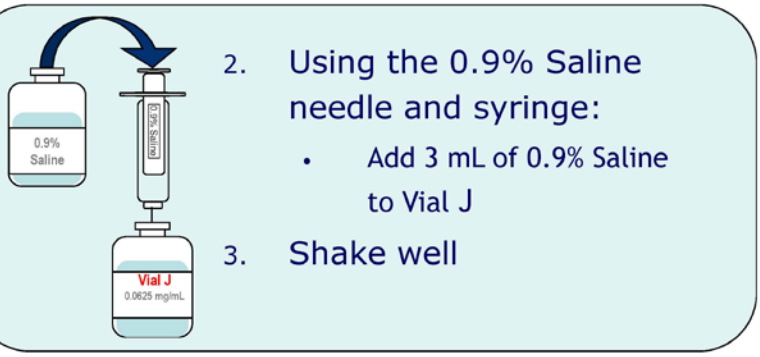
Step X: Preparing Vial J - Provocholine® **0.0625 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **I**
   - Transfer the 3 mL to Vial **J**



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial J
3. Shake well

After Completing Step X:

Vial I contains 3 mL of Provocholine® solution @ 0.125 mg/mL

-and-

Vial J contains 6 mL of Provocholine® solution @ 0.0625 mg/mL

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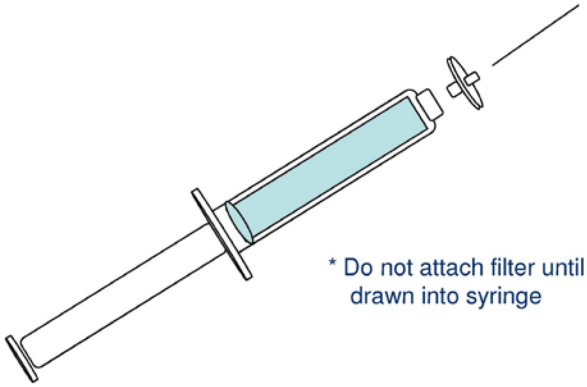
Final Step: Day of the Test

- Aseptically attach a 0.22 µm bacterial retentive filter (Millex GV®) to a 10 mL syringe*
- Transfer 2 to 3 mL of all dilutions (in vials A through J) to the nebulizer(s) through the 0.22 µm bacterial retentive filter (Millex GV®)



* Do not attach filter until AFTER solution has been drawn into syringe

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Storage Instructions

- Dilutions A through J (32 mg/mL through 0.0625 mg/mL) should be stored at 36° to 45°F (2° to 8°C) in a refrigerator for no more than 2 weeks
- Unreconstituted powder should be stored at 59° to 86 °F (15° to 30°C)
- Freezing does not affect the stability of dilutions

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Dilution Check Sheet and Control Record

PROVOCHOLINE® DILUTIONS FOR CHALLENGE TEST

Date: _____ Prepared by:_____ Checked by:_____

Provocholine® (see label on bottle): Expiration Date: _____

0.9% Saline: Expiration Date: _____

0.22 micron Filter: Expiration Date: _____

TAKE Provocholine® (Methacholine Chloride U.S.P. Powder for Inhalation)	**ADD** 0.9% Saline (SHAKE WELL!)	**OBTAIN DILUTION**	**VIAL NAME**	**COMPLETED**
Provocholine® 100 mg	3.125 mL	32 mg/mL	VIAL A - 32 mg/mL	
Provocholine® 100 mg	6.25 mL	16 mg/mL	VIAL B - 16 mg/mL	
3 mL from vial B	3 mL	8 mg/mL	VIAL C - 8 mg/mL	
3 mL from vial C	3 mL	4 mg/mL	VIAL D - 4 mg/mL	
3 mL from vial D	3 mL	2 mg/mL	VIAL E - 2 mg/mL	
3 mL from vial E	3 mL	1 mg/mL	VIAL F - 1 mg/Ml	
3 mL from vial F	3 mL	0.5 mg/mL	VIAL G - 0.5 mg/mL	
3 mL from vial G	3 mL	0.25 mg/mL	VIAL H - 0.25 mg/mL	
3 mL from vial H	3 mL	0.125 mg/mL	VIAL I - 0.125 mg/mL	
3 mL from vial I	3 mL	0.0625 mg/mL	VIAL J - 0.0625 mg/mL	

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Summary of Directions

Procedure:

1. Fill in information and attach labels to the vials.
2. Remove plastic cover from the Provocholine® and 0.9% Saline vials and line up all sterile empty vials. Wipe off all vial stoppers for Provocholine®, 0.9% Saline and sterile empty vials with alcohol swabs.
3. Attach needles to the syringes and label one for Provocholine® and one for 0.9% Saline.
4. Using the 0.9% Saline needle and syringe, withdraw 3.125 mL of 0.9% Saline* and insert into the first Provocholine® 100mg vial. SHAKE WELL. This produces 3.125 mL of Provocholine® 32 mg/mL, also known as **VIAL A – 32 mg/mL.**
5. Using the 0.9% Saline needle and syringe, withdraw 6.25 mL of 0.9% Saline* and insert into the second Provocholine® 100mg vial. SHAKE WELL. This produces 6.25 mL of Provocholine® 16mg/mL, also known as **VIAL B – 16 mg/mL.**
6. Using the Provocholine® needle and syringe, remove 3 mL from VIAL B 16 – mg/mL and transfer to the vial labelled VIAL C – 8 MG/ML. Using the 0.9% Saline needle and syringe, add 3 mL 0.9% Saline. Shake well. **THIS IS VIAL C – 8 mg/mL.**
7. Using the Provocholine® needle and syringe, remove 3 mL from VIAL C – 8 mg/mL and transfer to the vial labelled VIAL D – 4 MG/ML. Using the 0.9% Saline needle and syringe, add 3 mL of 0.9% Saline. Shake well. **THIS IS VIAL D – 4 mg/mL.**
8. Using the Provocholine® needle and syringe, remove 3 mL from VIAL D – 4 mg/mL, and transfer to the vial labelled VIAL E – 2 MG/ML. Using the 0.9% Saline needle and syringe, add 3 mL of 0.9% Saline. Shake well. **THIS IS VIAL E – 2 mg/mL.**
9. Using the Provocholine® needle and syringe, remove 3 mL from VIAL E – 2 mg/mL and transfer to the vial labeled VIAL F – 1 mg/mL. Using the 0.9% Saline needle and syringe, add 3 mL of 0.9% Saline. Shake well. **THIS IS VIAL F – 1 mg/mL.**

* Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

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1. METHACHOLINE CHALLENGE TESTING OVERVIEW

Methacholine challenge testing is performed to assess airway responsiveness. Airway responsiveness during a methacholine challenge is most often referred to as bronchial hyperresponsiveness (BHR), but can also be called airway(s) reactivity, or bronchial hyper reactivity. The vast majority of normal individuals show no change in lung function when they inhale low concentrations of substances such as histamine or methacholine. On the other hand, nearly all individuals with active asthma exhibit narrowing of their airways when they inhale concentrations of these substances. Methacholine challenge testing has a greater negative predictive power than a positive predictive power. However, there is a rough correlation between airway hyperresponsiveness and the severity of asthma – those with worse asthma tend to be more sensitive. There tends to be less bronchial hyperresponsiveness when the asthma is well controlled. It is important to understand, however, that there can be wide variability in the test results, both between people and within the same person, from day to day or season to season, so that the methacholine challenge test is not a substitute for other measures of asthma severity. In addition, bronchial hyperresponsiveness can also be seen in a wide variety of other diseases including COPD, bronchitis and allergic rhinitis.

Because an improvement in airway responsiveness is often associated with an improvement in asthma symptoms, clinical trials often use a change in PC20 as an objective study outcome or a characterization procedure.

1.1. Different Types of Bronchial Reactivity Testing

There are many methods to perform bronchial reactivity testing, most of which give similar results. Histamine is a naturally occurring substance released during allergic reactions and is particularly important in causing hives. When inhaled, histamine causes airway narrowing in asthmatics more easily than in non-asthmatics. Histamine is widely used, particularly in Europe, for bronchial reactivity testing, although it can cause flushing and headaches in some people. Other types of airway provocation challenges include: vigorous exercise, cold dry air, distilled water, hypertonic saline, or antigen challenges (inhaling specific allergens such as dust mite antigen or ragweed antigen).

Methacholine is most often used in North America, and is the testing substance used for the AsthmaNet trials. It is a derivative of acetylcholine, a naturally occurring substance released from nerves. This substance activates cholinergic or muscarinic nerve receptors on the airways and causes them to narrow by constricting the smooth muscle surrounding the airways. If given in excessive doses, methacholine may cause

a severe asthma attack. If too much methacholine is absorbed into the body, symptoms such as abdominal cramping, diarrhea, sweating, and salivation can occur.

The activation of the airway (or other organ) nerve receptors by the methacholine can be blocked by the drug atropine, which is the specific antidote for methacholine overdose.

1.2. Different Inhalation Methods Used In Methacholine Challenge Procedures

There are several inhalation methods that can be used to test reactivity to substances that provoke airway narrowing.

In the tidal breathing method, the individual performs quiet resting (tidal) breathing for two minutes while inhaling methacholine from a nebulizer. After the two minute breathing exposure, spirometry is performed. The procedure is repeated with increasing concentrations of methacholine until there is an adequate degree of airway narrowing or the maximum concentration is given.

The dosimeter method uses an electronically controlled valve to deliver a controlled amount of methacholine from a nebulizer to be inhaled during the course of an inspiratory capacity breath. Doses are followed by a short waiting period and then spirometry. Increasing concentrations of methacholine are administered until a PC20 is met or the maximum concentration is given. This is the method that will be used in the AsthmaNet protocols.

1.3. Measurement of Airway Responsiveness

A change in FEV_1 (forced expiratory volume in one second) will be used to determine the degree of airway narrowing during methacholine challenges in AsthmaNet studies.

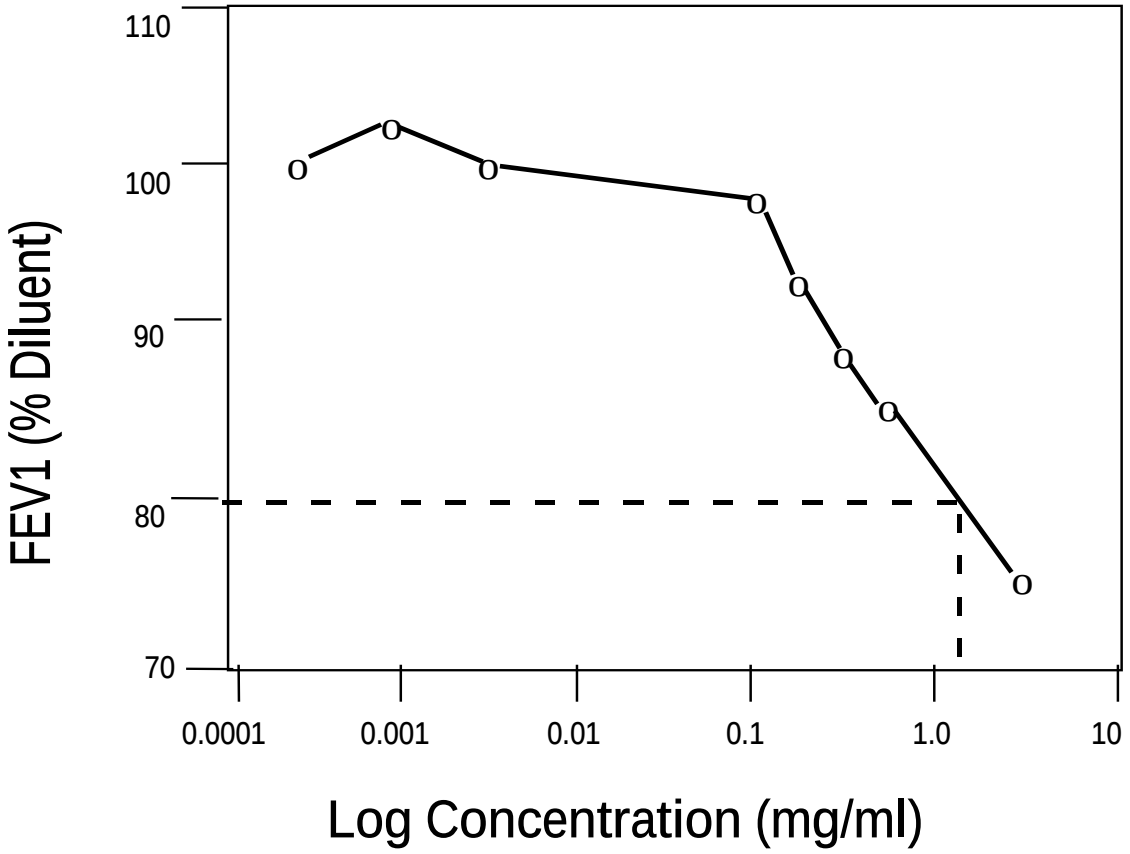
In the methacholine challenge study, increasing concentrations of methacholine are delivered until the FEV_1 falls by 20% or more compared to a reference (post-diluent) level. The Provocative Concentration that causes a 20% fall in FEV_1 is referred to as a PC_{20}.

When a PC_{20} is achieved (20% or greater reduction in FEV_1), the challenge is stopped. If the FEV_1 reduction is exactly 20%, the PC_{20} is equal to the last concentration of methacholine that was administered. If the reduction is greater than 20%, the PC_{20} is calculated from the last two concentrations administered. Since it is uncommon that a particular concentration causes exactly a 20% reduction in FEV_1 the PC_{20} is usually a calculated value.

Please enter the PC_{20} value calculated by the computer on the Methacholine Challenge Testing (METHA) data collection form.

The concentration of methacholine is plotted on a log scale. Note that the PC_{20} is interpolated between the changes of the last two concentrations. In this example PC_{20} = antilog (1.2) = 15.8 mg/ml.

Figure 1: Calculation of PC_{20} using the graphical method



In AsthmaNet trials, the computer will calculate the PC_{20} value for the majority of tests and the value will print on the graph on the methacholine challenge report. If a problem occurs and a PC_{20} needs to be recomputed, send an e-mail to the overreader and the protocol's scientific coordinator at the DCC, and download the challenge session. The PC_{20} will be re-calculated for you and communicated to you via e-mail. The e-mail message will serve as source documentation for data processing.

Note that the MedGraphics software will not compute the PC_{20} value if a participant's FEV_1 falls >20% after administering the first concentration (0.0625 mg/ml). In this case

the scientific coordinator at the DCC must be contacted to perform the calculation, as described above and in section 10 of this manual.

The lower the PC_{20}, the more sensitive an individual is to methacholine. Most people with active asthma have a PC_{20} less than 8-16 mg/ml methacholine.

2. CONTRAINDICATIONS AND SAFETY ISSUES

Methacholine challenge testing should ONLY be performed on study participants who can perform good quality spirometry.

If you have questions or concerns about whether you should proceed with a methacholine challenge, speak with your supervising physician and the protocol's scientific coordinator at the DCC.

It is important that the technician performing the test pay close attention to methacholine challenge contraindications and procedural details to avoid compromising the quality of the test results and/or subjecting the study participant to increased risk or discomfort. Technicians must be alert and attentive during this procedure. Technicians must closely observe the study participant for signs of respiratory distress while carefully, sequentially following the steps of the procedure. The greatest potential for inducing a severe asthma attack is to administer the test to a participant who already has severely impaired lung function, or to inadvertently administer too high a concentration of methacholine.

Contraindications to performing a methacholine challenge should be identified before the challenge is begun. Eligibility for performing a methacholine challenge test is assessed through the questions on the Methacholine Challenge Testing Checklists (METHACHK_ADULT and METHACHK_PED).

2.1. Pediatric Considerations

Study coordinators should use their best clinical judgment to determine if a pediatric study participant (defined as a participant under the age of 18) is able to understand directions and can remain on task for the duration of a methacholine challenge.

2.2. Pregnancy or Lactation

Methacholine testing should not be performed during pregnancy or lactation. A negative pregnancy test should be documented in females of childbearing potential on the day of testing.

2.3. FEV_1

Methacholine challenge testing is considered a safe procedure, even for people with severe asthma. However, methacholine testing should not be performed on a participant who has excessively low lung function or is having symptoms of an asthma attack. Dramatic drops in FEV_1 can occur and the risk of this is greater in individuals with low lung function.

The lower limit for FEV_1 varies even among the experts, but for the purposes of the AsthmaNet trials:

- Baseline (pre-diluent) FEV_1 must be at least 55% of predicted for ADULTS
- Baseline (pre-diluent) FEV_1 must be at least 1.0 liter for ADULTS
- Baseline (pre-diluent) FEV_1 must be at least 70% of predicted for PEDIATRICS

If a participant is currently having symptoms of an asthma attack (e.g., wheezing, coughing, report of chest symptoms), you should check with the supervising physician before proceeding with the testing, even if the participant's lung function exceeds the above parameters. Be prepared to stop the challenge if the participant demonstrates symptoms of worsening asthma during the procedure (wheezing, increased respiratory work, suprasternal tugging).

2.4. Blood Pressure

The possibility of cardiovascular stress related to induced bronchospasm may put individuals with hypertension at risk for a cardiovascular event. Therefore adult study participants with a systolic blood pressure >200 mm Hg or a diastolic blood pressure >100 mm Hg should not perform a methacholine challenge. The same blood pressure limits apply for children who are at least 12 years of age. Pediatric blood pressure limits for children under age 12 are systolic >180 mm Hg and diastolic >90 mm Hg.

2.5. Avoidance of Drugs and Substances in Preparation for Methacholine Testing

Certain medications and substances interfere with the spirometry and therefore methacholine testing. The participant should be informed to avoid these medications and substances for the specified time interval prior to lung function testing. In general, if there are no other contraindications, a methacholine challenge can be performed if the study participant correctly withheld medications and substances so that they are eligible for spirometry.

Of course there are exceptions to every generality. A methacholine challenge test should not be performed if the participant used 4 or more days of an oral or injectable steroid (i.e., prednisolone, prednisone, Solumedrol, Decadron) for treatment of an asthma exacerbation in the past 4 weeks. The results of a methacholine challenge may be hard to interpret and the participant may be at greater risk for an excessive response to the challenge. Spirometry can be performed but not challenge testing.

The methacholine challenge should be delayed or rescheduled, if possible within the protocol visit windows, for the appropriate time period if the study participant used a restricted medication or substance too close in time to the test.

Some AsthmaNet protocols may impose specific methacholine challenge medication holds or exceptions and those will be described in study specific MOPs.

2.6. Recent Illness

If the participant is currently having an acute asthma attack, he or she should not perform the methacholine test regardless of the baseline (pre-diluent) FEV_1.

If the participant has had an acute asthma attack requiring the use of prednisone in the previous 4 weeks, he or she should not perform the methacholine test.

If the participant has had any other severe acute illness in the previous 4 weeks, the supervising physician should be consulted regarding the appropriateness of testing. If methacholine challenge testing needs to be rescheduled due to acute illness, the date of the rescheduled visit should be based on the physician's discretion.

If the participant is less than 18 years of age and has had an upper respiratory infection or cold within the previous 4 weeks, he or she should be rescheduled, since even mild respiratory infections can increase bronchial reactivity for a prolonged time period. In some protocols this rule holds true for eligibility testing only, and once in the study, testing is done regardless if a cold has developed (see protocol specific MOP). In this case, the fact that the participant had a cold should be recorded and noted in the comments regarding the challenge. **However, if the participant is less than 18 years of age and has had an upper respiratory infection or cold within the previous 2 weeks, he or she should not perform the methacholine test, regardless of which study visit it is.**

2.7. Rescheduled Study Visit

In the case where methacholine challenge is performed at a rescheduled visit, all criteria to determine whether the participant is eligible for this test to be performed must

be verified, including a pregnancy test (if applicable), assessment of lung function, and a check on whether the participant has consumed medications or substances that should be withheld prior to this test.

2.8. Safety Measures

A physician should be available during methacholine challenge testing. In the unlikely event that there is a severe reaction to methacholine inhalation, it is important to have a physician on-site and readily available to evaluate the participant and supervise treatment. The physician, however, does not need to be present in the room during the actual challenge testing.

Emergency medications should be available. A clearly marked "Emergency Drug Box" should be kept accessible in the same room where methacholine challenge testing is performed so that reactions, should they occur, may be promptly treated. The box should include: a metered-dose inhaler bronchodilator (such as metaproterenol or albuterol) as well as ipratropium bromide, ampules of pre-measured bronchodilator and saline which can be administered via the same nebulizer as the methacholine, injectable epinephrine or terbutaline, and injectable atropine. The Emergency Drug Box should be checked on a regular basis to ensure that the drugs have not expired.

2.9. Summary

- Study participant must be able to perform good quality spirometry
- Documented negative pregnancy test for female study participants
- Baseline (pre-diluent) $FEV_1 \geq 55\%$ predicted (ADULTS)
- Baseline (pre-diluent) $FEV_1 \geq 1.0$ liter (ADULTS)
- Baseline (pre-diluent) $FEV_1 \geq 70\%$ predicted (PEDIATRICS)
- Stable blood pressure (Systolic ≤200 mm Hg, Diastolic ≤ 100 mm Hg for adults and children at least 12 years of age; Systolic ≤180 mm Hg, Diastolic ≤ 90 mm Hg for children under 12 years of age)
- No evidence of a concurrent asthma attack
- Meets all other exclusions (no recent illness that would preclude performing the challenge, no recent prednisone burst for an asthma exacerbation, etc)
- Physician available during test procedure
- Emergency drugs available
- Technician is proficient in spirometry, knowledgeable about procedure/equipment, knows contraindications to performing a methacholine challenge test, is proficient in administering a bronchodilator, is familiar with emergency procedures, and KNOWS WHEN TO STOP THE TEST

3. PREPARATION AND STORAGE OF THE METHACHOLINE SOLUTIONS

Methacholine will be prepared by a designated pharmacy. See Appendix A of this MOP.

Dry powder methacholine should be stored at 59°C to 86°C (15° to 30°C). Methacholine dilutions may be frozen for up to 6 months. Frozen dilutions should be stored in a typical frost-free freezer (temperature range, -20° to -7°C). Storage in a -80°C freezer is also acceptable. Once thawed, the reconstituted solutions should be refrigerated at 36° to 45°F (2° to 8°C) for no more than 2 weeks. Once thawed, the solutions may not be re-frozen.

Before conducting the challenge test, the vials should be removed from the refrigerator for at least 30 minutes to allow them to warm to room temperature. Since the temperature of the solution can affect the output of the nebulizer, the solutions should not be administered if they are either warm or cold.

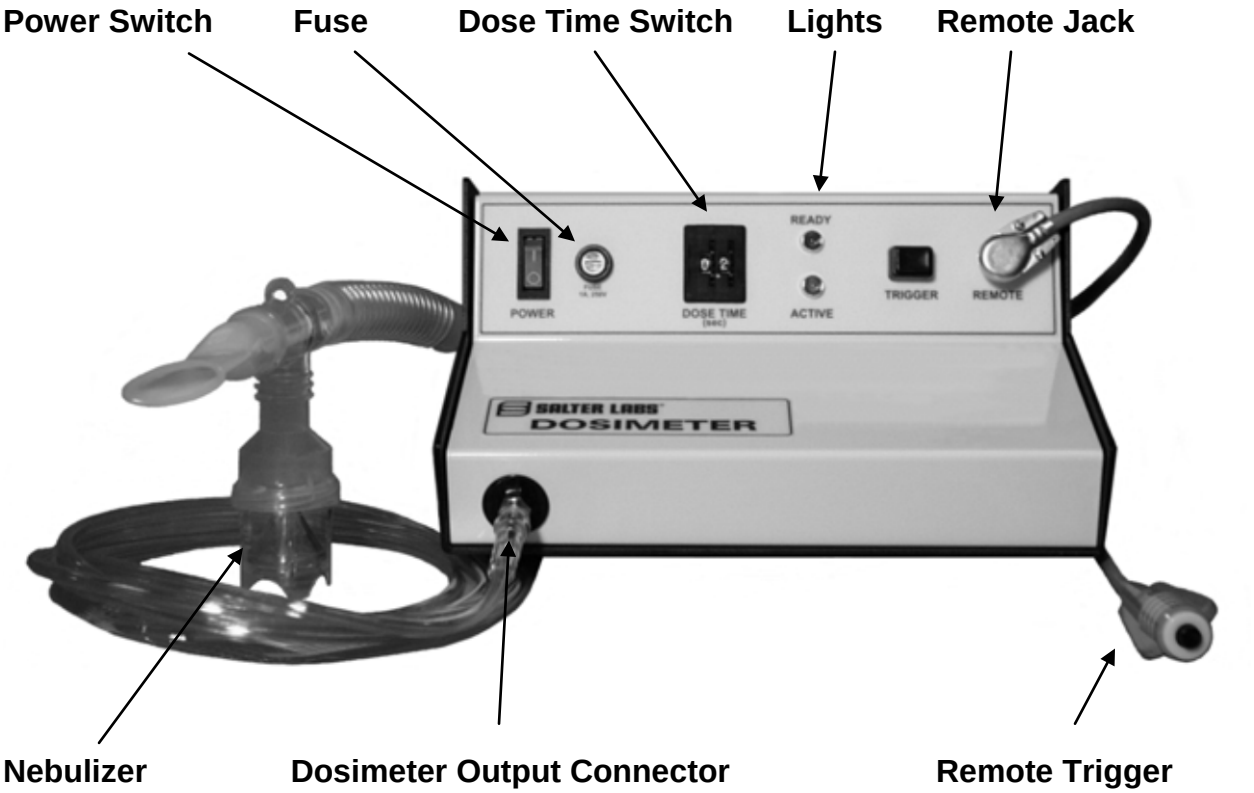
In preparation for a methacholine challenge, arrange the vials of methacholine in the order of administration starting with diluent. Great care should be taken to administer the lowest concentration of methacholine first, and then increase concentrations in the prescribed order. Never transfer solution from unmarked vials or syringes to the nebulizer, because it is possible to mix up the concentrations. The technician performing the methacholine challenge should always be able to identify which vial or syringe was used to fill the medicine cup of the nebulizer. If in doubt, it is better to discard the dose and draw up another. To maintain the correct sequence, it may be helpful to take the bottle out of the row and place it in front so that you always know which concentration you are delivering, and which one is next. When you go to the next concentration, place the vial just tested in back of the original row and move the next one in sequence in front.

Table 2. Methacholine Test Sequence

Stage
Pre
Diluent
Diluent II (if needed)
0.0625 mg/ml
0.125 mg/ml
0.25 mg/ml
0.5 mg/ml
1.0 mg/ml
2.0 mg/ml
4.0 mg/ml

8.0 mg/ml
16 mg/ml
32 mg/ml
Post
Post II (if needed)

4. SET UP OF THE SALTER LABS DOSIMETER



AC Adapter **Power Input** **Yellow Compressed Air Hose**

1. Place the dosimeter on a level, sturdy surface. Press the POWER switch to the O (off) position.

2. Plug the barrel connector from the AC adapter into the POWER INPUT connector on the rear of the unit.

3. Attach the power cord to the AC adapter. Plug the other end of the power cord into a wall outlet.

4. Plug the connector from the REMOTE TRIGGER switch into the REMOTE connector on the dosimeter's front panel.

5. Connect one end of the COMPRESSED AIR HOSE to the COMPRESSED GAS INPUT connector on the rear of the dosimeter.

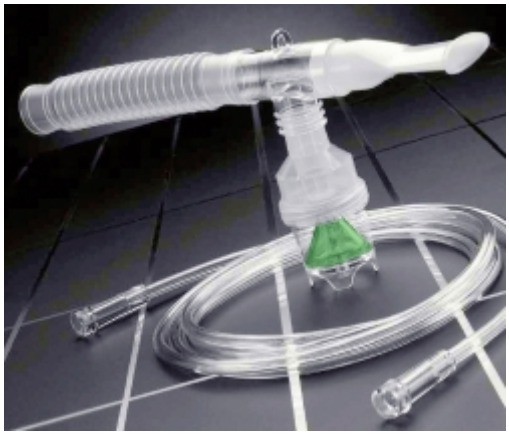
6. Connect the other end of the COMPRESSED AIR HOSE to an air source capable of delivering 7 lpm through the nebulizer.

CAUTION – The compressed air source must not exceed 60 psi into the dosimeter.

7. Unpack the Salter Labs nebulizer. Connect one end of the tubing to the dosimeter's outlet.

8. Assemble the nebulizer according to the instructions included in the package. Make sure that the green cone has been placed into the nebulizer cup before assembling the top to the cup.

CAUTION – Do not use a nebulizer if the green cone is missing.



9. Connect the tubing to the bottom inlet on the nebulizer.

10. Press the POWER switch to the I (on) position. The switch will illuminate, indicating that the dosimeter is turned on. The READY light will also illuminate, indicating that the unit is operating and ready.

11. Set the desired dose duration on the DOSE TIME switch to 0.5 seconds.

The dosimeter is now ready for use.

4.1. The Operation of the Dosimeter

1. Using sterile technique, draw up 2mL of diluent or methacholine.

2. Unscrew the nebulizer cup; add diluent or methacholine to the nebulizer cup taking care to keep the solution and equipment free of contamination.

3. Reassemble the filled nebulizer and hand to study participant.

4. Have the study participant place the mouthpiece in their mouth.

5. Instruct the study participant to breathe normally.

6. As the participant exhales a normal breath, press the REMOTE TRIGGER SWITCH and instruct the participant to take in a slow deep breath and hold it for five (5) seconds. (See Appendix B for further instructions/methods for methacholine administration).

7. The ACTIVE light illuminates and the READY light turns off. Nebulization begins as the dosimeter releases a timed pulse of compressed air to flow to the nebulizer.

8. Nebulization occurs for 0.5 seconds, the dosimeter shuts off the air flow, the ACTIVE light turns off, and the ready light illuminates.

9. Repeat this process five (5) times.

10. Begin a 90 second timer, and, using clean technique, empty any residual fluid from the nebulizer in preparation for the next dose.

5. STUDY PARTICIPANT PREPARATION

The purpose of the test should be explained to the participant and, if less than 18 years of age, to their caretaker. While explaining the purpose of the test, care should be taken not to cause undue alarm or concern. Experience has shown that those individuals who are told that the test is uncomfortable have more symptoms than those who are told that the test causes mild symptoms, if any. One can even induce bronchospasm in an asthmatic if one emphasizes that a placebo test solution causes bronchospasm. Your explanation of the test should be accurate but not alarming.

For example:

"This test measures whether your airways narrow when you breathe this medicine. We will have you breathe the medicine starting with a very low dose, and very gradually increase the dose. After each dose, you will do a breathing test to see whether the medicine has had any effect. Some people have coughing or a sensation in their chest from breathing the medicine, but it is usually mild. If we see that you have had a big change in your breathing, we will stop the test, and give you a bronchodilator, which will open up your airways."

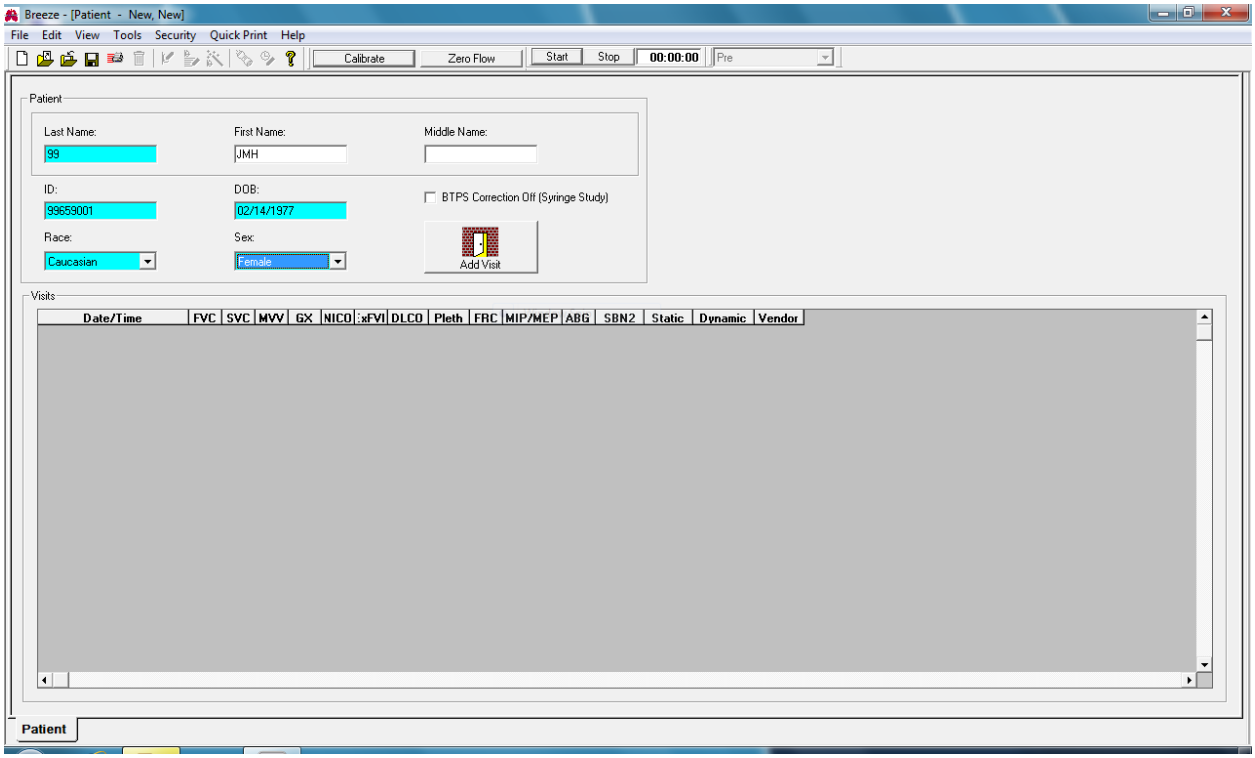
Ask the study participant if they would like to urinate before the test. Stress incontinence, especially in older women, can occur.

Study participants should have been given a list of items/medications to withhold before the test. Evaluate if the study participant meets eligibility criteria (has no

contraindications) for performing this procedure by completing the appropriate Methacholine Challenge Testing Checklist (METHACHK_PED, METHACHK_ADULT).

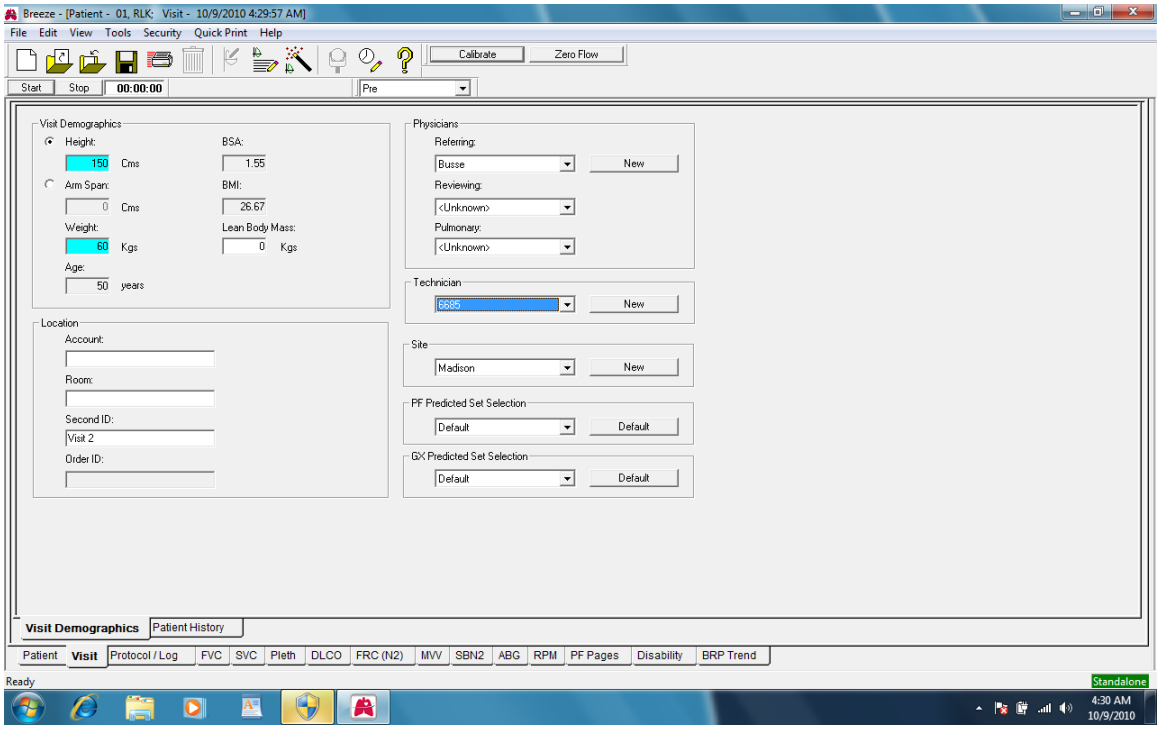
6. THE BRONCHIAL CHALLENGE

1. Enter a new participant's demographics or bring up a participant who is already in the system (see Spirometry MOP for these details).

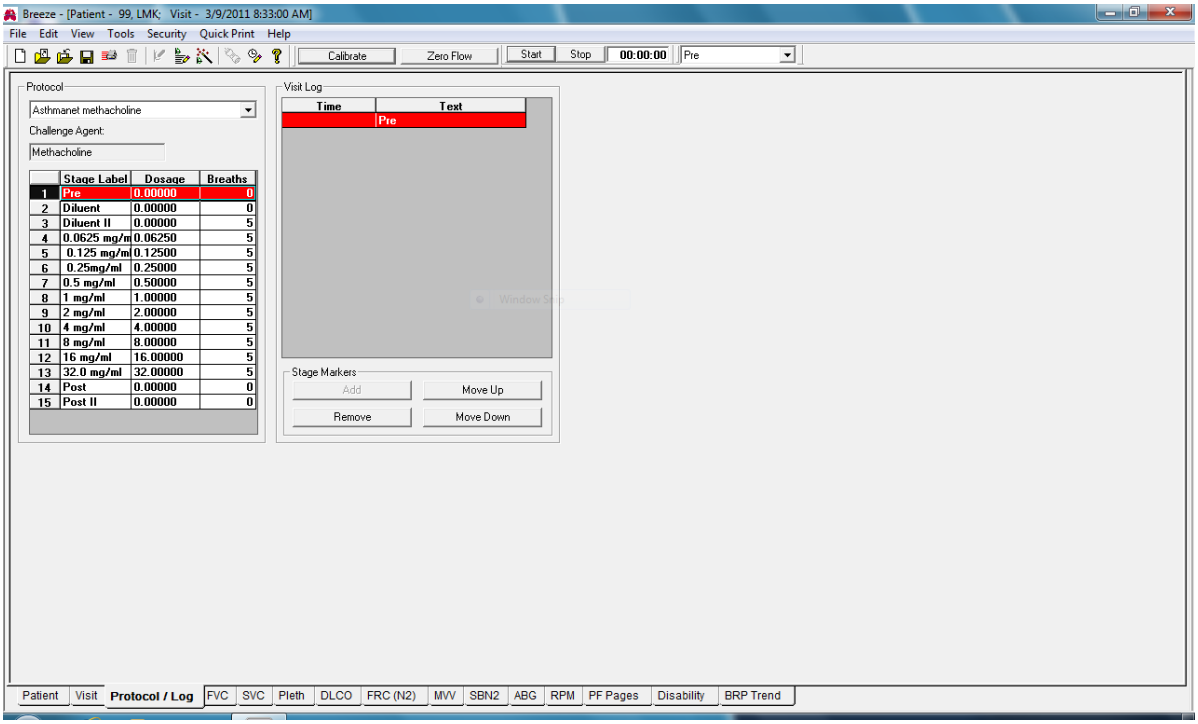


2. Click on "Add Visit"

You will now see this screen



3. Click on the “Protocol/Log” tab.



4. In the Protocol drop down menu, choose “AsthmaNet Methacholine”.

5. A message box will appear, “Changing the protocol will remove all stage markers from the visit log. Proceed anyway?” Click “OK”.

6. Click on the “FVC” tab along the bottom of the screen.

7. Click “Zero Flow”. (Zero Flow only needs to be performed at this point and not at every dose of methacholine.) Perform a complete baseline (pre-diluent) spirometry session (See AsthmaNet Spirometry MOP). The spirometry session needs 3 acceptable and 2 reproducible efforts.

STOP AND ASSESS YOUR BASELINE (PRE-DILUENT) SPIROMETRY SESSION.

If the baseline (pre-diluent) FEV_1 is less than 70% predicted (PEDIATRICS) or less than 55% predicted (ADULTS) or less than 1.0 liters (ADULTS), or the study participant is unable to perform acceptable and repeatable spirometry, then the methacholine test should be deferred. If the only requirement that is not met is that the FVC value is not repeatable, the procedure may continue if the technician is confident that the participant is breathing in all the way to total lung capacity (TLC) before each FVC maneuver and blowing out until completely empty. The FEV_1 value **must** be repeatable (i.e., within 0.15 liters) to continue with the challenge.

If the baseline (pre-diluent) spirometry session allows you to proceed with the methacholine challenge, you will need to perform the following calculations using the best baseline (pre-diluent) FEV_1 (also see METHA case report form).

- 90% of best (pre-diluent) FEV_1. The study participant must reverse to at least 90% of their baseline (pre-diluent) FEV_1 to be discharged.

- 80% of best (pre-diluent) FEV_1. Although uncommon, it is possible that a study participant will PC_{20} to the diluent. You will need to know 80% of the baseline (pre-diluent) FEV_1 to assess for this rare event.

NOTE:
Some participants with asthma develop bronchospasm from the effort of performing repeated FVC maneuvers. This is termed “spirometry-induced bronchospasm”(SIB) and is evidenced by a progressive decline in lung function with each maneuver despite apparently increasing effort. This phenomenon can create problems for repeatability, safety and study inclusion. It is very important that technicians understand and recognize this situation. It should not be confused with submaximal effort. Only acceptable maneuvers count toward the repeatability criteria. Any participant eligible for methacholine challenge where the technician observes or suspects spirometry-induced bronchospasm (SIB) at baseline or (pre-diluent) should:

- Consult study physician as this situation represents an “asthma symptom”.
- The study physician may decide that the participant can proceed with diluent.
- If the study physician feels that it is unsafe to proceed, then the testing will be stopped and a bronchodilator administered.
- NOTE the occurrence in the comment section to ensure that the overreader has a proper understanding of the situation.

If you are able to proceed with the methacholine challenge:

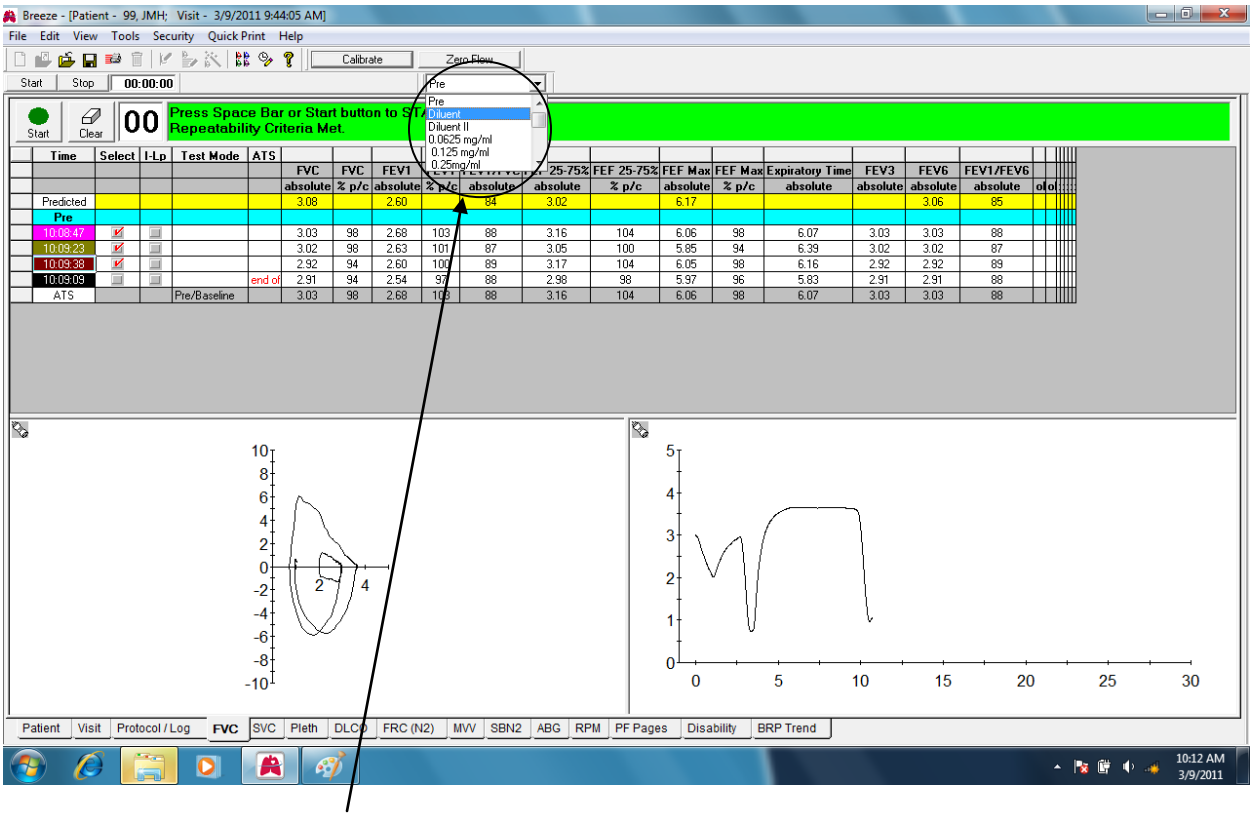
8. Using a sterile syringe and needle, draw up 2 mL of diluent solution and add it to the nebulizer.

NOTE. If possible, you should avoid having the study participant (especially pediatric) see you draw up the solution because they may assume that they will be given an injection.

9. Have the participant take five (5) inhalations of the diluent (See section 4.1 for an explanation on how to operate the dosimeter).

10. Begin a 90 second counter (there is one on the Medgraphics testing screen or you can use a separate device), and, using clean technique, empty any residual fluid from the nebulizer in preparation for the next dose.

11. Before performing spirometry following the administration of diluent (or any of the doses of methacholine) you must FIRST move to that stage on the computer. In the testing screen that now displays Pre, click on the drop-down menu and select “Diluent”. (On your screen, this may actually be located to the left above your green Start button.)



Select Diluent

12. 90-seconds after the last dose of diluent was administered, perform a complete spirometry session. The spirometry session needs 3 acceptable and 2 reproducible FEV_1 values. The challenge may proceed if FVC reproducibility is not attained, as long as FEV_1 reproducibility is met.

STOP AND ASSESS YOUR POST-DILUENT SPIROMETRY SESSION.

Post-diluent FEV_1 shows a ≥ 20% drop

- If the post-diluent FEV_1 is less than or equal to 80% of the baseline (pre-diluent) FEV_1, the study participant has PC_{20}'d to the diluent.
- The methacholine test should be stopped and the study participant reversed.
- The PC_{20} should be recorded as zero (0) in Q1050 on the METHA form.

Post-diluent FEV_1 shows a ≥ 10% drop.

- If the post-diluent FEV_1 is greater than 80% and less than or equal to 90% of the baseline (pre-diluent) FEV_1, the diluent should be repeated exactly as outlined above.
- In the testing screen that now displays Diluent, click on the drop-down menu and select "Diluent II" to perform spirometry 90 seconds after the second diluent was administered.
- The methacholine challenge PC_{20} will be calculated from the largest FEV_1 from all acceptable maneuvers post 2nd diluent.
- For participants over 18 years if age: if the participant develops symptoms of worsening asthma or an FEV_1 < 55% predicted or less than 1.0L after the 2nd diluent trial, contact the study physician to review the case.
- For participants less than 18 years if age: if the participant develops symptoms of worsening asthma or an FEV_1 < 70% predicted after the 2nd diluent trial, contact the study physician to review the case.

Post-diluent FEV_1 dropped to less than 70% predicted (PEDIATRICS) or less than 55% predicted (ADULTS):

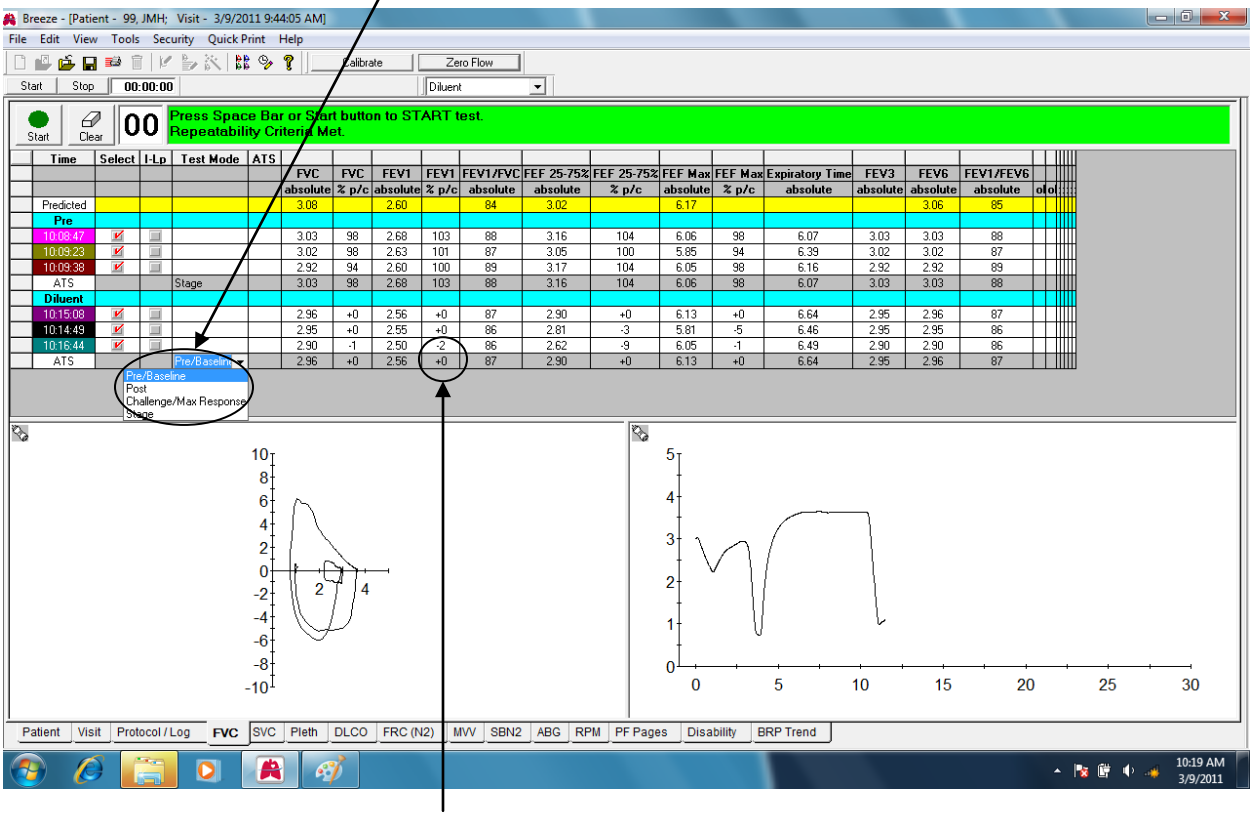
- You may proceed with the methacholine challenge test if the post-diluent FEV_1 has dropped to below test eligibility parameters as long as the FEV_1 has **not** dropped by **≥ 20% of baseline** and the study physician approves

13. You will need to tell the computer which session to use to determine a PC_{20}. So far you should have a pre-diluent and post-diluent (and possibly second post-diluent) session. Click on the cell that says “Stage” in the “Test” column after the Diluent session. A drop-down menu will appear when you select the “Stage” cell. Choose “Pre/Baseline”. This will mark the post-diluent maneuvers as the values from which the PC_{20} is calculated. If you needed to perform a second diluent session, you must click on the cell that says “Stage” in the “Test” column after the second diluent session. Check the cell under the column “FEV1 % p/c” in Diluent row, it should display “+0”.

As the methacholine challenge progresses, the percent change can be followed in the “FEV1 % p/c” column of the testing screen.

NOTE: If you do not assign the Pre/Baseline stage as outlined above, the PC_{20} will be calculated from the pre-diluent spirometry session and will be incorrect.

Click here and choose "Pre/Baseline" to mark the Diluent stage as the reference for the PC_{20} calculation.



This cell should be "+0" after you mark the Diluent Stage as the reference for the PC_{20} calculation.

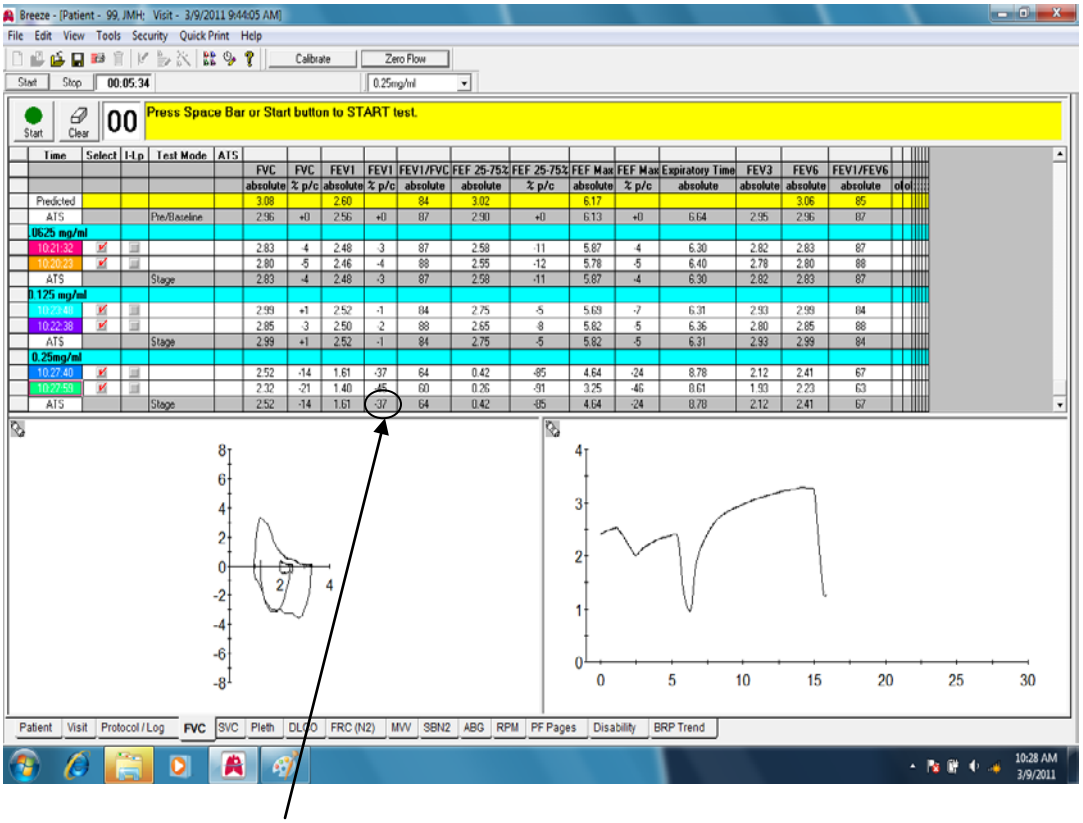
If the post-diluent spirometry session allows you to proceed with the methacholine challenge, using a new needle/syringe and sterile technique, draw up 2 mL of the first dose of methacholine (0.0625 mg/ml). You should use a new needle and syringe for each successive dose. Although the needle is not contaminated with blood products, it is a good habit not to recap the needle before discarding. Dispose of all the syringes and needles in the proper biohazard container.

14. Give the 0.0625 mg/ml dose of five (5) inhalations of the methacholine. Choose the 0.0625 mg/ml from the drop-down menu. Perform one (1) acceptable maneuver at 30 and one (1) acceptable maneuver at 90 seconds post dose. Perform more maneuvers if needed to achieve two (2) acceptable maneuvers. The FEV_1 does not need to be repeatable. Choose the largest FEV_1 of the acceptable maneuvers by clicking on the corresponding box under the "Select" column.

15. Check the cell under the column “FEV1 % p/c” in the 0.0625 mg/ml row.

- If this value is -19 or more, (less negative or positive) proceed to give the next methacholine dose following the steps outlined in step 14, above. Each administration of methacholine should be approximately 5 minutes after the previous administration. Continue administering doses of methacholine, following the methacholine dose sequence until either the FEV_1 drops by 20% or more from the “Post Diluent” FEV_1 (PC_{20}) or the last dose of methacholine has been given. When a dose displays a drop in FEV_1 by 20 or more, mark this stage as “Challenge” in the drop down menu under the “Stage” column. If this is not marked as “Challenge” from the drop down menu in the stage column, then no PC_{20} value will be displayed nor printed.

- If the value is -20 or less (more negative), the challenge is completed. Proceed to the Post stage by choosing “Post” from the drop-down menu. Give two (2) puffs of albuterol for standard reversal for most challenges. If the methacholine challenge is followed by sputum induction at the visit, standard reversal is four (4) puffs of albuterol. Wait 10 to 15 minutes and perform 1 to 5 spirometry maneuvers. As soon as the FEV_1 is within 90% of the baseline (pre-diluent) FEV_1, the procedure is completed. ***This stage must be marked as Post from the drop down menu in the test mode column.*** Only one acceptable post reversal maneuver that shows the participant has returned to at least 90% of baseline is needed. This is for safety only and does not need to show repeatability. If the FEV_1 does not return to within 90% of baseline, contact the supervising physician. You may need to administer additional bronchodilator. If you administer additional bronchodilator, subsequent spirometry would be performed under the Post II stage. Results of standard reversal (2 or 4 puffs of bronchodilator) are recorded on the Methacholine Challenge Testing (METHA) form. Additional treatment, if required, and corresponding FEV_1 values are recorded on the Additional Treatment Post Methacholine Challenge Testing (METHA_ADD_TRT) form.



When this value in -20 or more negative, the PC_{20} has been reached and 2 puffs of bronchodilator should be given at most study visits. If the methacholine challenge is followed by sputum induction at the visit, administer 4 puffs of bronchodilator as standard reversal treatment.

7. REVERSAL AND DISCHARGE POST METHACHOLINE CHALLENGE

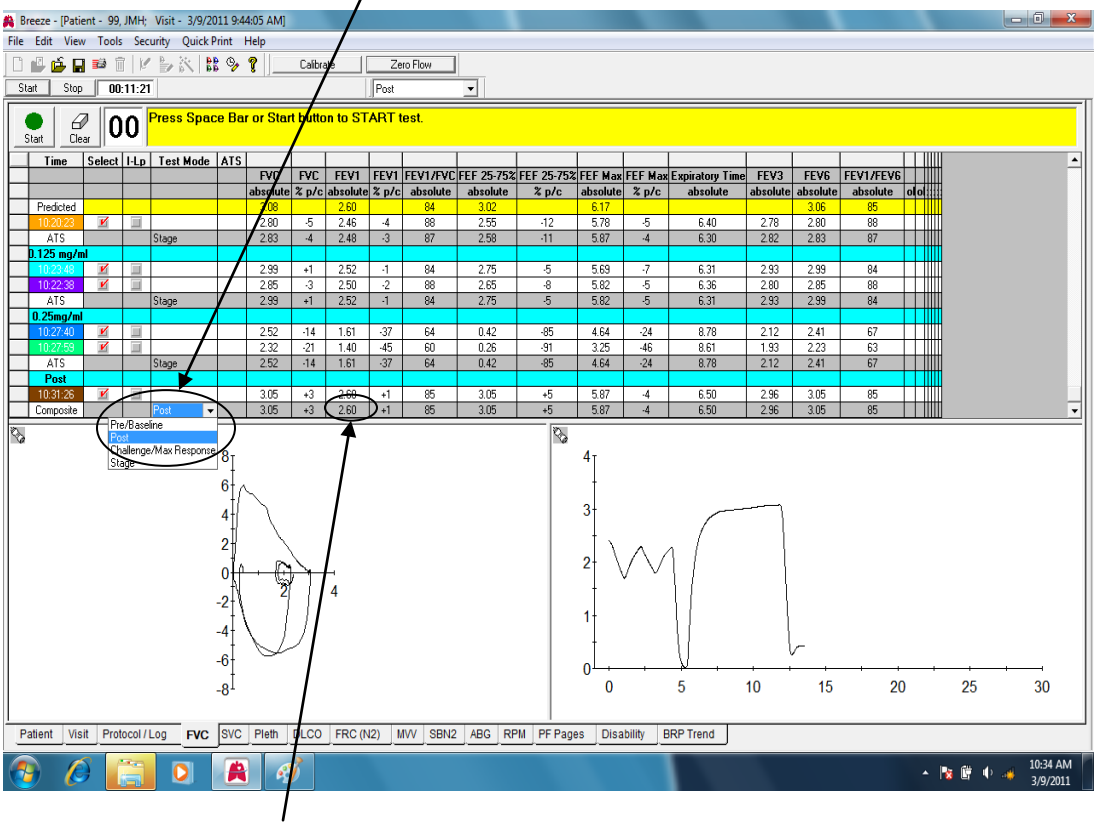
Although methacholine does not cause delayed-reactions, it is prudent to use an inhaled bronchodilator to reverse the effects of methacholine.

Most AsthmaNet methacholine challenges will use two (2) puffs of a bronchodilator for standard reversal. If the challenge is followed by sputum induction at a study visit, then four (4) puffs of bronchodilator should be administered as standard reversal treatment.

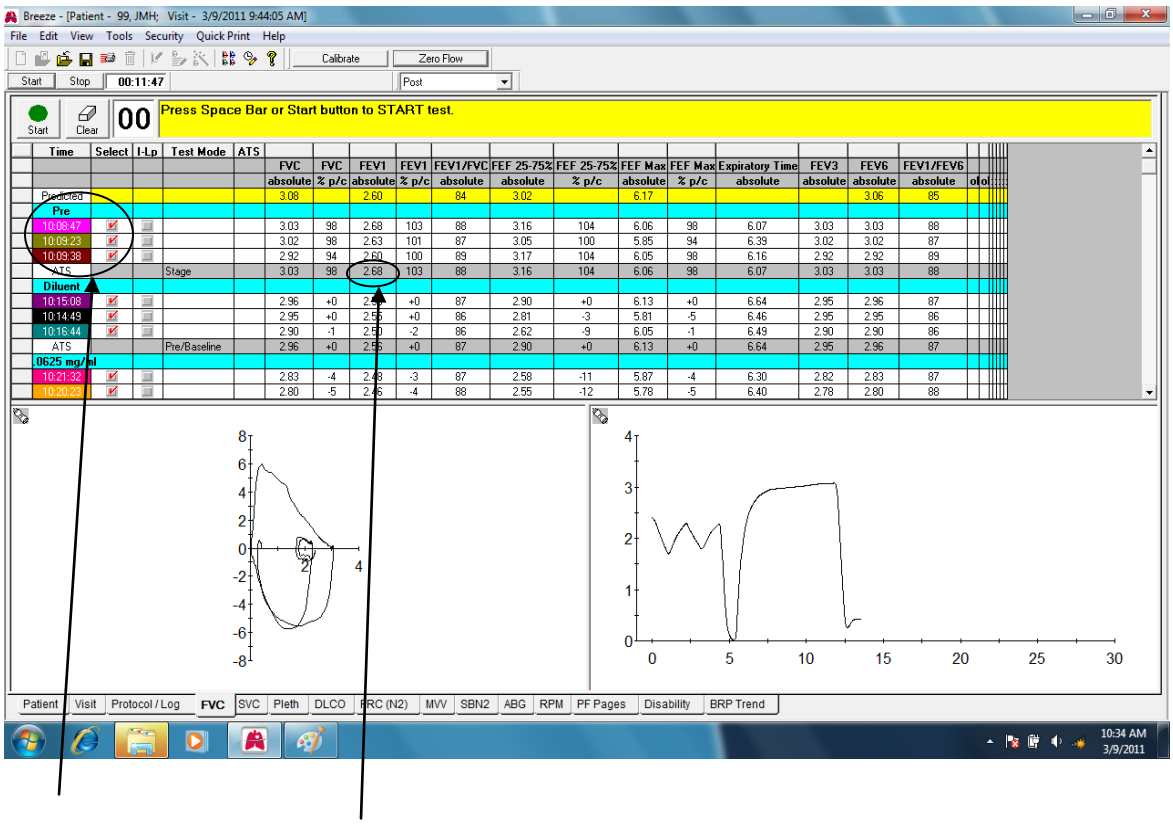
Upon completion of the challenge the participant must be given a bronchodilator even if no PC_{20} is achieved. Exposing the airway to methacholine may increase the chances of having airway reactivity after discharge.

The test participant should not be discharged from the clinic until the FEV_1 has returned to at least 90% of baseline (pre-diluent) FEV_1. Seek advice from the supervising physician.

Click here and choose "Post" to mark the Post stage.



Check this value and make sure it is at least 90% of the participant's Pre-Diluent FEV_1.



Pre-Diluent Maneuvers **This is the value that the "Post Albuterol" FEV_1 is compared to.**

Mark the reversal FEV_1 as "Reversal" from the drop down menu in the "Stage" column.

8. UNUSUAL OR SEVERE RESPONSES TO A METHACHOLINE CHALLENGE

Unusual or severe asthma symptoms should be reported to the supervising physician before discharging the participant from the clinic. Coughing and a sense of chest tightness are common during methacholine challenge testing, and therefore are <u>not</u> considered adverse events. However, if the event becomes more severe than expected or escalates, it can be considered an adverse event or serious adverse event.

Spirometry-induced bronchospasm may occur. In the methacholine challenge setting, this is **<u>not</u>** an adverse event unless it becomes more severe than expected.

Although uncommon, excessive methacholine doses may induce abdominal cramping, diarrhea, sweating, and salivation. These symptoms **are** considered adverse events unless they escalate to meet serious adverse event criteria.

If the participant experiences any unusual or particularly severe symptoms, document them on the Clinical Adverse Events (AECLIN) form, notify the supervising physician before discharging the participant from the clinic, and notify the DCC. If the participant has a severe reaction, the technician/coordinator should retain the inhaled test solutions and return them to their pharmacy for inspection. Do NOT use these solutions for other study participants.

9. TECHNICIAN SAFETY

Methacholine solution can be aerosolized into the atmosphere and cause asthma symptoms in technicians who are sensitive to it. The test should be conducted in a well-ventilated room, with at least two (2) complete exchanges of air per hour. In some circumstances, it may be necessary to use a portable safety hood or nebulization booth to minimize environmental exposure.

Each technician performing methacholine challenge testing will need to have their lung function measured (i.e., perform spirometry) as part of their certification for this procedure. Any time the technician feels they may be affected by exposure to the methacholine, they should repeat spirometry and compare the results to their baseline. If any significant differences are noted, the technician should have the results evaluated by their principal investigator.

Please use good clinical judgment when monitoring exposure to the methacholine and take appropriate measures

10. RECALCULATION OF PC_{20} VALUES

Occasionally it may be necessary to recompute a PC_{20} value recorded by the methacholine software. When a problem occurs during a methacholine challenge, note the circumstances in the comments associated with the challenge so that the overreader may adjust the grade accordingly. Send an e-mail to the overreader and the protocol's scientific coordinator at the DCC. Include a copy of the challenge report, along with an explanation of where the error or problem occurred. The corrected PC_{20} value will be calculated at the DCC via a computer program and the updated value will be sent to you via e-mail. Do not calculate PC_{20} values by hand or by using graphical techniques. Record the updated PC_{20} value on the Methacholine Challenge Testing (METHA) form and submit a copy of the e-mail with the corrected value with your visit packet for data processing.

11. OVERREADING METHACHOLINE CHALLENGES

All methacholine challenges are overread centrally, and reports are issued regularly. The review will primarily assess performance (i.e. adherence to the protocol), as well as the acceptability and reproducibility of the FVC and FEV_1.

Overreading Grading: The following 3 elements of the methacholine challenge procedure will be evaluated. The procedure is approved only if the criteria for all 3 elements are met.

- Baseline and diluent(s) ≥ 3 trials that meet ATS/ERS criteria
- Two (2) acceptable trials after each concentration of methacholine
- Methacholine challenge procedure terminated at the appropriate step and the challenge was acceptable

12. CERTIFICATION

Candidates shall view the MedGraphics training modules and successfully complete the ten (10) Self Checks.

Candidates shall perform three (3) mock methacholine challenge procedures and have them reviewed and approved by the overreader. The procedures for certification should have a participant ID number that begins with 99. Note: These are practice procedures; therefore, no methacholine should be given. Just “pretend” to give the doses. Proceed through the first 3 doses and stop after spirometry for the 0.125mg/ml stage, then go to post and perform 1 acceptable spirometry maneuver at that stage.

Alternatively, individuals who already possess spirometry certification may perform protocol-related methacholine challenges on study participants to meet the methacholine challenge certification requirements. The participant’s assigned study ID number should be recorded on these challenges following normal procedures. Any challenge that is performed by an individual who does not possess methacholine challenge certification must be supervised by a certified technician/coordinator. The ID number of the certified individual must be recorded on the Methacholine Challenge Testing (METHA) form and the appropriate Methacholine Challenge Testing Checklist (METHACHK_ADULT, METHACHK_PED) in the supervisor ID field. The ID number of the person performing the challenge should be completed in the technician ID field.

13. CONTACT INFORMATION

13.1. Procedural Questions:

Rick Kelley
rlk@medicine.wisc.edu
1-215-594-1891 (cell)
1-715-282-7972 (landline)

13.2. Software and Hardware Questions

Medical Graphics Tech Support
Primary: 1-800-333-4137
Secondary: Lisa Knepper, 1-800-950-5597, ext 1308

Sites are responsible for any additional supplies. Contact MedGraphics sales and service department (800-950-5597) for consumable orders and be sure to state that you are participating in the AsthmaNet study. You will need a purchase order or credit card number when ordering supplies.

13.3. PC_{20} Recalculation

Contact the scientific coordinator for the protocol at the DCC and the overreader via e-mail. Attach a copy of the methacholine challenge report and give details of where the problem occurred.

13.4. Ordering Methacholine (Provocholine®)

To order methacholine supplies, please contact either Ms. Tracey Feely or Ms. Allison Sinclair at Methapharm using the contact information included below.

Tracey Feely
Clinical Services
Direct: 1-519-751-3602 ext. 7223
tfeely@methapharm.com

Allison Sinclair
Operations Manager
Direct: 1-519-751-3602 ext. 7217
asinclair@methapharm.com

13.5. Ordering Methacholine Equipment/Supplies

To order Methacholine supplies, please contact Ms. Lisa Knepper at MedGraphics using the contact information included below:

Lisa Knepper
Direct: 1-651-766-3308
lknepper@medgraphics.com

13.6. Salter Dosimeter/Nebulizer Equipment Failure

In the event that a problem is experienced with the Salter dosimeter, please contact Ms. Tina Hernandez at Salter Labs to arrange for service:

Tina Hernandez, Customer Service Representative
100 W. Sycamore Road
Arvin, CA 93203
800-421-0024, extension 845
thernandez@US.SalterLabs.com

The DCC is securing an additional dosimeter to serve as a backup while a site's equipment is being serviced. Contact Ron Zimmerman at the DCC to arrange for use of the loaner dosimeter.

APPENDIX A: PREPARATION OF METHACHOLINE SERIAL DILUTIONS

A.1. Preparation of Serial Dilutions Up To 32 mg/ml, Using Two of the 100mg Vials

Please refer to the specific AsthmaNet protocols as to exactly what methacholine concentrations will be needed to complete visit requirements, for either determination of participant eligibility or to determine study outcomes. AsthmaNet serial dilutions are noted in the table below. **Per the AsthmaNet Equipment Committee, the sterile bacterial-retentive filter referred to in the first sentence under the table is not required in AsthmaNet studies because pharmacies are making the dilutions under sterile technique. 0.4% phenol may be added as a preservative if dilutions are shipped or frozen. Whether 0.9% saline OR 0.9% saline plus 0.4% phenol is used is a matter of local discretion. According to the ATS statement on methacholine challenge, there are no differences in testing when phenol is used. Phenol does not affect the safety of the test or the procedural outcome. The decision whether or not to use phenol should be made by the local pharmacist in concert with the AsthmaNet Principal Investigator at that site.**

PREPARATION OF SERIAL DILUTIONS UP TO 32 mg/mL USING 2 - 100 mg Vials

	TAKE Provocholine	ADD 0.9% Saline	OBTAIN DILUTION	Total Volume
Step 1	100 mg ***	3.125 mL to vial	32 mg/mL - VIAL A	3.125 mL
Step 2	100 mg****	6.25 mL to vial	16 mg/mL - VIAL B	3.25 mL
Step 3	3 mL from VIAL B	3 mL	8 mg/mL - VIAL C	3 mL
Step 4	3 mL from VIAL C	3 mL	4 mg/mL - VIAL D	3 mL
Step 5	3 mL from VIAL D	3 mL	2 mg/mL - VIAL E	3 mL
Step 6	3 mL from VIAL E	3 mL	1 mg/mL - VIAL F	3 mL
Step 7	3 mL from VIAL F	3 mL	0.5 mg/mL - VIAL G	3 mL
Step 8	3 mL from VIAL G	3 mL	0.25 mg/mL - VIAL H	3 mL
Step 9	3 mL from VIAL H	3 mL	0.125 mg/mL - VIAL I	3 mL
Step 10	3 mL from VIAL I	3 mL	0.0625 mg/mL - VIAL J	6 mL

A STERILE BACTERIAL-RETENTIVE FILTER (POROSITY 0.22µm) SHOULD BE USED WHEN TRANSFERRING A SOLUTION FROM EACH VIAL TO A NEBULIZER

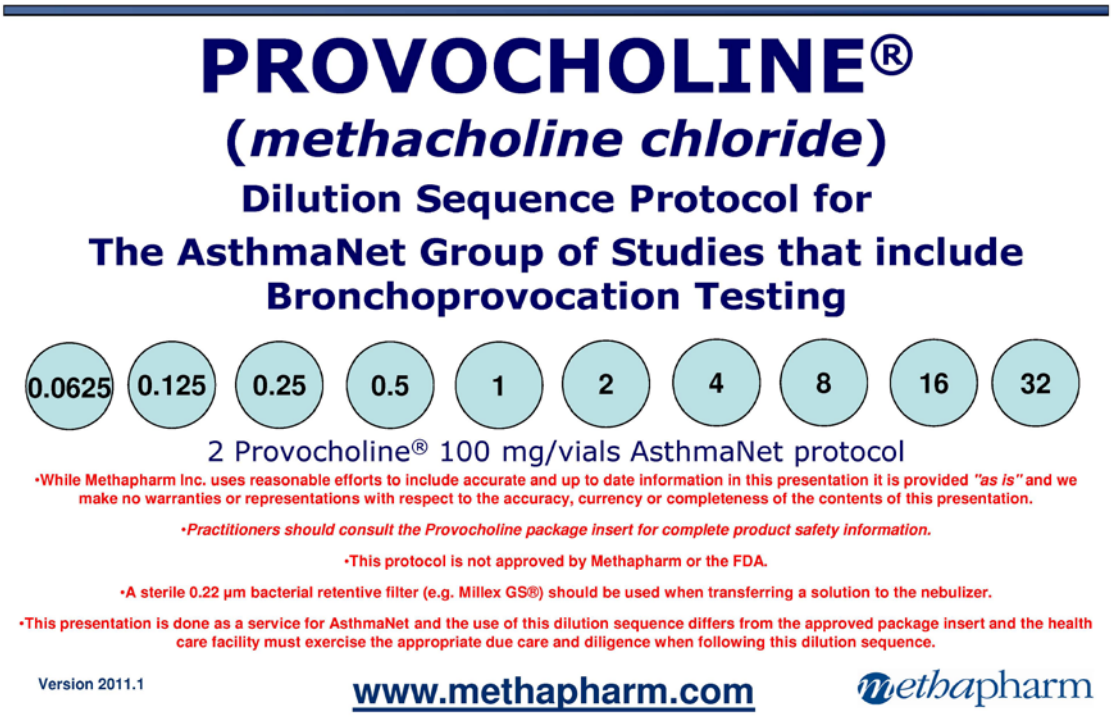
****** CONSULT PROVOCHOLINE® PACKAGE INSERT FOR ALL SAFETY INFORMATION *******

****** THIS DILUTION SEQUENCE HAS NOT BEEN APPROVED BY METHAPHARM OR THE FDA AND THEREFORE SAFETY INFORMATION IS UNAVAILABLE ******

A.2. Provocholine Dilution Sequence Protocol for the Provocholine Challenge Test

ALL METHACHOLINE WILL BE PREPARED BY A DESIGNATED PHARMACY. PLEASE PROVIDE THE ATTACHED METHAPHARM SLIDE SET AS A REFERENCE TO YOUR PHARMACISTS.

The preparation of Provocholine®, including the quantity of material prepared for the AsthmaNet methacholine challenges, is up to the purview of the site pharmacies. The MOP provides guidance as to dilution preparation methodology that is compatible with MethaPharm Provocholine® instructions. If a site wishes to make up more than the amount required for a single challenge it may do so as long as the correct AsthmaNet MOP-defined dilutions are available to the study coordinators for specific visits and meet site standards for storage and expiration date.



Introduction

Dilution Sequence Protocol

- These instructions are for the preparation of Provocholine and prepared according to the protocol provided by AsthmaNet
- The following are step-by-step instructions to dilute two 100 mg vials of Provocholine® into the 10 (ten) requisite concentrations for use in a bronchoprovocation challenge test
- **Please refer to the Package Insert for full instructions and safety precautions**
- Accurate sterile mixing is essential for the accuracy of the test results and to maintain patient safety
- Only trained individuals should mix and label methacholine solutions
- The following protocol is used to prepare two 100 mg vials of Provocholine® for testing a single patient only

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Important Notes

- Do not inhale powder during preparation of dilutions
- Do not handle Provocholine® if you have asthma or hay fever
- Provocholine® dilutions should be mixed by a pharmacist or other well-trained individual using sterile technique
- All vials should be labeled with the appropriate Lot Number, concentration, diluent used (in this case 0.9% Saline), preparation, initials of person preparation, and expiration dates.
- To reduce back pressure, vent vials with an extra needle as needed
- All dilutions should be made with:
  - 0.9% Saline
  - Sterile, empty USP Type I borosilicate glass vials
- When transferring solution from each vial (at least 2 mL) to a nebulizer use a sterile bacterial-retentive filter (porosity 0.22 µm)
- Practitioners should consult the Provocholine package insert for complete product safety information.

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Supplies Required

Suggested supplies for the dilutions of Provocholine® 100 mg

Quantity	Description
2	100 mg/vials of Provocholine®
1	100 mL 0.9% Saline
8	10 mL Sterile empty USP Type I borosilicate glass vials
1	10 mL Syringe* ** (labeled for diluent)
1	3 mL Syringe* ** (labeled for methacholine)
1	1 mL Syringe* ** (labeled for diluent)
3	20 Gauge, 1" Syringe Needles*
1	Millex GV 0.22 µm filter (Millipore)*
11	Alcohol Preparation Swabs*
1 set	Labels for vials
X	Set of Directions
X	Provocholine® Dilution Sequence Check Sheet and Control Record
X	Package Insert for Provocholine®

* Quantity of supplies subject to facility protocol

** Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

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Overview of the Dilution Process

TAKE Provocholine® (Methacholine Chloride U.S.P. Powder for Inhalation)	**ADD** 0.9% Saline (SHAKE WELL!)	**OBTAIN DILUTION**	**VIAL NAME**
Provocholine® 100 mg	3.125 mL	32 mg/mL	VIAL A - 32 mg/mL
Provocholine® 100 mg	6.25 mL	16 mg/mL	VIAL B - 16 mg/mL
3 mL from vial B	3 mL	8 mg/mL	VIAL C- 8 mg/mL
3 mL from vial C	3 mL	4 mg/mL	VIAL D - 4 mg/mL
3 mL from vial D	3 mL	2 mg/mL	VIAL E - 2 mg/mL
3 mL from vial E	3 mL	1 mg/mL	VIAL F - 1 mg/mL
3 mL from vial F	3 mL	0.5 mg/mL	VIAL G - 0.5 mg/mL
3 mL from vial G	3 mL	0.25 mg/mL	VIAL H - 0.25 mg/mL
3 mL from vial H	3 mL	0.125 mg/mL	VIAL I - 0.125 mg/mL
3 mL from vial I	3 mL	0.0625 mg/mL	VIAL J - 0.0625 mg/mL

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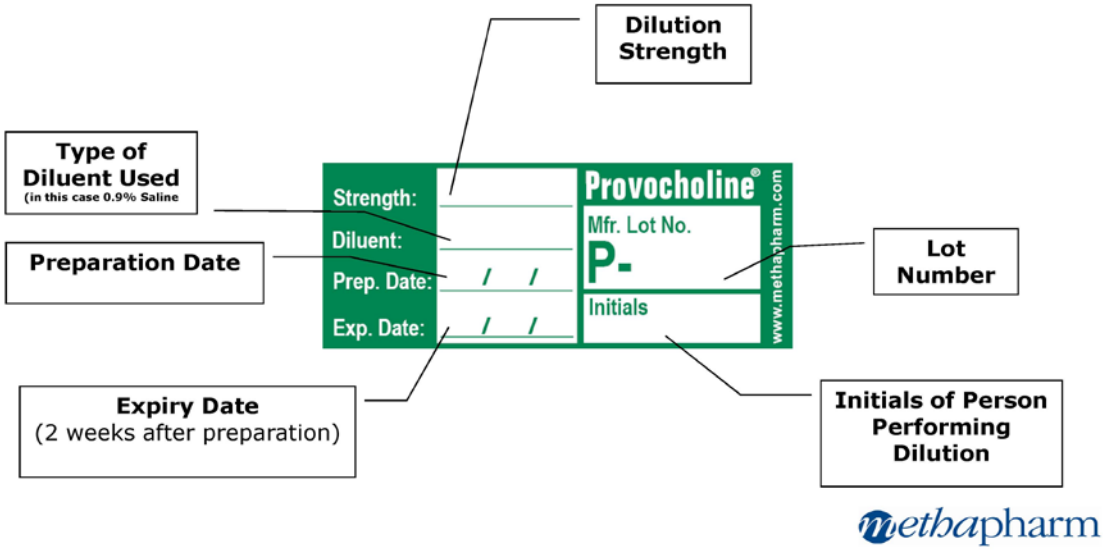
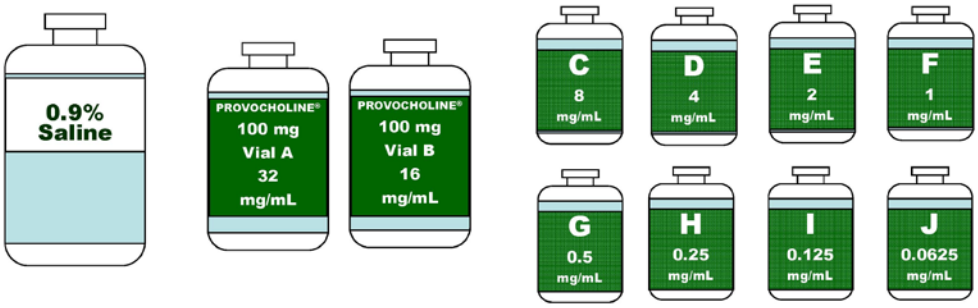
Getting Started –Labels

- Fill in all information on **labels**
- Include **14 day expiration** date on vials A – J

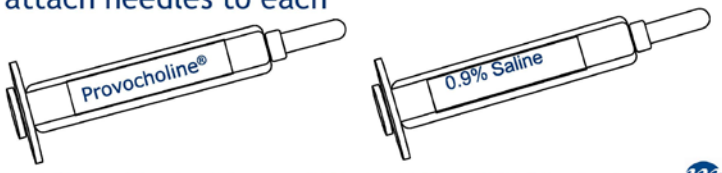


Getting Started

1. Attach labels to sterile empty vials
2. Wipe down the tops of the Sterile Empty vials, Provocholine®, and Saline vials with alcohol swabs:



3. Label syringes, (one for Provocholine® and one for 0.9% Saline) and attach needles to each*



*Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

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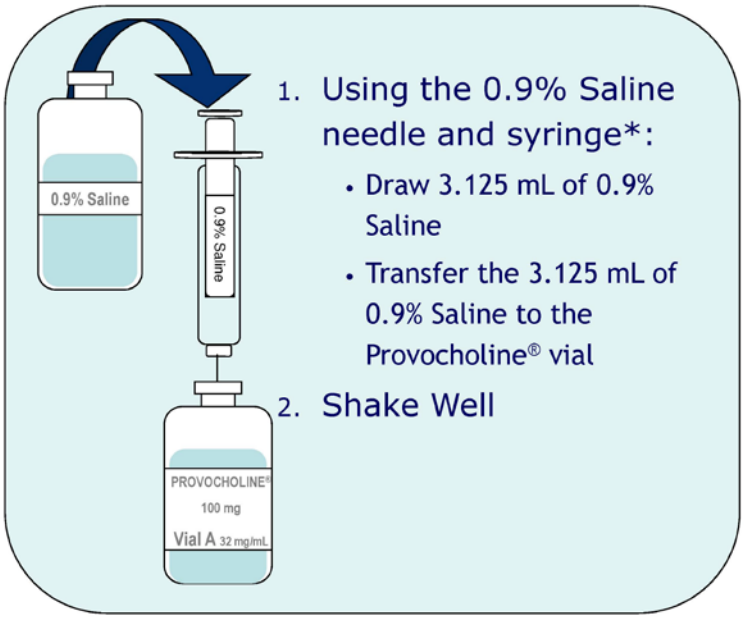
Step I: Preparing Vial A - Provocholine® **32 mg/mL** Solution



1. Using the 0.9% Saline needle and syringe*:
   - Draw 3.125 mL of 0.9% Saline
   - Transfer the 3.125 mL of 0.9% Saline to the Provocholine® vial
2. Shake Well

After Completing Step I:

Vial A contains 3.125 mL of Provocholine® Solution @ 32 mg/mL

* Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

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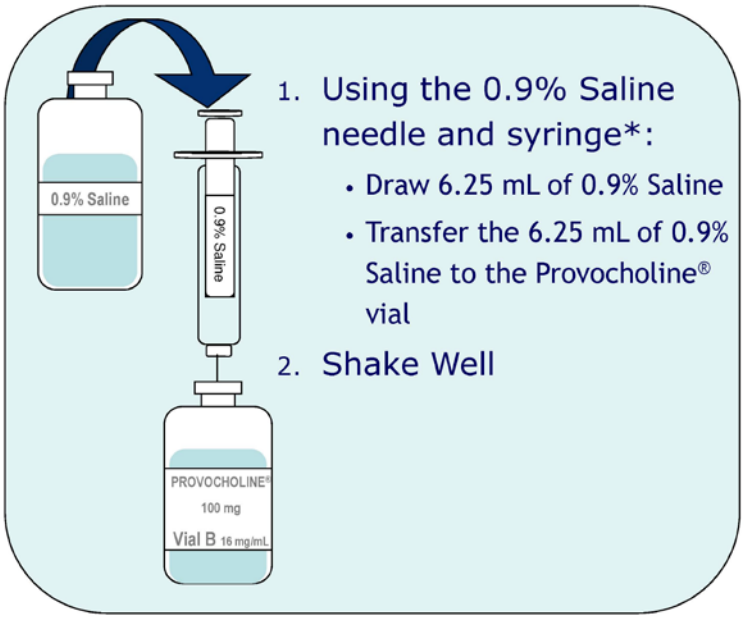
Step II: Preparing Vial B - Provocholine® **16 mg/mL** Solution



1. Using the 0.9% Saline needle and syringe*:
   - Draw 6.25 mL of 0.9% Saline
   - Transfer the 6.25 mL of 0.9% Saline to the Provocholine® vial
2. Shake Well

After Completing Step II:

Vial B contains 6.25 mL of Provocholine® Solution @ 16 mg/mL

* Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

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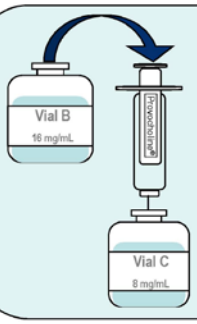
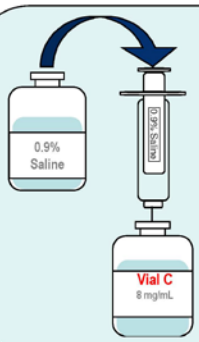
Step III: Preparing Vial C - Provocholine® **8 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial B,
   - Transfer the 3 mL to Vial C



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial C
3. Shake well

After Completing Step III:

Vial B contains 3.25mL of Provocholine® solution @ 16 mg/mL

-and-

Vial C contains 6 mL of Provocholine® solution @ 8 mg/mL

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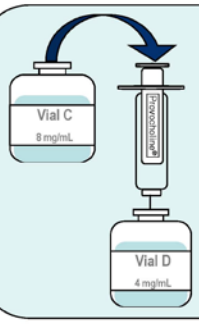
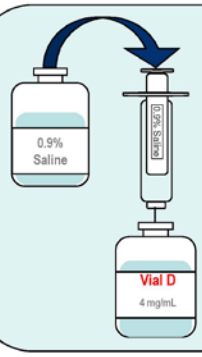
Step IV: Preparing Vial D - Provocholine® **4 mg/mL** solution



1. Using the Provocholine® needle and syringe,
   - Remove 3 mL from Vial C
   - Transfer the 3 mL to Vial D



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial D
3. Shake well

After Completing Step IV:
Vial C contains 3 mL of Provocholine® solution @ 8 mg/mL

-and-

Vial D contains 6 mL of Provocholine® solution @ 4 mg/mL

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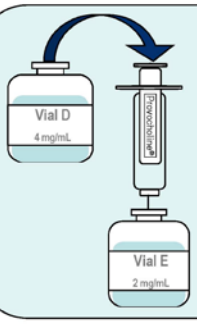
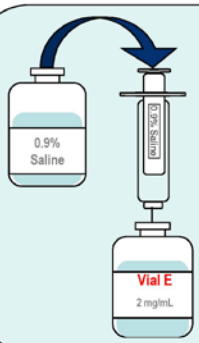
Step V: Preparing Vial E - Provocholine® **2 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **D**
   - Transfer the 3 mL to Vial **E**



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial E
3. Shake well

After Completing Step V:

Vial D contains 3 mL of Provocholine® solution @ 4 mg/mL

- and-

Vial E contains 6 mL of Provocholine® solution @ 2 mg/mL

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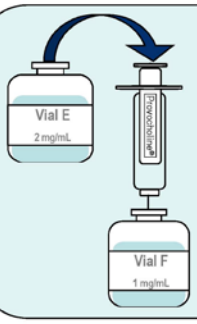
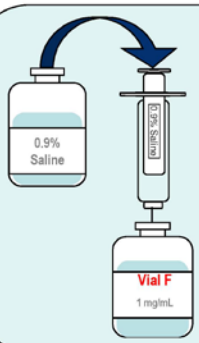
Step VI: Preparing Vial F - Provocholine® **1 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **E**
   - Transfer the 3 mL to Vial **F**



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial F
3. Shake well

After Completing Step VI:

Vial E contains 3 mL of Provocholine® solution @ 2 mg/mL

-and-

Vial F contains 6 mL of Provocholine® solution @ 1 mg/mL

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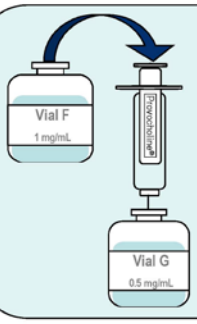
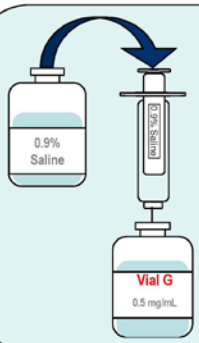
Step VII: Preparing Vial G - Provocholine® **0.5 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **F**
   - Transfer the 3 mL to Vial **G**



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial G
3. Shake well

After Completing Step VII:

Vial F contains 3 mL of Provocholine® solution @ 1 mg/mL

-and-

Vial G contains 6 mL of Provocholine® solution @ 0.5 mg/mL

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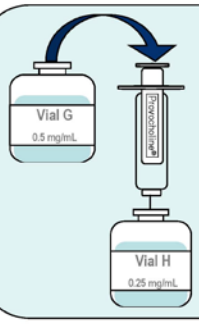
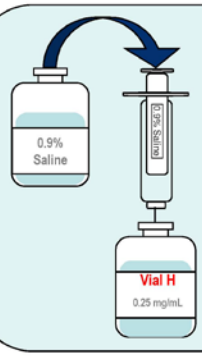
Step VIII: Preparing Vial H - Provocholine® **0.25 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **G**
   - Transfer the 3 mL to Vial **H**



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial H
3. Shake well

After Completing Step VIII:

Vial G contains 3 mL of Provocholine® solution @ 0.5 mg/mL

-and-

Vial H contains 6 mL of Provocholine® solution @ 0.25 mg/mL

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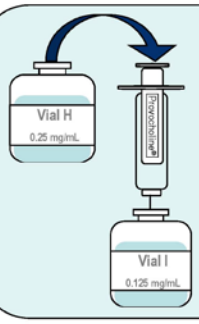
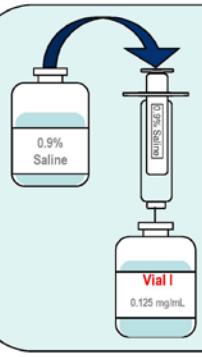
Step IX: Preparing Vial I - Provocholine® **0.125 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial **H**
   - Transfer the 3 mL to Vial I



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial I
3. Shake well

After Completing Step IX:

Vial H contains 3 mL of Provocholine® solution @ 0. 25 mg/mL

-and-

Vial I contains 6 mL of Provocholine® solution @ 0.125 mg/mL

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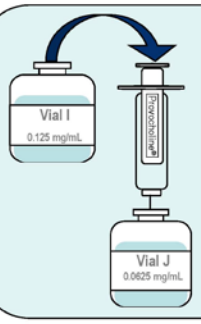
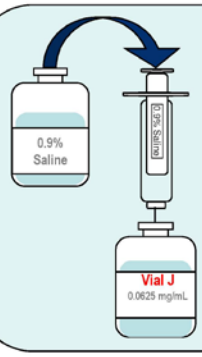
Step X: Preparing Vial J - Provocholine® **0.0625 mg/mL** solution



1. Using the Provocholine® needle and syringe:
   - Remove 3 mL from Vial I
   - Transfer the 3 mL to Vial J



2. Using the 0.9% Saline needle and syringe:
   - Add 3 mL of 0.9% Saline to Vial J
3. Shake well

After Completing Step X:

Vial I contains 3 mL of Provocholine® solution @ 0.125 mg/mL

-and-

Vial J contains 6 mL of Provocholine® solution @ 0.0625 mg/mL

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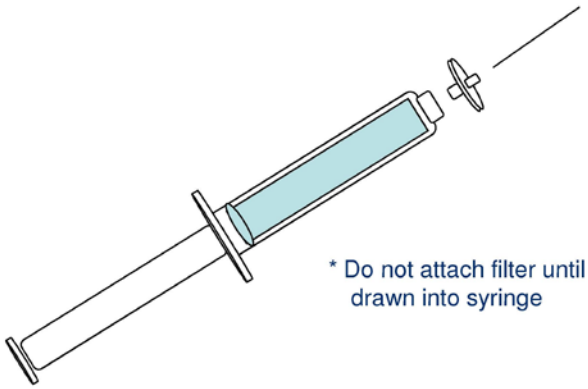
Final Step: Day of the Test

- Aseptically attach a 0.22 µm bacterial retentive filter (Millex GV®) to a 10 mL syringe*
- Transfer 2 to 3 mL of all dilutions (in vials A through J) to the nebulizer(s) through the 0.22 µm bacterial retentive filter (Millex GV®)



* Do not attach filter until AFTER solution has been drawn into syringe

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Storage Instructions

- Dilutions A through J (32 mg/mL through 0.0625 mg/mL) should be stored at 36° to 45°F (2° to 8°C) in a refrigerator for no more than 2 weeks
- Unreconstituted powder should be stored at 59° to 86 °F (15° to 30°C)
- Freezing does not affect the stability of dilutions

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Dilution Check Sheet and Control Record

PROVOCHOLINE® DILUTIONS FOR CHALLENGE TEST

Date: _____ Prepared by:_____ Checked by:_____

Provocholine® (see label on bottle): Expiration Date: _____

0.9% Saline: Expiration Date: _____

0.22 micron Filter: Expiration Date: _____

TAKE Provocholine® (Methacholine Chloride U.S.P. Powder for Inhalation)	**ADD** 0.9% Saline (SHAKE WELL!)	**OBTAIN DILUTION**	**VIAL NAME**	**COMPLETED**
Provocholine® 100 mg	3.125 mL	32 mg/mL	VIAL A - 32 mg/mL	
Provocholine® 100 mg	6.25 mL	16 mg/mL	VIAL B - 16 mg/mL	
3 mL from vial B	3 mL	8 mg/mL	VIAL C - 8 mg/mL	
3 mL from vial C	3 mL	4 mg/mL	VIAL D - 4 mg/mL	
3 mL from vial D	3 mL	2 mg/mL	VIAL E - 2 mg/mL	
3 mL from vial E	3 mL	1 mg/mL	VIAL F - 1 mg/Ml	
3 mL from vial F	3 mL	0.5 mg/mL	VIAL G - 0.5 mg/mL	
3 mL from vial G	3 mL	0.25 mg/mL	VIAL H - 0.25 mg/mL	
3 mL from vial H	3 mL	0.125 mg/mL	VIAL I - 0.125 mg/mL	
3 mL from vial I	3 mL	0.0625 mg/mL	VIAL J - 0.0625 mg/mL	

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Summary of Directions

Procedure:

1. Fill in information and attach labels to the vials.
2. Remove plastic cover from the Provocholine® and 0.9% Saline vials and line up all sterile empty vials. Wipe off all vial stoppers for Provocholine®, 0.9% Saline and sterile empty vials with alcohol swabs.
3. Attach needles to the syringes and label one for Provocholine® and one for 0.9% Saline.
4. Using the 0.9% Saline needle and syringe, withdraw 3.125 mL of 0.9% Saline* and insert into the first Provocholine® 100mg vial. SHAKE WELL. This produces 3.125 mL of Provocholine® 32 mg/mL, also known as **VIAL A – 32 mg/mL.**
5. Using the 0.9% Saline needle and syringe, withdraw 6.25 mL of 0.9% Saline* and insert into the second Provocholine® 100mg vial. SHAKE WELL. This produces 6.25 mL of Provocholine® 16mg/mL, also known as **VIAL B – 16 mg/mL.**
6. Using the Provocholine® needle and syringe, remove 3 mL from VIAL B 16 – mg/mL and transfer to the vial labelled VIAL C – 8 MG/ML. Using the 0.9% Saline needle and syringe, add 3 mL 0.9% Saline. Shake well. **THIS IS VIAL C – 8 mg/mL.**
7. Using the Provocholine® needle and syringe, remove 3 mL from VIAL C – 8 mg/mL and transfer to the vial labelled VIAL D – 4 MG/ML. Using the 0.9% Saline needle and syringe, add 3 mL of 0.9% Saline. Shake well. **THIS IS VIAL D – 4 mg/mL.**
8. Using the Provocholine® needle and syringe, remove 3 mL from VIAL D – 4 mg/mL, and transfer to the vial labelled VIAL E – 2 MG/ML. Using the 0.9% Saline needle and syringe, add 3 mL of 0.9% Saline. Shake well. **THIS IS VIAL E – 2 mg/mL.**
9. Using the Provocholine® needle and syringe, remove 3 mL from VIAL E – 2 mg/mL and transfer to the vial labeled VIAL F – 1 mg/mL. Using the 0.9% Saline needle and syringe, add 3 mL of 0.9% Saline. Shake well. **THIS IS VIAL F – 1 mg/mL.**

* Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

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Summary of Directions, continued

Procedure continued:

11. Using the Provocholine® needle and syringe, remove 3 mL from VIAL F – 1 mg/mL and transfer to the vial labeled VIAL G – 0.5 mg/mL. Using the 0.9% Saline needle and syringe, add 3 mL of 0.9% Saline. Shake well. **THIS IS VIAL G – 0.5 mg/mL.**
12. Using the Provocholine® needle and syringe, remove 3 mL from VIAL G – 0.5 mg/mL and transfer to the vial labeled VIAL H – 0.25 mg/mL. Using the 0.9% Saline needle and syringe, add 3 mL of 0.9% Saline. Shake well. **THIS IS VIAL H – 0.25 mg/mL.**
13. Using the Provocholine® needle and syringe, remove 3 mL from VIAL H – 0.25 mg/mL and transfer to the vial labeled VIAL I – 0.125 mg/mL. Using the 0.9% Saline needle and syringe, add 3 mL of 0.9% Saline. Shake well. **THIS IS VIAL I – 0.125 mg/mL.**
14. Using the Provocholine® needle and syringe, remove 3 mL from VIAL I – 0.125 mg/mL and transfer to the vial labeled VIAL J – 0.0625 mg/mL. Using the 0.9% Saline needle and syringe, add 3 mL of 0.9% Saline. Shake well. **THIS IS VIAL J – 0.0625 mg/mL.**

NOTE:

- **A sterile bacterial-retentive filter (porosity 0.22 µm) should be used when transferring a solution from each vial to a nebulizer.**
- **To avoid back pressure when injecting the contents of a syringe into a vial, you may wish to vent the vial with another needle.**

* Please note that the dilutions in Step 1 and 2 requires that you draw 3.125 mL and 6.25 mL of 0.9% Saline, therefore you should use an additional 1 mL syringe containing 0.9% Saline for Step 1 and 2

methapharm

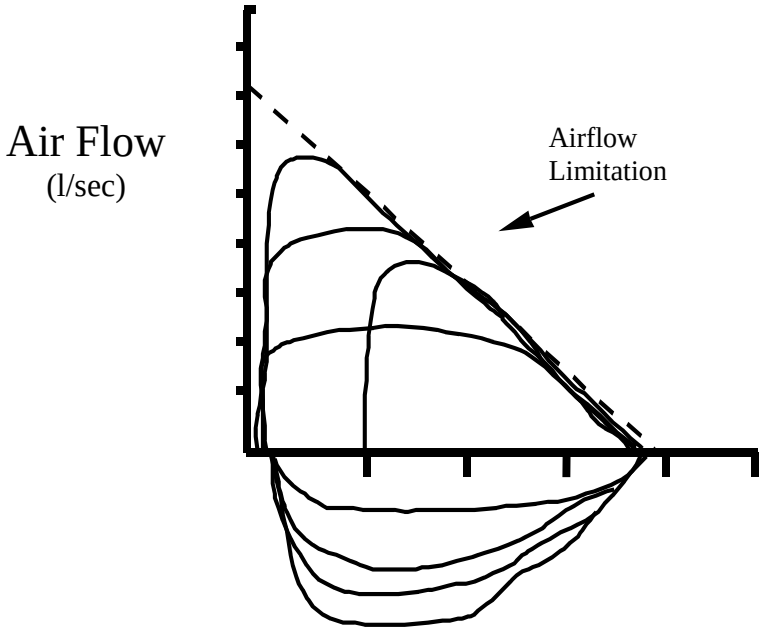
Please note that the instructions below assume that the methacholine is made with a 0.9% normal saline diluent and sterile filter. A sterile filter is not required. 0.4% phenol may be added per local pharmacist/investigator discretion. Dilutions may also be frozen for up to 6 months prior to use in a typical frost-free freezer (temperature range, -20° to -7°C). Storage in a -80°C freezer is also acceptable. Once thawed, dilutions must be discarded within 14 days; they may not be refrozen.

APPENDIX B: FURTHER INSTRUCTIONS (METHOD) FOR METHACHOLINE ADMINISTRATION

- Instruct the participant to begin breathing normally on the nebulizer set mouthpiece.
    - The coordinator should observe the participant's respirations to ensure full inspiration and expiration and quality of administration. In addition, it is important for the coordinator to reinforce to the participant that there should be no coughing or clearing of the airways as this may result in coughing out the dose and not generating a true reduction in FEV_1 or true reaction.
- The participant should wear nose clips and maintain a tight seal on the nebulizer set mouthpiece.
- Coach the participant through normal breathing exercises to prepare them for a deep inhalation. This observation period will also give the coordinator an opportunity to examine the participant's breathing patterns to better gauge when a full expiration has taken place.
- When the participant exhales a complete normal breath, press the REMOTE TRIGGER SWITCH and prompt the participant to take in a slow, deep breath and hold that breath for 5 seconds.
- The coordinator will count off 5 seconds aloud to the participant with the aid of a stopwatch to ensure 5 seconds have passed.
- At the end of the 5 second hold, instruct the participant to let their breath out and take a normal inhalation and exhalation.
- At the end of this normal exhalation, repeat this procedure 4 more times.
    - In summary, the participant should inhale a dose of methacholine every other inhalation, alternating with a normal inhalation and exhalation.

Example:

"Mr. Jones, please place the nose clips on your nose and insert the mouthpiece in your mouth. Form a tight seal and begin breathing normally. (The participant will breathe normally for a few inhalations and exhalations to acclimate to the instrument.) Breathe in, breathe out, breathe in, breathe out. Ok, on your next breath in I want you to take a big breath in and hold it for 5 seconds. Ready…Take a big breath in and hold for 1..2..3..4..5. Ok, release your breath and take a normal breath in and then out. Ready…Take a big breath in and hold for 1..2..3..4..5." Repeat this procedure until 5 inhalations receiving the methacholine dose have been completed.



SPIROMETRY MANUAL OF OPERATIONS

SPIROMETRY MANUAL

This manual is intended to serve as both a training document and a reference document for persons who perform spirometry for AsthmaNet, as well as for those involved in other aspects of the AsthmaNet trials. It is written to be useful for people who have never performed pulmonary function testing before, as well as for those who are experienced in performing these tests, but who are not familiar with the procedures used in AsthmaNet trials. Proficiency in performing spirometry for AsthmaNet, however, is best acquired by practice and experience. You are encouraged to practice spirometry on yourself, your friends and your co-workers.

1.0 OVERVIEW

1.1. INTRODUCTION TO SPIROMETRY

1.1.1 WHAT IS SPIROMETRY?

Spirometry is the time-based measurement of the amount of air that can be forcefully exhaled from the lungs after a full inspiration. In this manual, we will use the term spirometry more specifically to refer to the measurement of the forced expiratory vital capacity (FVC) maneuver. In this maneuver, a person inhales as big a breath as possible and then blows it out as fast and as long as possible until no more air can leave the lungs.

The volume of air leaving the lungs is recorded continuously throughout the maneuver in order to calculate important measures of lung function. This recording is called a spirogram.

When properly performed with maximum effort and attention to the details of calibration of the instrument, spirometry maneuvers are among the most reproducible of biomedical measures. Indeed, in a highly trained, experienced subject a coefficient of variation of 2-3% is not unusual. Values measured from the spirogram are important indicators of the type and severity of disease and the response to treatment, and they can even predict life expectancy. Spirometry is also utilized during methacholine challenge, which is a measure of the severity of bronchial reactivity. In AsthmaNet trials, we use these measures not only to determine the severity of asthma in the study subjects, but also to determine the effects of the different experimental treatments that will be used in the various protocols. As part of the AsthmaNet trials, spirometry is an essential component of the inclusion criteria and frequently constitutes primary outcome measures. Therefore, it is essential that this measurement be conducted with care,

patience, enthusiasm and attention to detail. Your role in performing the collection of this important data cannot be over emphasized.

1.1.2. FLOW LIMITATION

The reason that spirometry is a very reproducible test is that people develop maximum flow rates of air leaving the lungs during a forced vital capacity maneuver. Once this point is reached the rate of flow of air cannot be increased, no matter how hard the person tries to force the air out of the lungs.

This phenomenon is called flow-limitation and is thought to be due to the fluid dynamics of flow through collapsible airways. Clearly then, in order to obtain reproducible results, it is important to obtain maximum effort from the participant each time an FVC is performed.

Once a certain amount of effort has been exerted, the speed that air can be forced out of the lungs depends upon three factors: the elastic properties of the lung, i.e. the lung elastic recoil pressure; the diameter of the airways; and the ease which airways tend to collapse. In asthma, worsening disease causes narrowing of airways due to constriction of airway smooth muscle and inflammation of the airways as well as an increase in the tendency of airways to collapse. The elastic properties of the lungs are a major driving force for movement of air out of the lungs during a maximal expiration. The elastic recoil pressure of the lungs changes with the volume of air in the lung; i.e., it is greatest at full lung inflation and decreases at lower lung volumes. Therefore, there is greater airflow out of the lung at higher lung volumes than at lower lung volumes

About 1 in 5 people show a phenomenon of negative effort dependence. This means that the flow of air from the lungs can actually fall with increasing effort. In these people, it is important to obtain maximum effort because less than maximum effort can cause a "falsely" high measure of lung function, which is also difficult to reproduce.

1.1.3. ASSESSMENT OF EFFORT DURING SPIROMETRY

Determining whether a person is giving a maximum effort on a forced expiratory maneuver requires both experience and judgment. An important clue is whether the tests are reproducible, since less than maximum effort is difficult to reproduce.

Another measure of the effort generated during a spirogram is the magnitude of the Peak Flow (also called PEFR for peak expiratory flow rate, or PEF for peak expiratory flow). The PEFR is the maximum flow generated during the test; it occurs during the very early part of the FVC maneuver. It is determined by two factors: how deep a breath has been taken in and how hard the person blows out. For this reason, this

measurement is said to be "effort dependent". This is a useful characteristic as it means that the PEFR can be used as an index of effort, but it also means that it has more variability than other measures taken from the spirometry maneuver.

In order to determine that all of the air has been blown out of the lungs, it is necessary that the participant continue to blow air out of the lungs until no more air can exit. As a rule of thumb, it takes about six seconds for a normal person to empty their lungs. In persons with asthma, it can take considerably longer. For this reason, the goal of testing is to continue the expiratory effort until the flow of air out of the lungs falls to zero, i.e., no more air can leave the lungs. During spirometry, the duration of expiration should be adequate so that the final flow rates should be close to or zero.

In addition to the reproducibility of efforts, high peak flows, and long duration of expiration, it is important to closely observe the subject being tested. The body language of the test subject is often a better guide to maximum effort than the recording of the maneuver. An experienced pulmonary function technician observes the test subject more closely than the spirometer or computer screen.

1.2 ASSESSMENT OF MAXIMAL EFFORT IN FORCED EXPIRATION

- **The maneuvers are reproducible**
- **The peak flows are high**
- **The onset of expiration is sudden and forceful**
- **The duration of expiration is at least 6 seconds**
- **The flow at the end of test is zero, even though the subject is still exerting effort**
- **The subject appears to be producing a maximal effort.**

1.3 GUIDELINES FOR MANEUVER ACCEPTABILITY

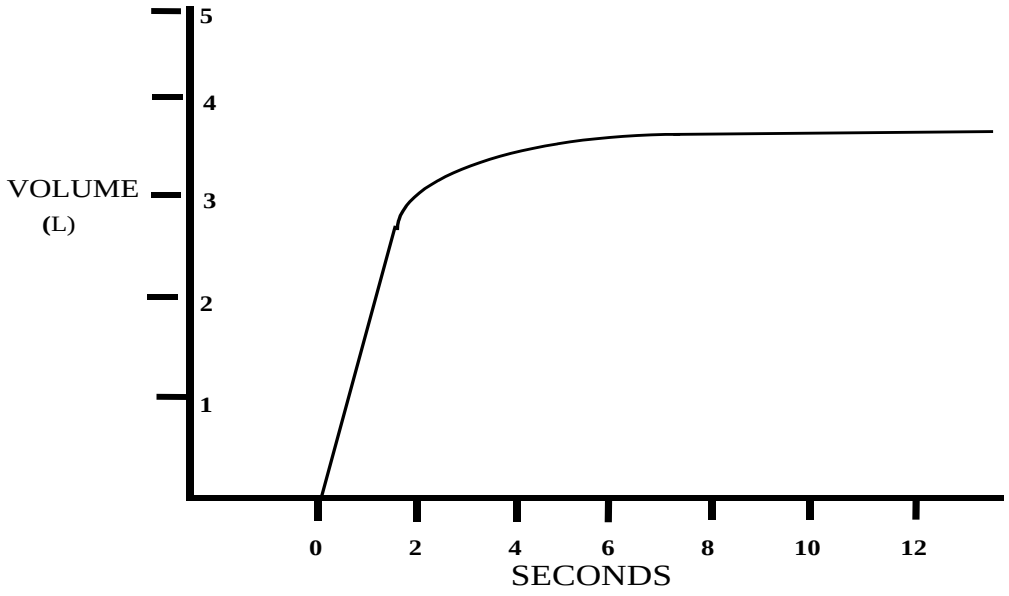
For the purposes of AsthmaNet, the following table will serve as a guideline for accepting flow-volume maneuvers in study participants. We have chosen to apply ATS preschool criteria to the children under eight years of age and the standard ATS criteria in children eight years of age and older and adults. Choose the correct criteria based on the participant’s age at the time of the visit.

Maneuver Acceptability Criteria	ATS (age ≥8 at time of visit)	ATS Preschool Guidelines (age <8 at time of visit)
START- OF -TEST	Extrapolated volume < 5% of FVC or 0.15L, whichever is greater	Visually satisfactory start; rapid onset of expiration; extrapolated volume < 12.5% of FVC or 0.08L, whichever is greater
PEAK FLOW	15% reproducibility; no criterion per ATS	Clearly determined Peak Flow; a single distinct peak in the MEFV curve
MANEUVER ARTIFACT	No cough/glottic closure during first second of exhalation, leak or early termination	No cough/glottic closure during first second of exhalation, leak or early termination
END-OF-TEST	No early termination or cutoff WITH volume-time tracing showing obvious plateau	No abrupt ending or truncation with sharp drop/cessation in flow from a point where flow was >10% of the PEF
REPEATABILITY	Minimum of 3 maneuvers with 2 of the maneuvers having FVC and FEV_1 within 150 ml	Minimum of 3 maneuvers; shapes of flow-volume curves visually repeatable with FVC and $FEV_{0.5}$ within 10% if the FVC is $\geq$ 1.0L. If the FVC is < 1.0L, the FVC and $FEV_{0.5}$ should be reproducible within 0.1L.
NUMBER OF MANEUVERS	Minimum of 3 Maximum of 8	Minimum of 3 Maximum of 8
FET **(Forced Expiratory Time)**	Minimum of 6 seconds unless there is an obvious plateau in the volume-time display; shorter times acceptable in children FVC has to be greater than FEV_1 FVC cannot equal FEV_1	Minimum of 1 second FVC has to be greater than FEV_1 FVC cannot equal FEV_1

1.3.1. VOLUME-TIME AND FLOW-VOLUME TRACINGS

The forced expiratory maneuver measures the volume of air leaving the lung over time. The tracing inscribed is called the spirogram. One type of spirogram is the timed vital capacity or the volume-time tracing. When performed well, this tracing shows a steep initial slope, which smoothly and gradually becomes less steep until the slope is completely flat indicating that no further flow is leaving the lung. The slope of the volume-time tracing is equal to the flow that is leaving the lung. The flow leaving the lung is maximized during the early part of forced expiration (Peak Flow) and falls gradually to zero at the end of the effort. The advantage of the volume-time tracing is that it emphasizes events at the end of the maneuver and it is easy to detect the end of test or zero flow point.

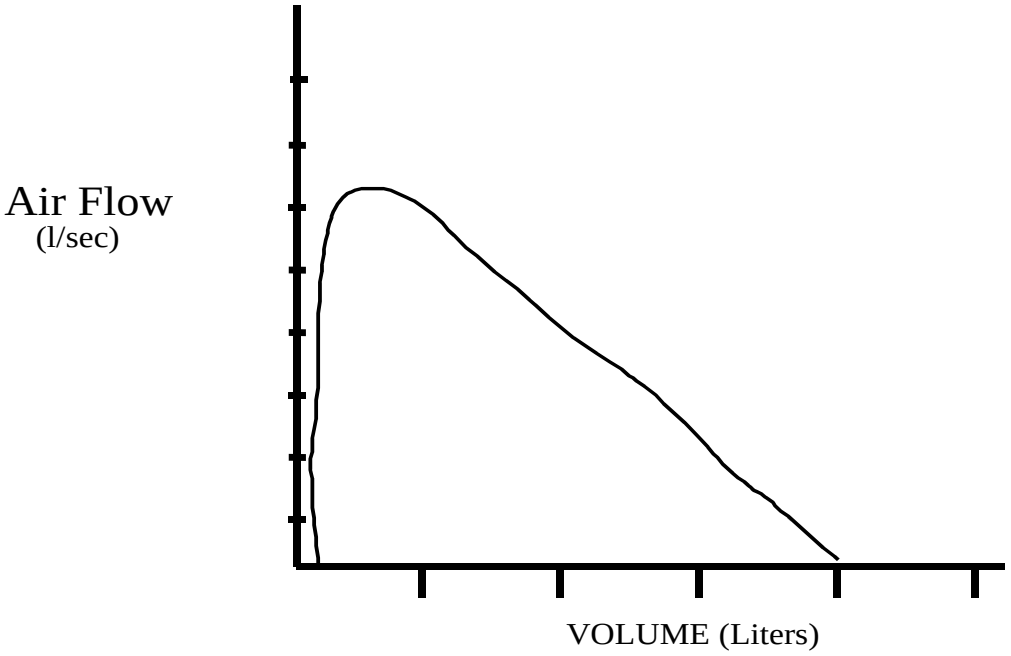
Volume-Time Tracing



This is a volume-time tracing obtained on a normal participant. Note the clean start and the clear end of test and zero flow point.

Another spirogram used to display the forced expiratory maneuver is the flow-volume curve which plots flow against volume. Because it is easier to assess effort and reproducibility from a flow-volume tracing, this is the format that will most often be used to display the spirometry maneuvers in the AsthmaNet trials.

Flow-Volume Tracing



This is a normal, good quality flow-volume tracing. There is an early and high peak flow and a continuous contour of the curve.

1.3.2. MEASUREMENTS OBTAINED FROM SPIROMETRY

The spirogram provides many parameters that are used by respiratory physiologists and physicians to measure lung function. In the AsthmaNet trials, we are mostly concerned with three important measurements: Peak Flow (PEF), FEV_1, and FVC.

The peak flow is the maximum flow that occurs during the maneuver.

The FEV_1 is the volume of air that is blown out during the first second of the maneuver. The FEV_1 cannot be measured directly from the flow-volume curve. It can be determined by the computer, however, and can also be measured by hand from a paper recording of the volume-time tracing.

The FVC is the total amount of air that leaves the lung during the FVC maneuver. An additional useful calculation is the fraction or percent of the total volume of air expired that can be exhaled in one second. This is called the FEV_1/FVC ratio. Normally, children can blow 85-90% of their vital capacity out of the lung in one second, whereas adults can blow about 80% of their vital capacity out of the lung in one second. With normal aging, this ratio falls slightly such that healthy elderly adults have an FEV_1/FVC ratio of about 75%.

In asthma or other disorders that are characterized by airflow limitation or "obstruction" of the lung, the FEV_1 is reduced. The FVC may be normal in these conditions, but it is also often reduced due to increased "trapping" of air behind closed airways.

The hallmark of an "obstructive ventilatory defect" or airflow limitation is a reduction in the FEV_1/FVC ratio. Emphysema, chronic bronchitis, and bronchiectasis, in addition to asthma, are the common causes of obstructive ventilatory defects. However, asthma is a disease with variable obstruction. Therefore, much attention is paid to the FEV_1 and other measures of airflow as an indicator of disease activity and severity, as well as response to treatment.

In "restrictive" disorders of the lung, the FEV_1 and the FVC are both reduced by about the same amount due to destruction or stiffening of the lung tissue. Thus in these disorders, the FEV_1/FVC ratio generally remains normal. Typical restrictive lung diseases include idiopathic pulmonary fibrosis, asbestosis, and sarcoidosis. This is also seen with surgical resection of lung tissue.

It is possible for a participant to develop maneuver-induced bronchospasm during spirometry efforts. In this case, the first PEF and FEV_1 are larger than subsequent trials and usually not reproducible. The values and their order must be considered in order to detect maneuver-induced bronchospasm. There is usually a gradual decrease in both values with the next effort. Please note that if a participant has a large PEF and FEV_1, and the next maneuver is low, and the third and/or fourth maneuvers are high, this is not bronchospasm; that is indicative of variable effort. If you suspect a participant displayed maneuver-induced bronchospasm, please make a note to the overreader.

1.3.3. KEY TERMS & DEFINITIONS

FVC: The difference between the lowest and highest volumes obtained during a forced maneuver.

FEF_{max}: The highest flow value measured during forced expiration.

FEFx%: The flow measured at a specific percent of exhaled volume (e.g. FEF_{25}%).

FEFx-y: The average exhaled flow between two selected volume points (e.g. FEF25-75%).

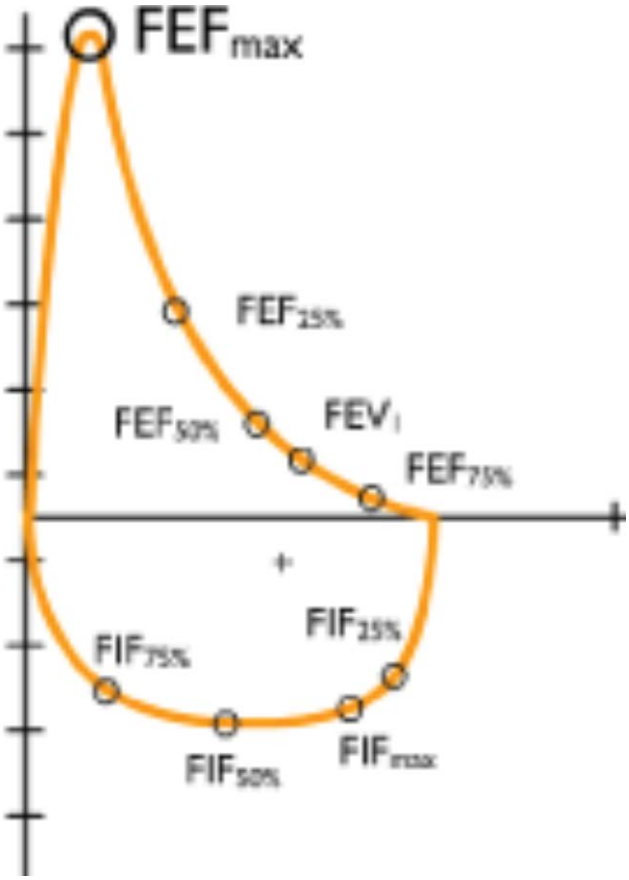
FEV_1: The volume exhaled during the first second of a forced expiratory maneuver started from the level of total lung capacity.

FIF_{max}: The highest flow value measured during forced inhalation.

FIFx%: The flow measured at a specific percent of inhaled volume (e.g. FEF_{25}%).

FIFx-y%: The average inhaled flow between two selected volume points (e.g. FEF25-75%).

FEV_1/FVC: The proportion of total air volume that can be expired in the first second of expiration.



1.3.4. FORCED VITAL CAPACITY (FVC) TESTING

A Forced Vital Capacity (FVC) test measures the maximal volume of gas that can be expired as forcefully and rapidly as possible after a maximal inspiration to total lung capacity.

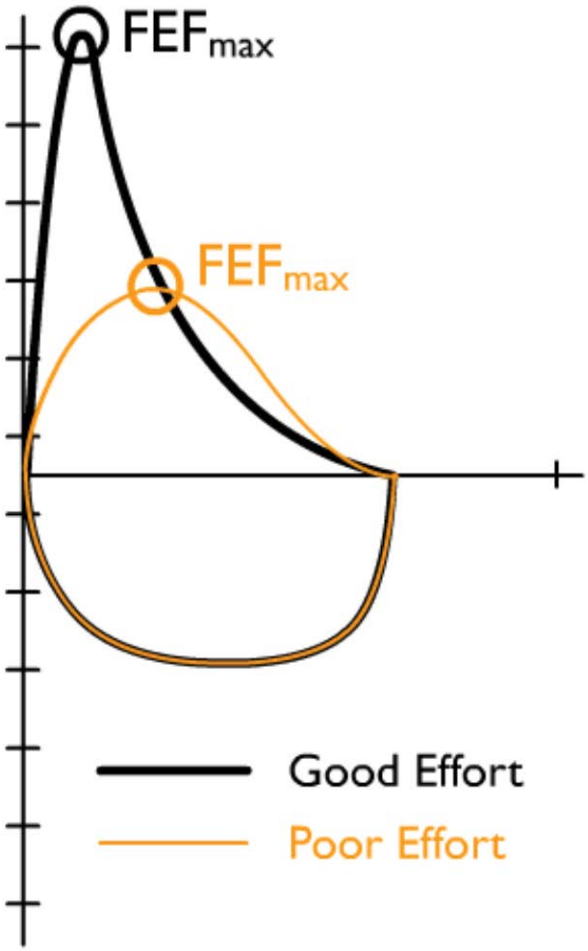
Caution: FVC testing can cause fatigue and may be dangerous for some patients. Some patients may be at risk for vertigo, arrhythmia or fainting. A maximum of eight (8) tests is recommended.

1.3.5. PHYSIOLOGY

A Forced Vital Capacity Test begins with even tidal breaths. After tidal breathing, the participant inhales maximally then exhales as rapidly, forcefully, and completely as possible (a minimum of six seconds of exhalation is recommended).

The flow volume loop, as a picture, can be very informative. The trained eye can easily identify different pulmonary function patterns without using numbers or predicted values.

FEF_{max} is the highest flow achieved during the FVC maneuver. It is a very effort-dependent value. A FEF_{max} that is not reproduced indicates inconsistent patient efforts. A good effort shows a sharp point, or peak, versus the rounded pattern of a poor effort.



1.4 BASIC INTERPRETATION OF SPIROMETRY

Airflow Limitation (Obstructive) Defects (asthma, bronchitis, emphysema)

- Reduced FEV_1
- Normal or reduced FVC
- Reduced FEV_1/FVC ratio

Restrictive Ventilatory Defects (idiopathic pulmonary fibrosis, asbestosis, sarcoidosis)

- Reduced FEV_1
- Reduced FVC
- Normal FEV_1/FVC ratio

1.5 BTPS CORRECTION

When the air is in the lung it is warmed to body temperature (37°Celsius or 98.6°Fahrenheit). When the air leaves the lung, it cools off to room temperature and becomes about 10% smaller. This is described by Charles' Law, which states that the volume of gas is directly proportional to its absolute temperature (V/T = V'/T'). The absolute temperature (Kelvin degrees) is equal to Celsius degrees + 273.

The amount of reduction of gas volume when air leaves the lungs depends upon how cool the room is. In order to make consistent measurements, we must always correct spirometry measurements to refer to the amount of air that would be in the lungs under conditions of normal body temperature (BTPS Correction).

We also make a small correction that takes into account the barometric pressure (Boyle's Law: PV = P'V') and humidity so that changes in the weather or altitude do not affect spirometry measurements. This standard method of expressing spirometry measurements is called BTPS meaning "Body Temperature and Pressure of Saturated gas".

The standard body temperature is 37°Celsius, the standard pressure is barometric pressure at sea level (760 mm Hg), and saturated gas means that it contains 100% humidity. For average room temperature, the BTPS correction factor is about 1.07 to 1.08.

2.0 EQUIPMENT AND PREPARATION

2.1. MEDGRAPHICS SYSTEM OVERVIEW

- Power-up/Warm-up
- Power-down
- preVent Flow Sensor
- Testing Components
- Flow Sensor Testing Circuit Assembly with Barrier Filter

2.1.2. POWER-UP/WARM-UP

1. Verify the connection from the CPFS/D USB to the computer's USB port.
2. Power on the computer.
3. Password: A$thm@n3t! Click on right pointing arrow.
4. Launch the BreezeSuite software
5. User Name: admin Password: "leave blank", click "OK".

NOTE: The system should be powered on for at least five (5) minutes before testing.

2.1.3. POWER-DOWN SEQUENCE

1. Exit the BreezeSuite software.
2. Shut down the computer.

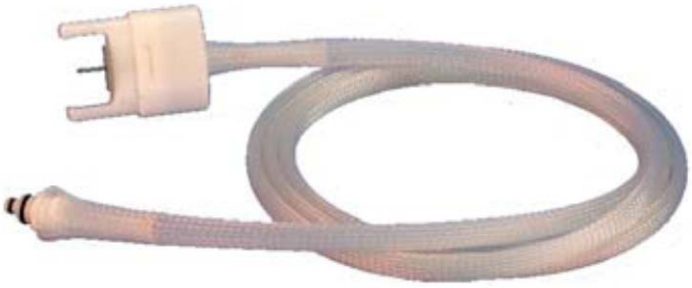
2.1.4. TESTING COMPONENTS

preVent Flow Sensor

The flow sensor is the clear component of the flow sensor testing assembly that a participant forces air through during testing to measure inspiratory and expiratory airflow.

Umbilical Cable

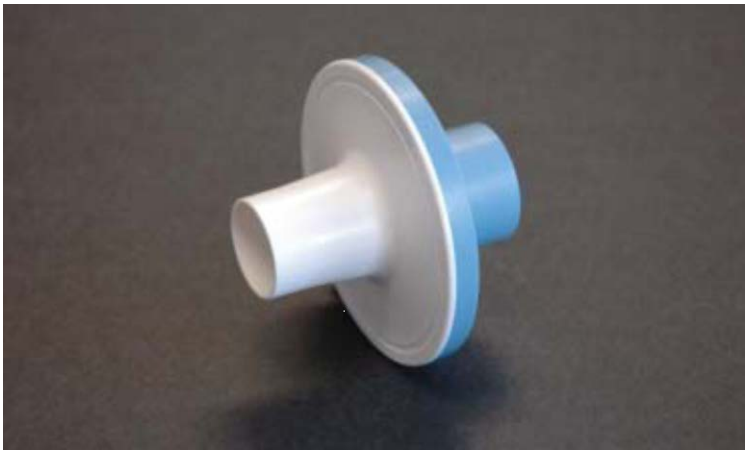
The umbilical cable connects to the CPFS/D USB of the computer. Its two pronged handle or clip connects to the flow sensor. It is the means by which the airflow measurements are transferred thru the flow sensor into the system for processing.



preVent Barrier Filter

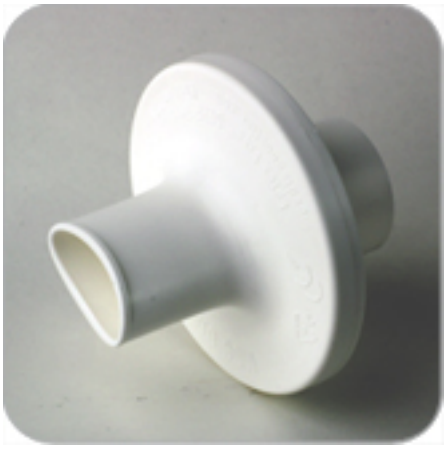
A barrier filter will be used, and it must be connected to the honeycomb side of the preVent flow sensor.

The preVent Filter (shown) provides a minimal resistance of 0.23 cmH2O/L/sec.



preVent II Barrier Filter

This barrier filter has an oval mouthpiece with lip grip, which may provide better testing results when working with pediatric participants. This filter may be used in place of the preVent Barrier Filter.



Participant Mouthpiece (optional)

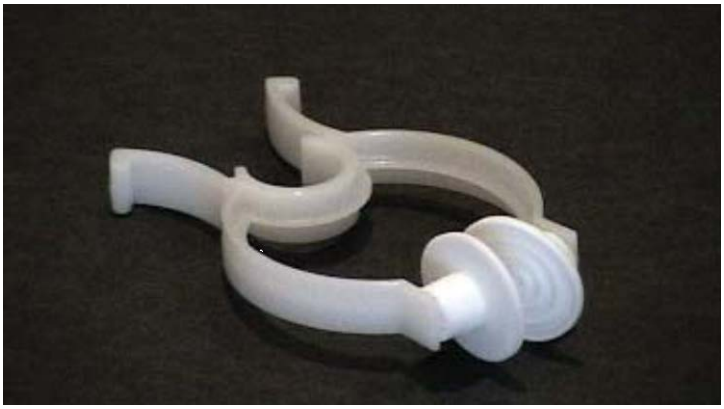
The mouthpiece is placed in the participant's mouth while testing is being performed.

preVent Handle

This handle may be used to assist participants with holding the Flow Sensor.

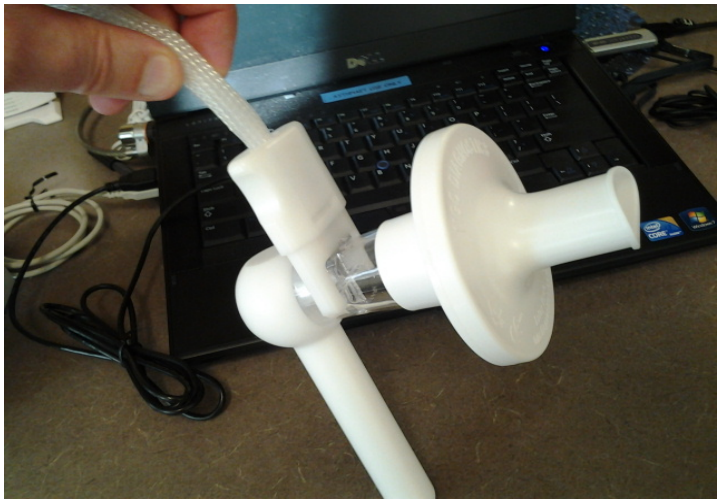
Noseclips

Noseclips are placed over the participant's nose during testing to prevent leaks.



Flow Sensor Testing Circuit Assembly Options with Barrier Filters

preVent Flow Sensor Testing Circuit



preVent II Flow Sensor Testing Circuit with preVent Handle

Refer to the photos above, and follow the steps below for assembling the Flow Sensor Testing Circuit:

1. Gather the following parts: (preVent Flow Sensor, preVent or preVent II Filter, optional preVent Handle, optional Mouthpiece.) These parts comprise the Flow Sensor Testing Circuit.

2. If you chose to use it, firmly push the optional mouthpiece onto the white end of the preVent Filter.

3. Firmly push the preVent Filter onto the honeycomb side of the preVent Flow Sensor.

4. Slip the preVent Flow Sensor onto the two prongs of the Umbilical clip making sure that the clip notch slides into the preVent Flow Sensor indentation. They should snap together and form a tight fit. Further details and pictures follow.

ATTENTION! CORRECT ASSEMBLAGE IS VERY IMPORTANT.

1. The base of the preVent Flow Sensor has a tab protruding outward and an indented notch. The base of the Umbilical clip also has a tab protruding outward. When the two pieces are connected, the Umbilical tab snaps into the notch of the preVent Flow Sensor

2. To connect the two units, align and insert the metal probes of the Umbilical clip into the holes at the base of the preVent Flow Sensor. The units should snap together and form a tight fit.

3. Ensure that the honeycomb end of the preVent Flow Sensor is facing the participant.

4. The flat end of the Umbilical clip faces the participant and the tab side faces away from the participant.

5. The flat end of the Umbilical clip always faces the honeycomb side of the preVent Flow Sensor.

2.1.5. FLOW SENSOR CALIBRATION

Enter BreezeSuite.

1. To access the calibration button at the top of the Open Patient Screen, click the “Done” button.

2. Click the Calibrate button on the BreezeSuite computer screen to calibrate the preVent Flow Sensor then enter the appropriate values for temperature, barometric pressure and humidity at the bottom of the calibration screen.

3. Each site will have a MedGraphics 3-liter calibrating syringe. Attach the 3-liter syringe to the participant side (white) of a preVent Barrier Filter using the blue rubber adapter. HINT: you may keep the calibration syringe-blue rubber adapter-preVent Barrier Filter assembled and use it daily. You may label this preVent Barrier Filter as the calibration filter. Replace preVent Barrier Filter as needed.

4. Attach the preVent Barrier Filter to the participant end (honeycombed side) of the preVent Flow Sensor.

5. Click the Zero Flow button (circled at the top of the screen).

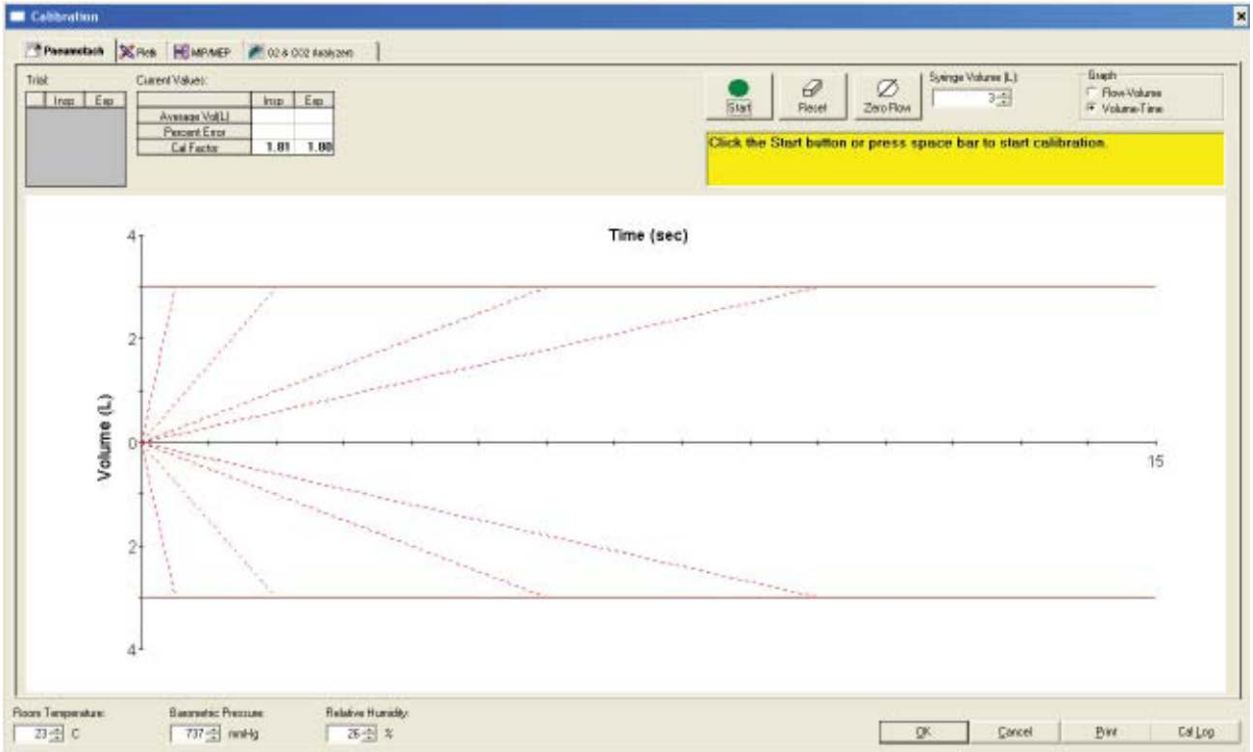
6. Chose either the Flow-Volume or Volume-Time graph in the box at the top of the screen labeled “Graph”.

7. Click Start, then withdraw and inject the syringe according to varied rates as indicated on the calibration screen. Follow the computer prompts to know when to withdraw and inject.

8. Vary the speed of subsequent strokes to test the linearity of the flow system. The “flow lines are suggestions to vary the speed of withdrawal and injection”. Always provide smooth consistent strokes.

9. After 5 complete strokes, the BreezeSuite confirms when the calibration is successful. Click OK to save the calibration. The tab labeled “Print” is available to print the calibration (although this is not necessary), and “Cal Log” will allow a table of previous calibrations (this table can also be printed if needed).

10. Do not click Reset. If calibration fails, click “Cancel” and repeat. If calibration continues to fail, replace preVent Flow Sensor.



NOTE: If the syringe sticks during calibration, do the following:

1. Click Stop
2. Push the syringe in
3. Click Start
4. Fully withdraw the syringe
5. Wait for the inject prompt to appear, then
6. Inject the syringe
7. Click Stop
8. Return to step three of calibration process above

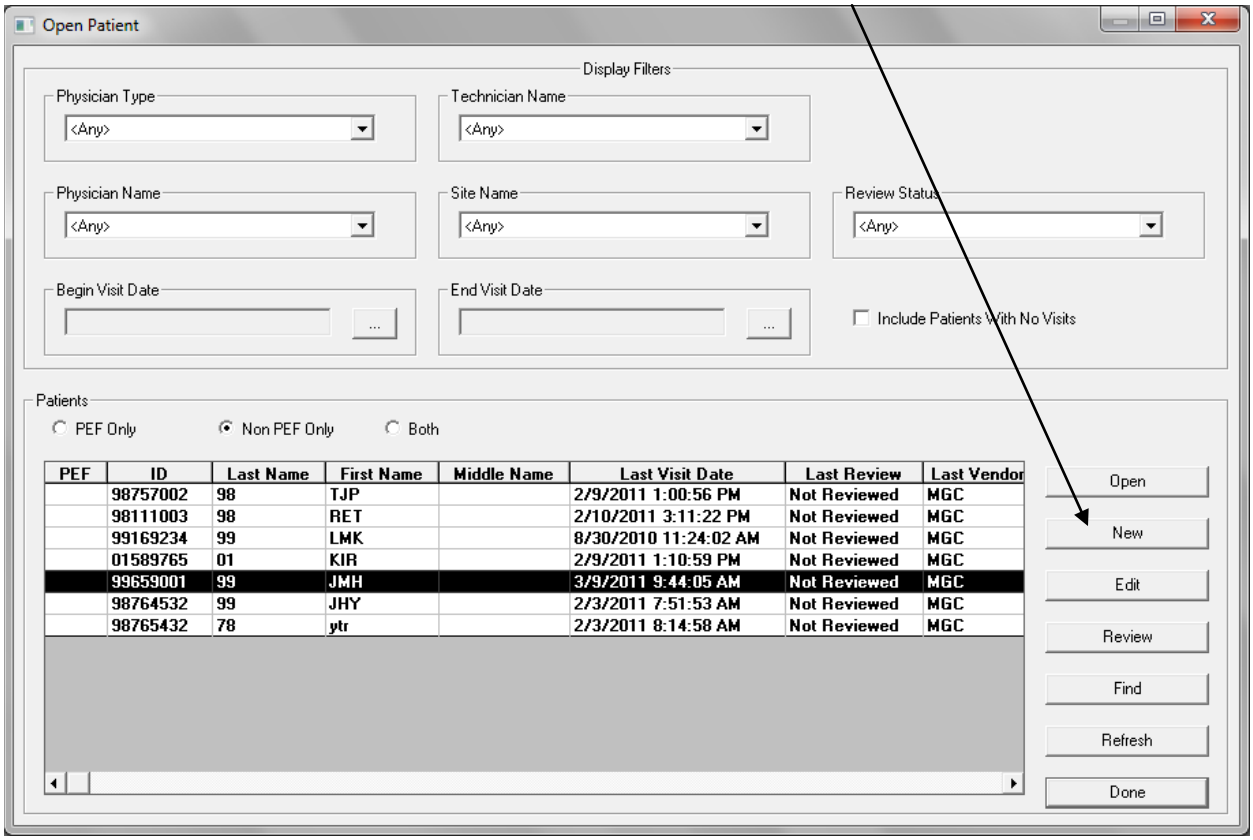
2.1.6. CALIBRATION REPORT

To print a Calibration Report, click the Print button on the bottom of the calibration screen. To save an electronic copy only, click OK.

All calibrations are stored electronically and can be pulled up at a later date by clicking on the Cal Log button at the bottom of the calibration screen. It is not necessary to print the calibration report each day.

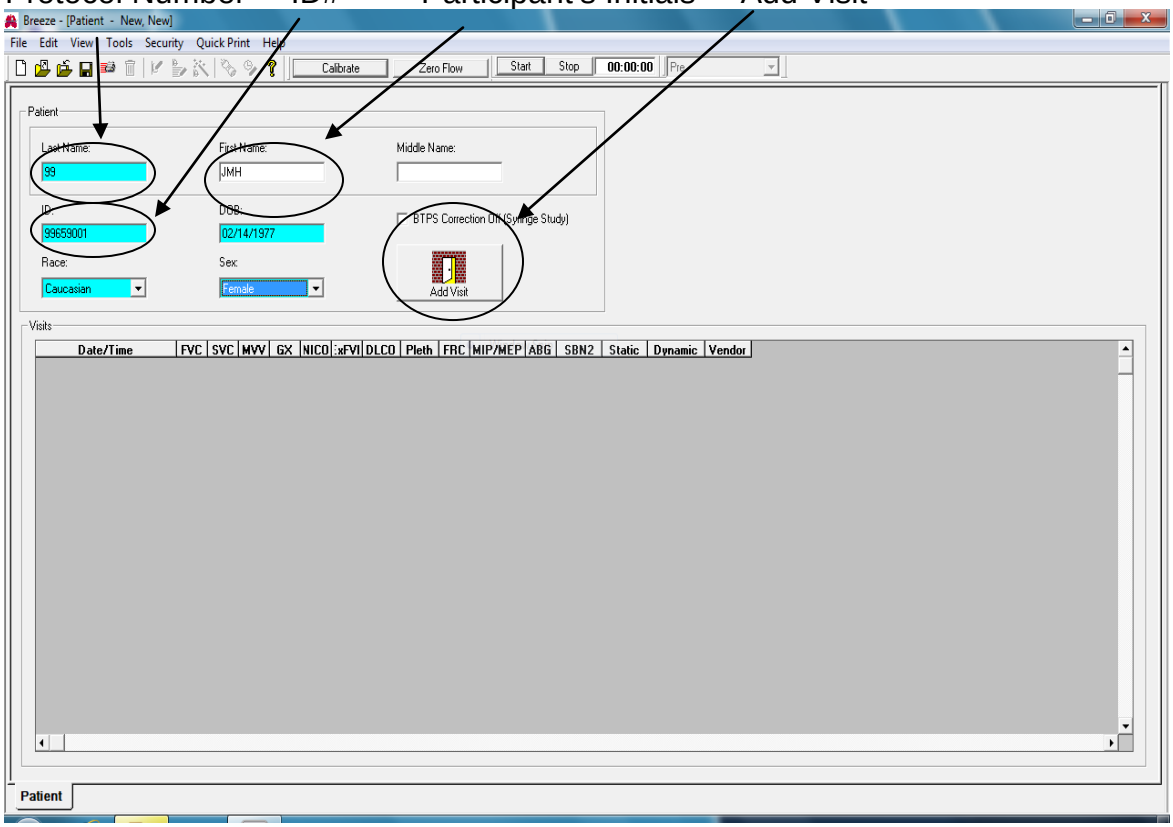
2.1.7. PATIENT/PARTICIPANT INFORMATION

To create a new patient/participant, select the “NEW’ button.



The following screen should appear:

Protocol Number ID# Participant's Initials Add Visit



All information in the colored fields is required to create a new patient/participant. Use the Tab key or mouse to advance to the next field.

- In the "Last Name" field, enter the 2 digit protocol number. For single digit protocols, enter a zero prior to the protocol number.

- In the "First Name" field, enter the participant's three initials (first, middle, last); use an 'X' if a person does not have a middle name.

- Leave the "Middle Name" blank.

- Enter the patient ID in the ID field. The ID must be unique in the following format protocol number (two digits) + site number (three digits) + subject number (three digits.) Do not use dashes.

  NOTE: If spirometry is being performed on a "practice" subject or for non-AsthmaNet purposes, use 98 as the first two numbers in the ID. These tests will not be overread or graded. If spirometry is being performed for certification purposes, use 99 as the first two numbers in the ID.

- Enter the date of birth in the Date of Birth field (MM/DD/YYYY). The software will calculate the age from the date of birth.

  NOTE: During some protocols, the participant may have a birthday that results in predicted values being calculated from a different author set. The software will automatically calculate the new predicted sets.

- Use the black arrows of the drop down menus to choose values for Race (Black, Caucasian, Asian, or Hispanic) and Sex, or type the first letter of your selection.

  NOTE: The race that is entered into the MedGraphics system must be the participant's 'spirometry race/ethnicity' listed on his/her AsthmaNet Registry report.

1. Select the Add Visit button. You will be taken to the "Visit Demographics" screen (see below).

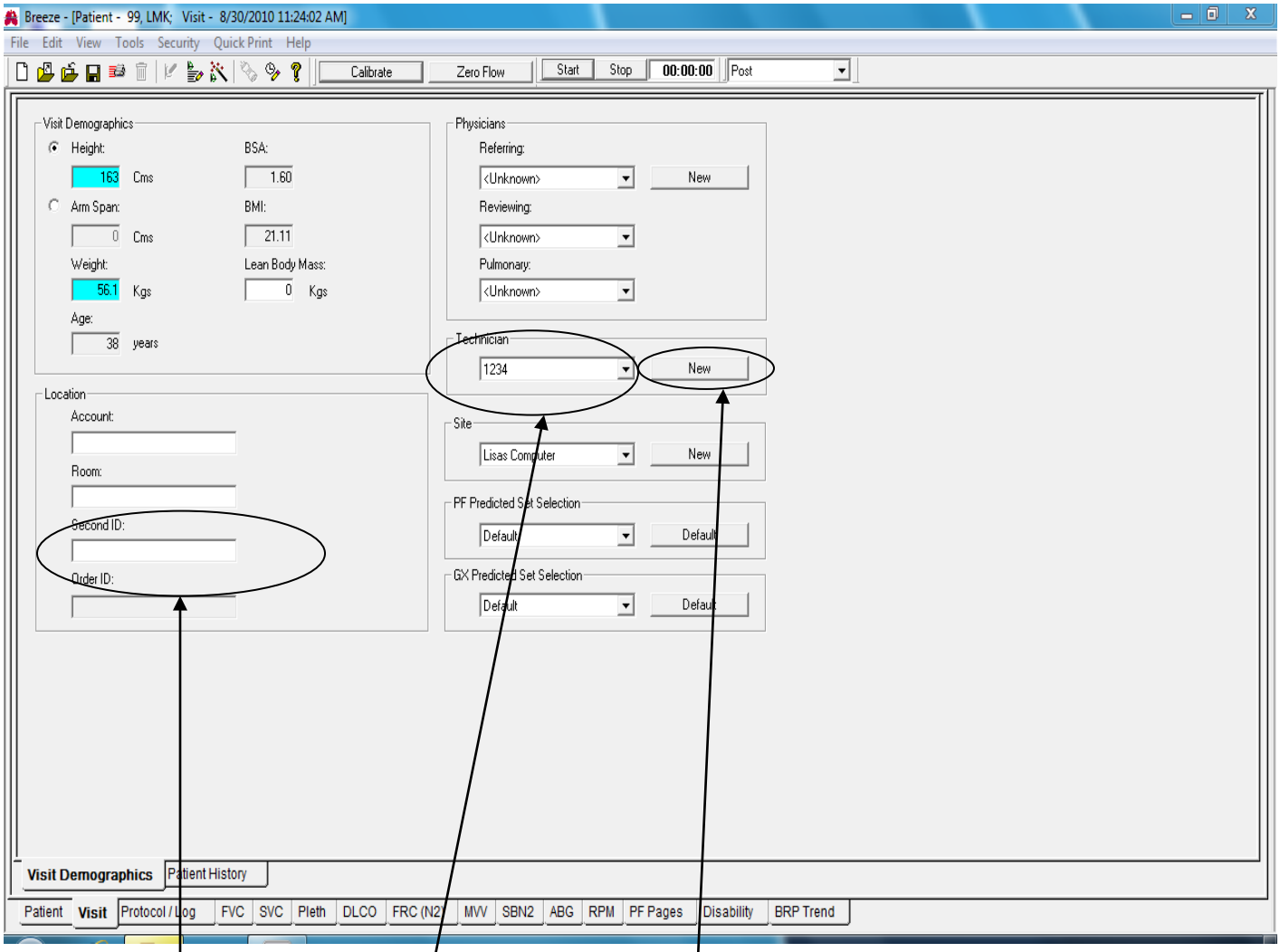
2. Enter the participant's height and weight. The height and weight parameters (cm & kg) cannot be changed while this screen is open. Height should be entered in full centimeters for individuals 18 years of age and older (round to the nearest cm) as reflected on the Adult Body Measurements form (BODYMEAS_ADULT). Height should be entered in centimeters rounded to the nearest tenth for individuals under the age of 18 as reflected on the applicable Pediatric Long or Short Physical Exam form (LEXAM_PED or SEXAM_PED). Weight should be entered to one decimal place as recorded on the relevant physical exam or body measurements form.

3. In the technician field, click the black arrows of the appropriate drop-down menu to choose the four digit tech ID.

4. If a new technician needs to be added, click on "New." Enter the tech's ID number in both the Last Name box and in the ID box. Then click on "File" located in the upper left-hand corner and Save and Exit. Click "Yes" to save the information. Then click on Breeze and recall the current participant.

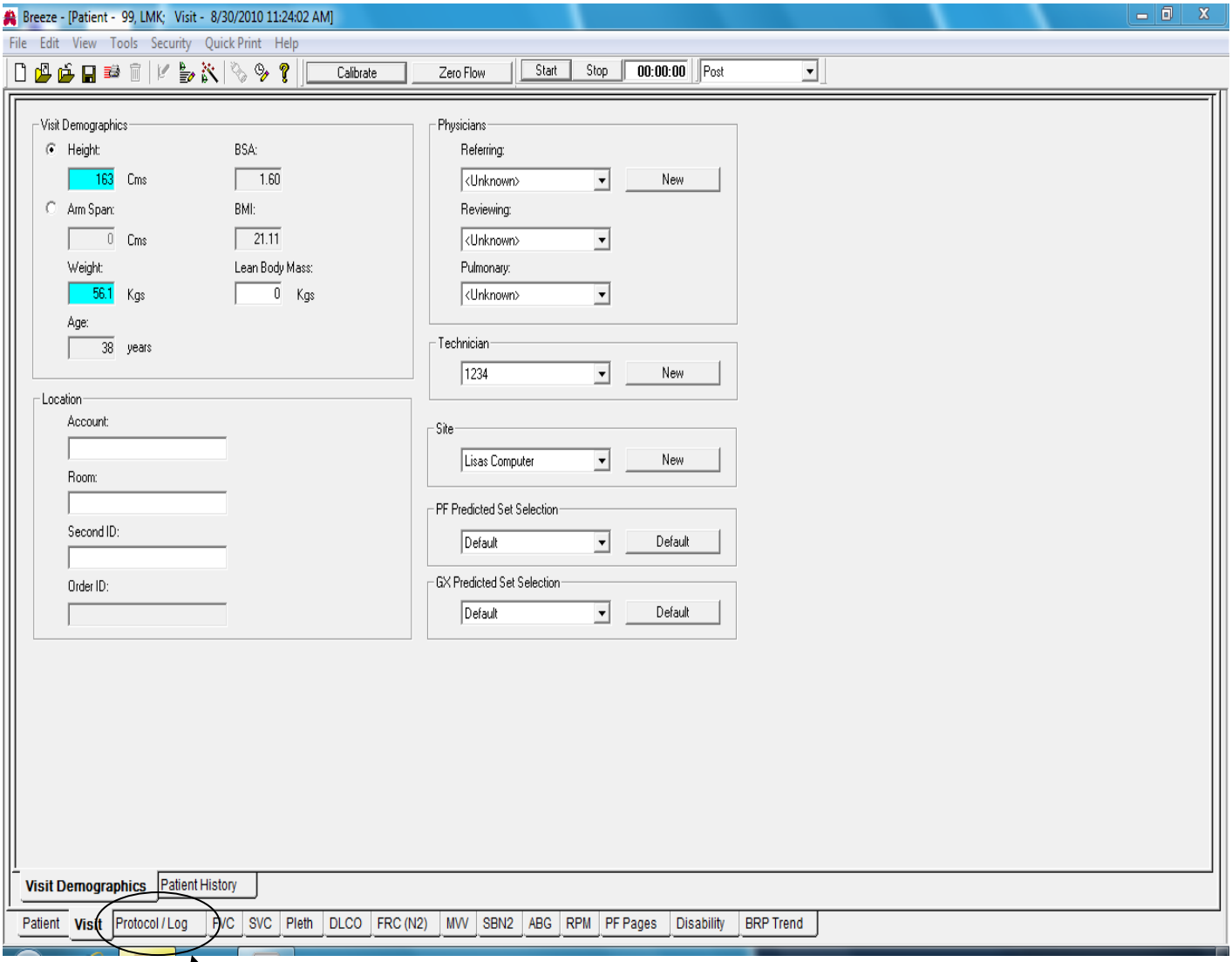
5. In the "Location" panel, you will find the "Second ID" In this field, type the visit number. On the final report, the information in this field will be displayed as Visit.

Visit Demographics screen



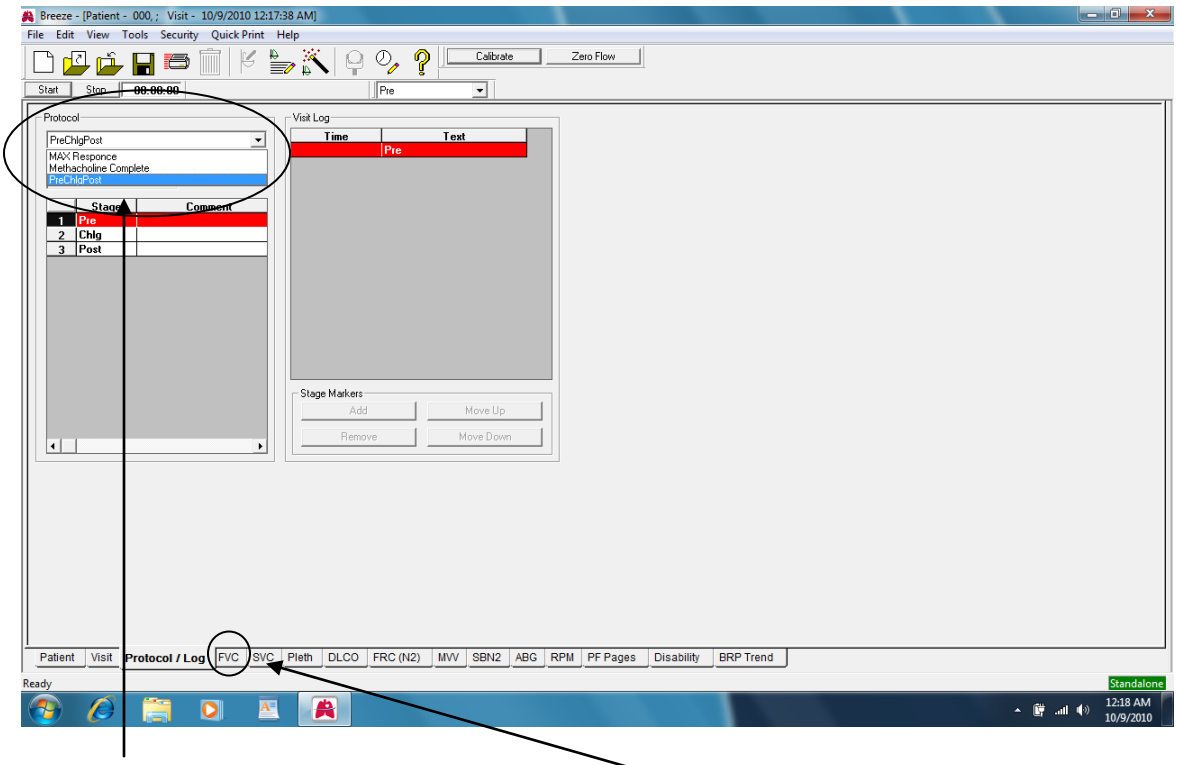
Visit Number Technician Enter New Technician ID#

Select the specific procedure by using the Protocol/Log tab (see below).



To Select Procedure (Spirometry, Max Response, Methacholine Challenge)

You will then see this screen. Use the drop down menu to select the procedure.

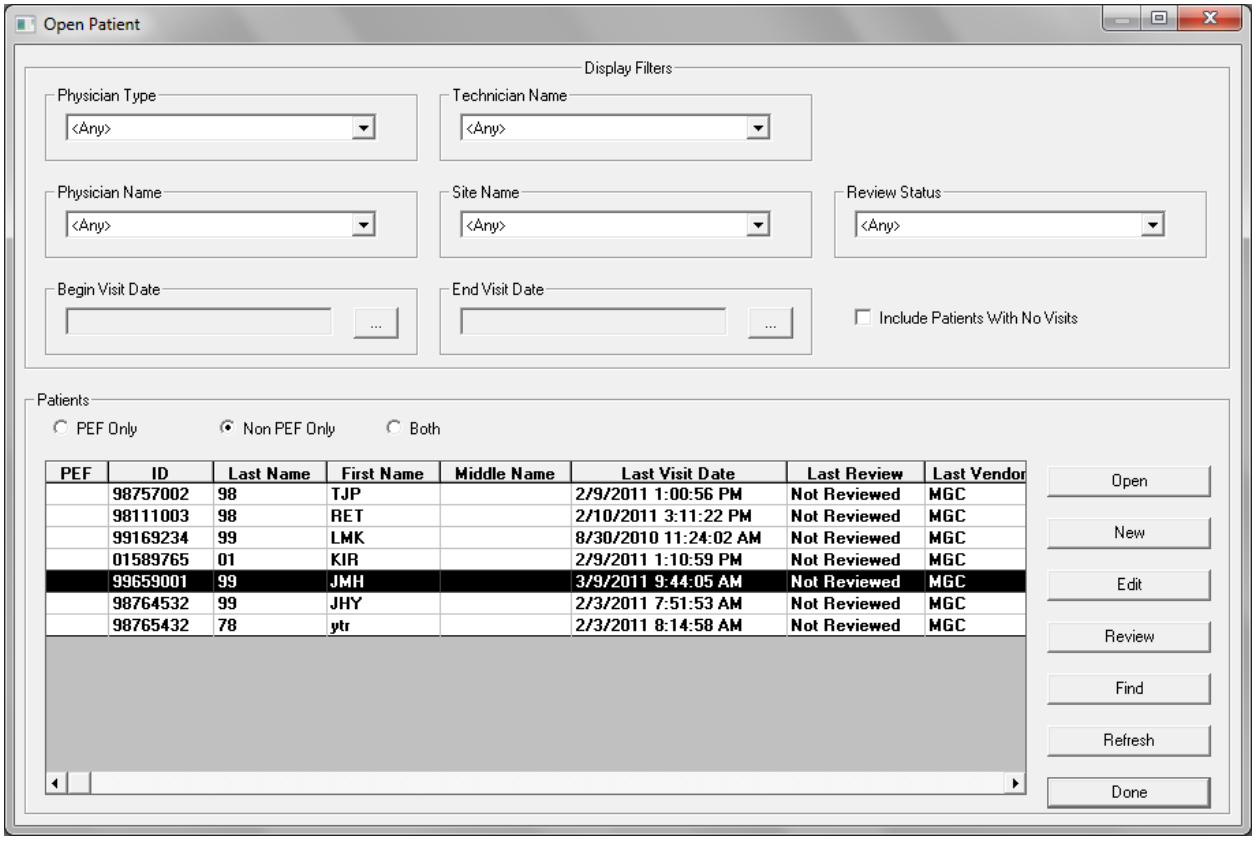


6. Click the desired test tab from the Protocol drop down menu. “PreChlgPost” is the option for spirometry. BreezeSuite will calculate predicted values.

7. Click on the tab “FVC” to open the testing screen.

2.1.8. OPENING AN EXISTING PATIENT

If a participant has previously been seen, highlight the participant's ID in the Open Patient screen and click Open. Previous information and visit dates will be displayed. If the Open Patient Screen is not displayed, click the Open Patient icon to display the participant list.



2.1.9. SEARCHING THE DATABASE

If the list of participants is long, it may be difficult to find a given person. Rather than scrolling through a long list, use the search tool to find a participant.

1. Select the FIND button.

2. From the FIND IN pull down menu, select whether to search by Last name (protocol number), First name (subject initials), ID# or Last Visit Date.

3. Fill in the “FIND WHAT” line with enough of information to narrow the search.

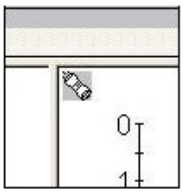
4. Click Find to run the search. The first match will be highlighted. Click Find again to move to the next match in the list.

5. To open a participant record, either double click on the participant or select it and click Open.

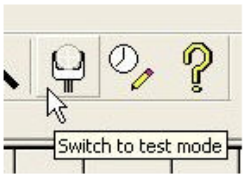
2.10. TEST AND REVIEW MODE

Test Mode

To perform a test, BreezeSuite must be in Test Mode. When creating a new visit and starting the test, the system is automatically in test mode. BreezeSuite displays the Test Mode icon in the corner of the test graphs and the test pad. Click the Test Mode icon at the top of the screen to switch from Review Mode to Test Mode.



Test Mode Icon in Test Mode



Large Icon Test Mode View

Small Icon Test Mode View

Review Mode

Review Mode offers additional analysis options. In review mode, the user can draw graphs of multiple effort graphs, zoom graphs, show graph labels, etc. After opening an existing visit, BreezeSuite automatically operates in Review Mode. To switch from Test Mode to Review Mode, click the Review Mode icon.

Review Mode Icon

TIP: When clicking a time stamp of an effort to draw it, BreezeSuite switches to Review Mode. Click the Test Mode icon to re-enter Test Mode.

3.0 PERFORMING SPIROMETRY

3.1. SPIROMETRY (FVC TEST) REQUIREMENTS

The following are requirements for the FVC test procedure:

1. Full inspiration, smooth and continuous exhalation until complete.

2. An abrupt and unhesitating start (the patient cannot cough during the first second of the test).

3. Six second expiratory time or flow plateau.

4. A minimum of three acceptable efforts. No more than 8 attempts.

5. The two largest FVCs must be within 150 ml

6. The two largest FEV_1s must be within 150 ml

Caution: The opening at the end of the flow sensor must not be obstructed. Make sure that the participant does not block it with his/her fingers. This will result in volume and flow that are erroneously high.

3.2. PERFORMING SPIROMETRY (FVC TESTING)

Avoidance of Drugs and Substances in Preparation for Spirometry Testing

Certain medications and substances interfere with spirometry testing. To make sure the participant is properly prepared for the test, the coordinator should inform the participant of any medications and substances that should be avoided prior to spirometry as well as the specified time interval of holding them. Refer to study specific MOP and study specific Pulmonary Procedure Checklist for medications and substances to avoid. Spirometry should be delayed or rescheduled, if possible, for the appropriate time period if the study participant used a restricted medication or substance too close in time to the test.

Before starting spirometry, make sure that the participant is comfortable (feet on the floor) and fully understands the maneuvers necessary to complete the procedure. Explain and demonstrate the breathing techniques and provide continuous, enthusiastic feedback during the test, especially near maximum inspiration and expiration levels.

1. Click the FVC tab to initiate a Forced Vital Capacity (FVC) Test.

2. Click Zero Flow to zero the flow sensor and ensure that there is no airflow through the flow sensor. This only needs to be done prior to a spirometry session, not before each maneuver.

3. Click Start or press the spacebar to begin the test. Instruct the participant to take normal, relaxed breaths.

4. Instruct the participant to inhale fully, then exhale as rapidly, forcefully, and completely as possible (try to have the participant exhale until the yellow indicator box turns green, indicating end of test criteria), then inhale fully.

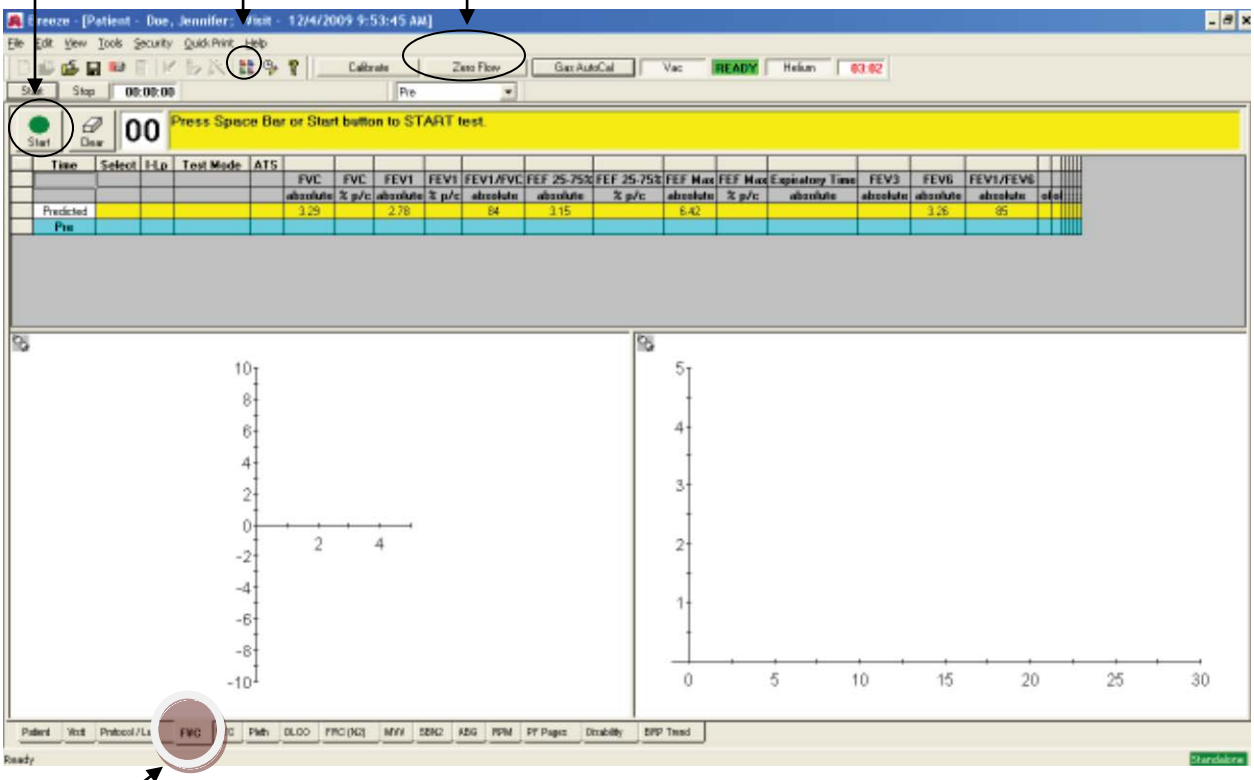
5. Click Stop or press the space bar to stop the test. After stopping the test, the completed test results appear in the data table. The BreezeSuite software automatically selects all efforts that meet American Thoracic Society (ATS) criteria.

6. To manually select or deselect efforts, click in the Select column to add or remove the red check mark. Data in the gray row display the values used in the final report.

Start/Stop Button

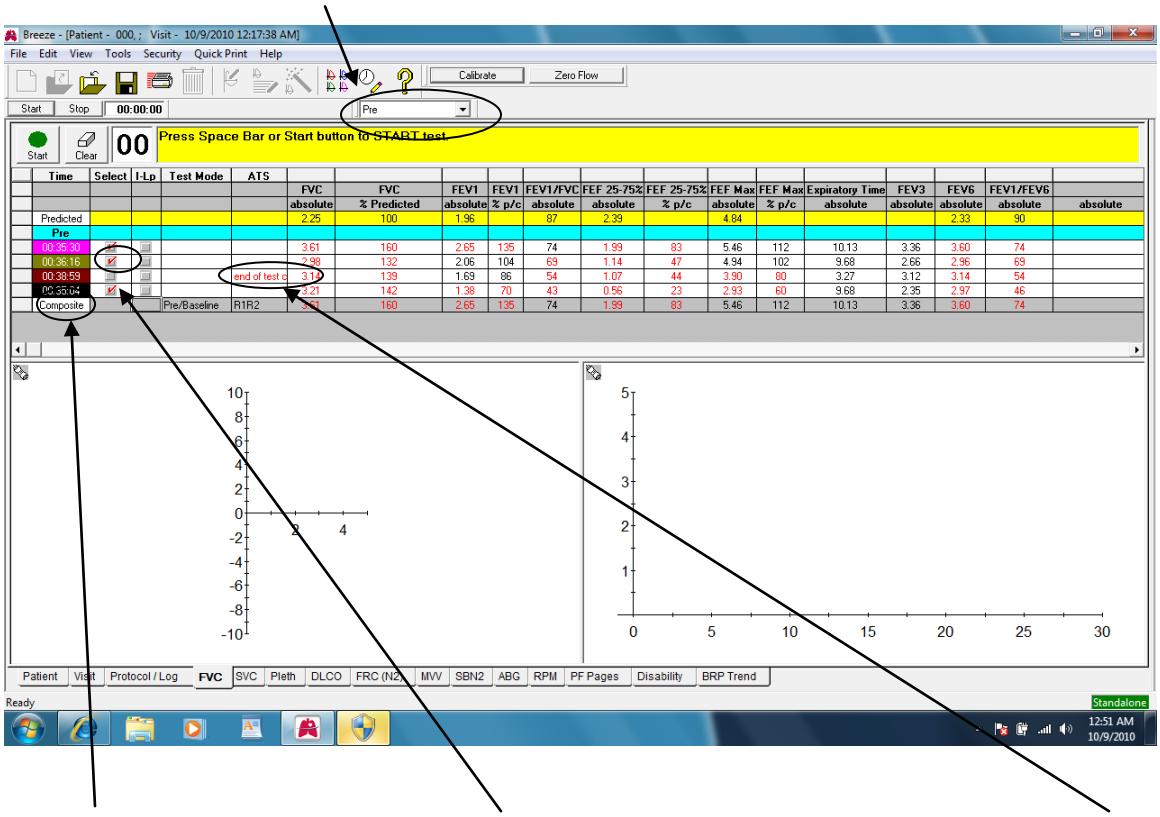
Review Mode

Zero Flow Button



Some protocols will require spirometry before and after a bronchodilator. If that is the case, you will need to change to the Post stage when doing post-bronchodilator spirometry.

To Change Stages (Pre & Post) [on your screen, this button might actually be located more to the left, above the green start button]



Composite
(Row Displays Best Values)

To Select/Deselect Maneuvers

ATS Errors

Note: Composite tab will change to ATS when standards are met.

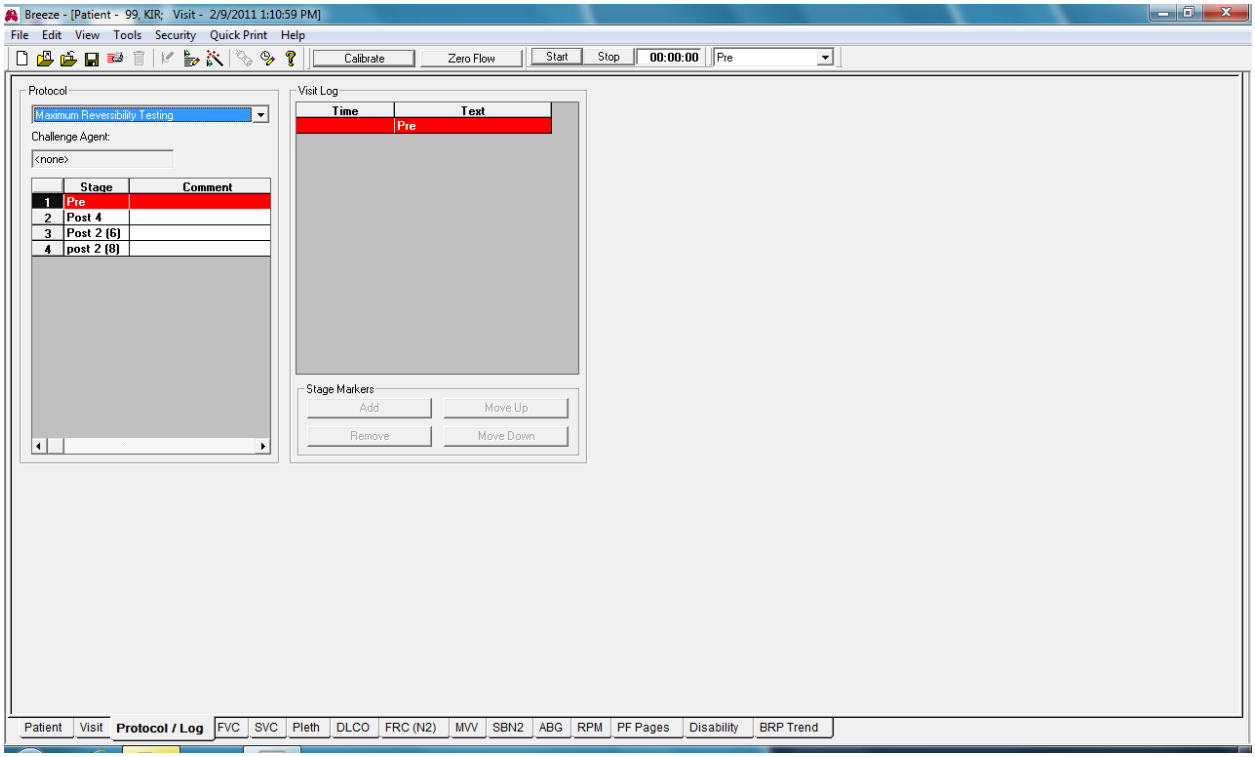
3.3. MAXIMUM REVERSIBILITY TESTING

The purpose of the Maximum Reversibility Testing is to document the maximum bronchodilation that can be achieved from use of a short-acting beta agonist.

First enter the participant's demographics.

Click on the "Protocol/Log" tab.

In the Protocol section, choose the "Maximum Reversibility Testing" from the drop-down menu.



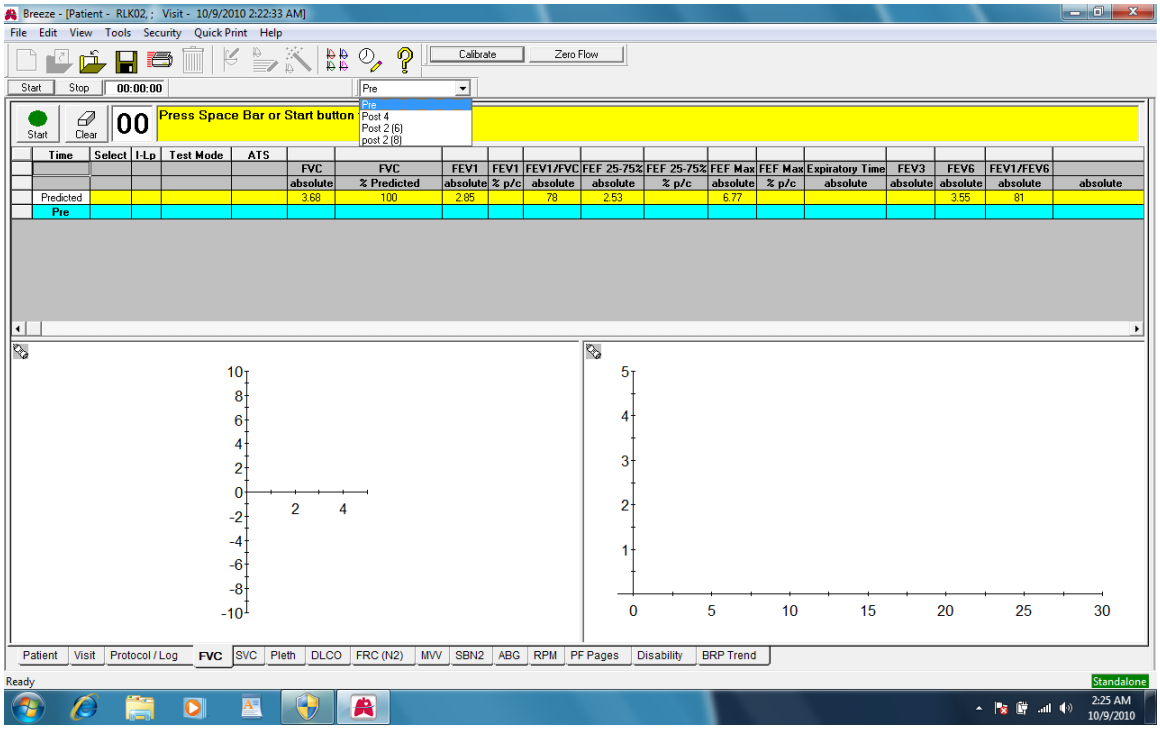
The message "Changing the protocol will remove all stage markers from the visit log. Proceed anyway?" will appear. Click "OK".

Click on the "FVC" tab.

Click on "Zero Flow". Then perform spirometry according to the AsthmaNet (ATS/ERS) guidelines. Perform at least three (3) and not more than eight (8) acceptable maneuvers with FVC and FEV_1 reproducible volumes.

Give four (4) inhalations of a bronchodilator. Wait 10-15 minutes before repeating spirometry.

To perform spirometry post four (4) inhalations of bronchodilator, click on the drop-down menu in the middle of top of the screen that displays “Pre”. From the drop-down menu, choose “Post 4”. Perform spirometry.



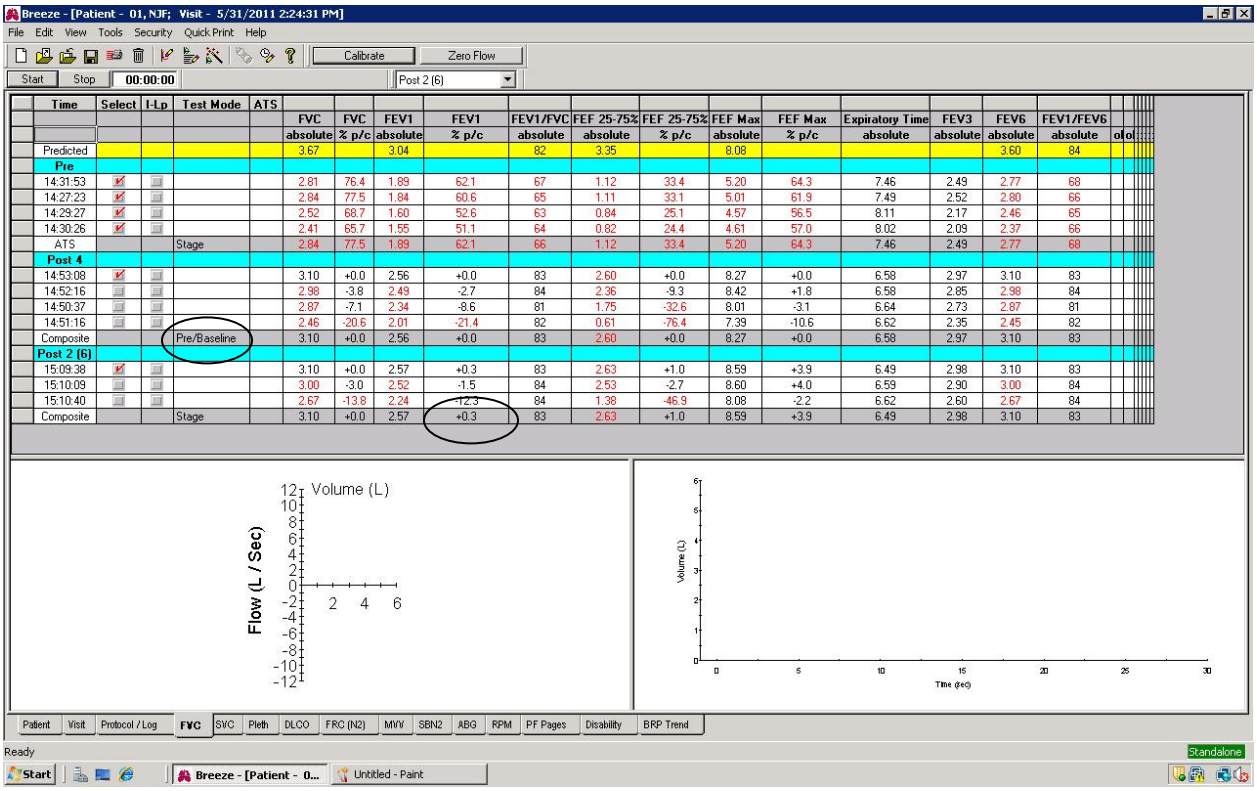
Select the maneuver with the largest FEV_1 (from all acceptable maneuvers) by clicking on the box in the “Select” column that corresponds with that maneuver. If the session meets AsthmaNet/ATS criteria, including reproducibility, then the procedure should continue to the next stage with two (2) additional puffs of bronchodilator. If the session does not meet ATS criteria, then the procedure should stop and the largest FEV_1 from the acceptable maneuvers should be reported on the Maximum Reversibility Testing (MAXREV) form. A comment indicating why the procedure was stopped prematurely should be included on the MAXREV form.

If the post four (4) session was completed successfully, then give two (2) more inhalations of the bronchodilator. Wait 10 -15 minutes. From the drop-down menu that currently displays “Post 4”, now select “Post 2 (6)”.

Perform spirometry. This session must also meet AsthmaNet/ATS criteria.

Select the maneuver with the largest FEV_1 volume (from all acceptable maneuvers) by clicking on the box in the “Select” column that corresponds with that maneuver.

PROCEDURE TO DETERMINE THE NEED TO GIVE THE POST 2 (8) DOSE.



Time	Select	I-Lp	Test Mode	ATS	FVC absolute	FVC % p/c	FEV1 absolute	FEV1 % p/c	FEV1/FVC absolute	FEF 25-75% absolute	FEF 25-75% % p/c	FEF Max absolute	FEF Max % p/c	Expiratory Time absolute	FEV3 absolute	FEV6 absolute	FEV1/FEV6 absolute
Predicted					3.67		3.04		82	3.35		8.08				3.60	84
Pre																	
14:31:53	☑				2.81	76.4	1.89	62.1	67	1.12	33.4	5.20	64.3	7.46	2.49	2.77	68
14:27:23	☑				2.84	77.5	1.84	60.6	65	1.11	33.1	5.01	61.9	7.49	2.52	2.80	66
14:29:27	☑				2.52	68.7	1.60	52.6	63	0.84	25.1	4.57	56.5	8.11	2.17	2.46	65
14:30:26	☑				2.41	65.7	1.55	51.1	64	0.82	24.4	4.61	57.0	8.02	2.09	2.37	66
ATS			Stage		2.84	77.5	1.89	62.1	66	1.12	33.4	5.20	64.3	7.46	2.49	2.77	68
Post 4																	
14:53:08	☑				3.10	+0.0	2.56	+0.0	83	2.60	+0.0	8.27	+0.0	6.58	2.97	3.10	83
14:52:16					2.98	-3.8	2.49	-2.7	84	2.36	-9.3	8.42	+1.8	6.58	2.85	2.98	84
14:50:37					2.87	-7.1	2.34	-8.6	81	1.75	-32.6	8.01	-3.1	6.64	2.73	2.87	81
14:51:16					2.46	-20.6	2.01	-21.4	82	0.61	-76.4	7.39	-10.6	6.62	2.35	2.45	82
Composite			Pre/Baseline		3.10	+0.0	2.56	+0.0	83	2.60	+0.0	8.27	+0.0	6.58	2.97	3.10	83
Post 2 (6)																	
15:09:38	☑				3.10	+0.0	2.57	+0.3	83	2.63	+1.0	8.59	+3.9	6.49	2.98	3.10	83
15:10:09					3.00	-3.0	2.52	-1.5	84	2.53	-2.7	8.60	+4.0	6.59	2.90	3.00	84
15:10:40					2.67	-13.8	2.24	-12.3	84	1.38	-46.9	8.08	-2.2	6.62	2.60	2.67	84
Composite			Stage		3.10	+0.0	2.57	+0.3	83	2.63	+1.0	8.59	+3.9	6.49	2.98	3.10	83

Mark the Post 4 stage as “Pre/Baseline” in the Test Mode column. Now, check the value in the “FEV1 % p/c” column for the Post 2 (6) stage in the Composite row.

If the value in the “FEV1 % p/c” column is +5.0 or less, stop the procedure. Click on “Quick Print”. Click on “Report Switchboard”. Click on “Maximum Reversibility Testing”. Click “Print”.

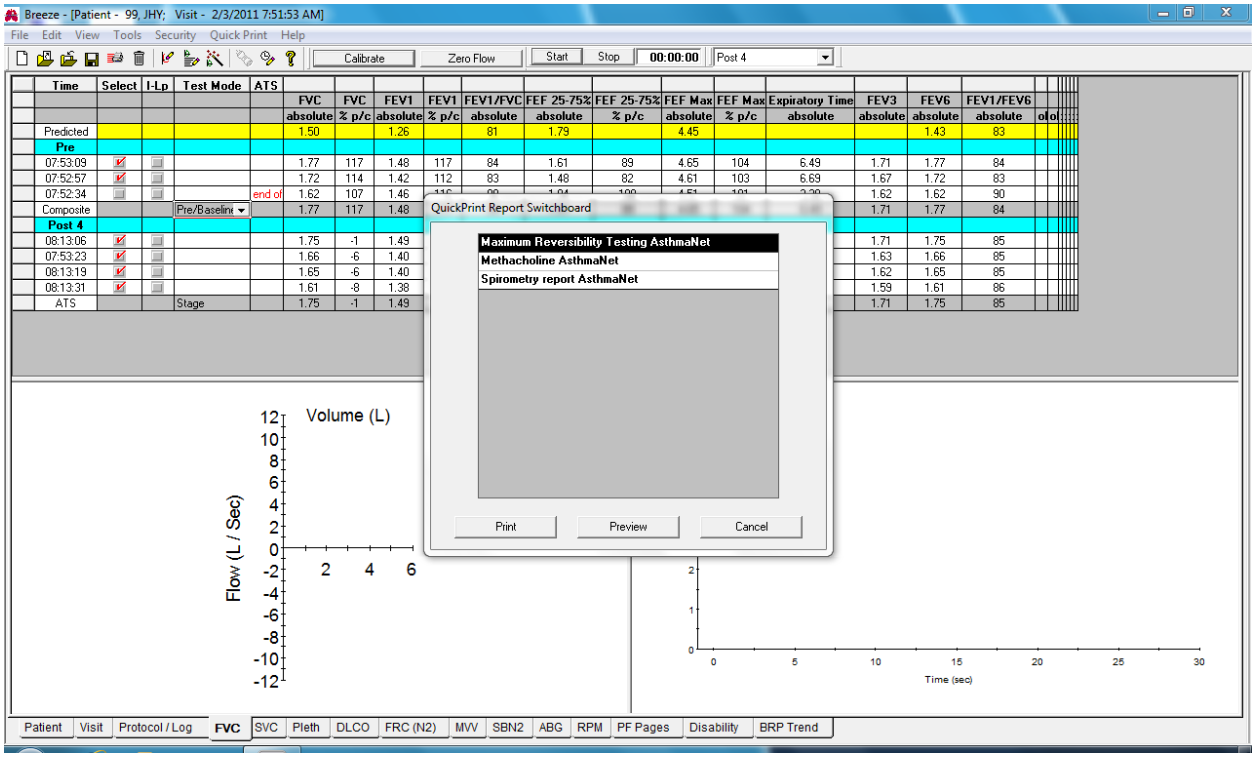
If the “FEV1% p/c” value is more than +5.0 and the session meets AsthmaNet/ATS criteria, give the next two (2) inhalations of bronchodilator. Wait 10 -15 minutes.

From the drop-down menu that currently displays “Post 2 (6)”, now choose “Post 2 (8)”.

Perform spirometry.

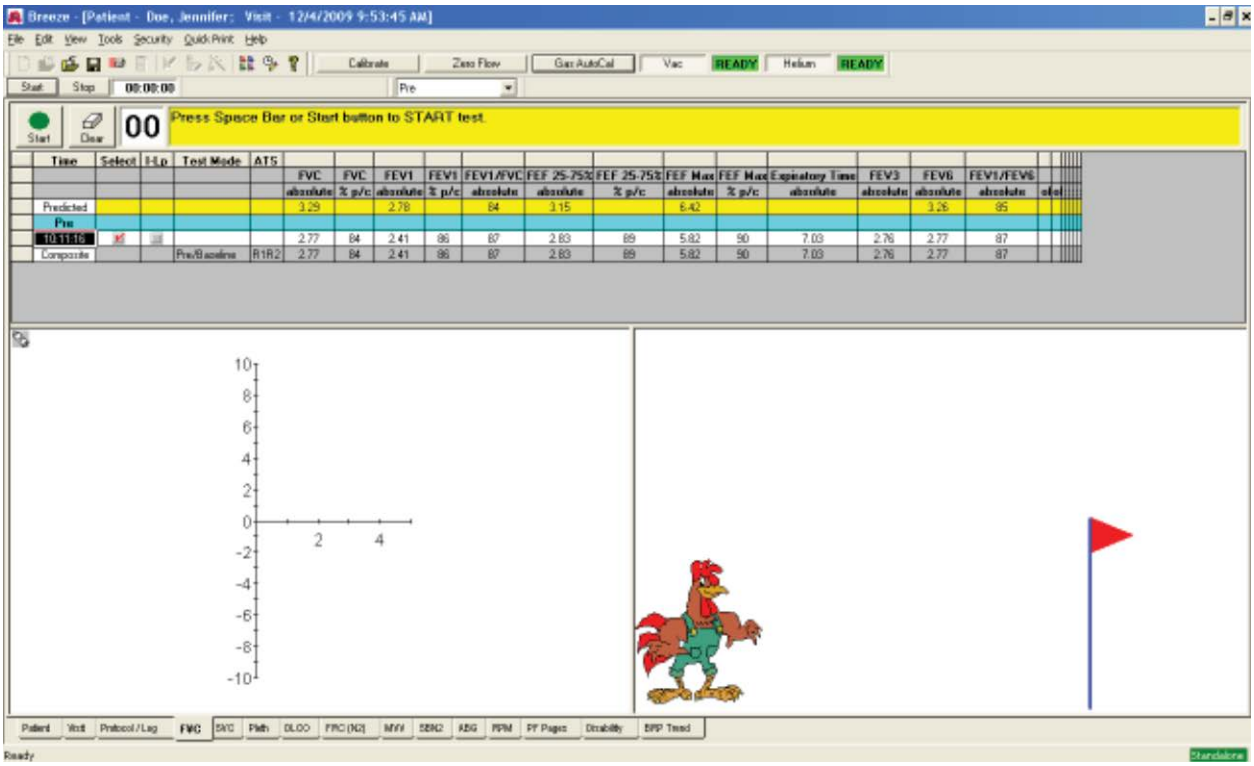
Choose the maneuver with the largest FEV_1 volume (from all acceptable maneuvers) by clicking on the box in the “Select” column that corresponds with that maneuver.

Click on “Quick Print”. Click on “Report Switchboard”. Click on “Maximum Reversibility Testing”. Click “Print”.



Always include any notes that may help the overreader’s evaluation.

3.4. PATIENT INCENTIVES



1. To display participant incentive animations, right-click the FVC graph and select Properties.

2. Under Graph Type, choose from four incentive types:

    - Clown
    - Rooster
    - Balloon
    - Birthday Cake

3. In this example, as the participant inhales, the rooster runs toward the flag until the patient reaches Vital Capacity (VC).

4. REPORTS

4.1. ACCESSING THE REPORT SWITCHBOARD

Quick Print brings together the features of a default report switchboard and a report design function into a single application.

To access the Report Switchboard from the toolbar:

1. Select Quick Print > Report Switchboard; the Report Switchboard contains a selection of final reports.

4.2. PRINTING A REPORT

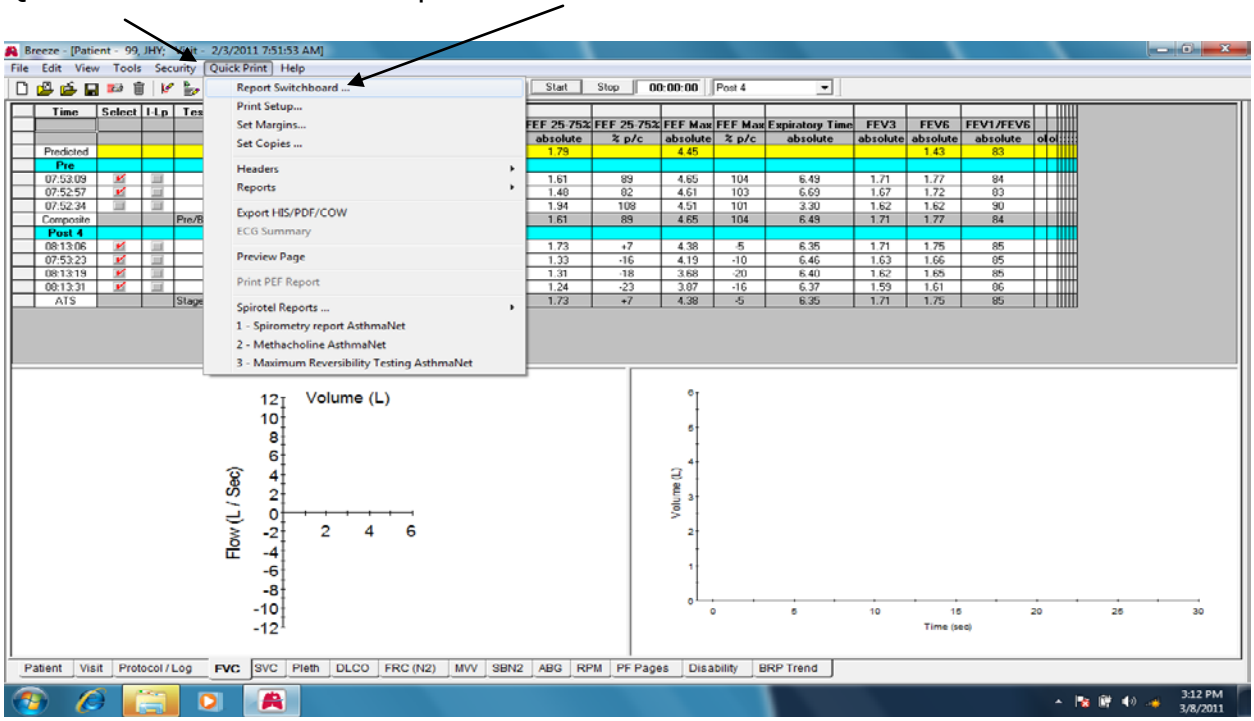
To print a report:

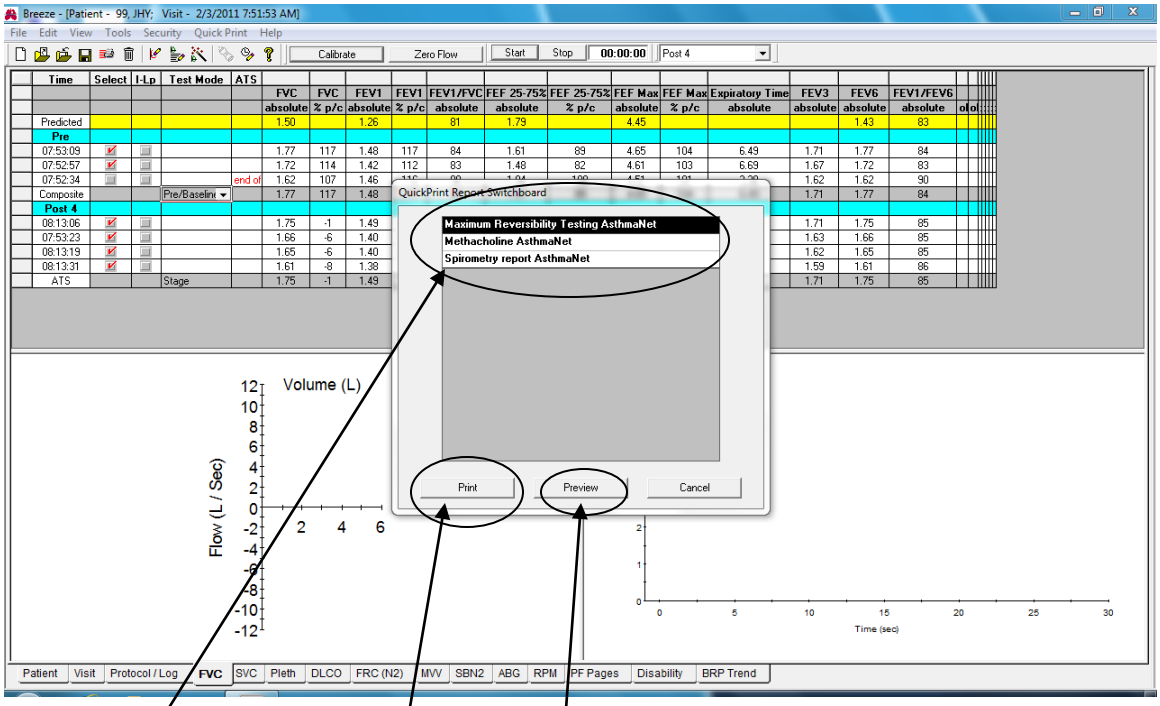
1. Select Quick Print > Report Switchboard.

2. Choose the report to be printed and select Print.

3. Post-test comments may be added to a printed report by typing the comments into the Post Test Comments dialog box.

Quick Print Report Switchboard





5. QUALITY CONTROL

5.1. INTRODUCTION

Quality Control (QC) is the periodic testing of a system to verify that it meets MEDGRAPHICS specifications. It is recommended that Biologic QC be performed monthly following a complete calibration, to ensure optimal performance of the system. To perform a Biological QC session, please select someone in the lab area who does not smoke and does not have asthma. Enter “Bio QC” in the “Last Name” field. In the First Name field, enter the device serial number that can be found on the bottom of the CPFS/D module. In the “ID:” field, enter 99 plus the last four digits of the subject’s social security number. The remaining fields should be completed based on the Bio QC subject’s information. Leave the “Second ID:” field (visit number) blank. Perform a complete spirometry session on this Biological QC subject. Please keep these reports in a separate binder.

Ideally, each lab has two Bio QC subjects each month. So, if one of the Bio QC subject’s is ill or leaves the area, the site will still have a back up subject. Please notify

the overreader if the Bio QC subject changes for any reason. The overreader will notify the lab and the DCC if there is any abnormal drift in the Bio QC results.
The QC technician should be familiar with all procedures to ensure that no technique errors are made. All QC testing data should be saved in the BreezeSuite database, and a log book should be kept, to include the following:

1. Date of any service (Phone or Onsite)
2. Date of biologic QC
3. Date of annual syringe calibration

The Biologic Quality Control is a procedure that uses a healthy, non-asthmatic, non-smoker practice subject. Once a month, this practice subject needs to perform a complete spirometry session, meeting all ATS/ERS guidelines. This Bio QC subject should be someone who will be available each month. It is advised that each site have two (2) Bio QC subjects to ensure that at least one will be available every month.

These tests should be downloaded at the end of the day. The overreader will document the results (FVC & FEV_1). After the first 10 Bio QC sessions, the SD and CV will be monitored. If a device's results are outside of acceptable ranges, the site will be notified.

6.0 CLEANING, MAINTENANCE AND TROUBLESHOOTING

6.1. PREVENT FLOW SENSOR

The sites will be supplied with numerous preVent Flow Sensors.

The flow sensors are made of polystyrene, which can be damaged by heat. Prior to use, always inspect the flow sensor for cracks, chips, and/or loose screen. If any of these conditions exists, replace with a new flow sensor.

When using a Barrier Filter, the flow sensor may be reused between participants. Important: After each use, a flow sensor must be cleaned and disinfected before it is reused.

Under no circumstances may heat > 120°F/49°C be used to disinfect or to dry the flow sensor.

The following flow sensor disinfecting technique is suggested before reuse of the flow sensor; however it is also permissible to use any cleaning technique required by your institution:

1. Wash flow sensor using a cleaning brush to loosen and remove any foreign matter.

2. To disinfect the flow sensor, soak the flow sensor in a solution of 10% household bleach and water for 60 minutes, or soak in a 2.0% gluteraldehyde solution for 45 minutes, or use the sterrad sterilization (hydrogen peroxide) system. Excessive soaking will degrade the flow sensor material and reduce accuracy.

3. Rinse thoroughly in cool water

4. To ensure test accuracy, no water may remain in the flow sensor before using. Air dry for a minimum of 24 hours. Fan drying at room temperature or low-pressure air through the holes in bottom of flow sensor may be used to hasten drying.

6.2. FLOW DRIFT AND ZERO FLOW ERRORS

The following components are involved in the flow system:

- preVent Flow Sensor
- Umbilical Cable
- CPFS/D USB PC Board
- Pressure Sensors
- Zero Flow Solenoids

If flow successfully zeros but flow drifts in either calibration or testing, complete the following procedure:

1. Click the SVC (Slow Vital Capacity) tab and click zero flow.

2. With umbilical and flow sensor stabilized, start a SVC test.
    a. If the flow drift occurs, stop test and remove the flow sensor from the umbilical cable.

3. Re-Zero Flow and start a SVC test.
    a. If flow drift occurs, call Technical Support.
    b. If no drift occurs, replace the flow sensor and attempt calibration.

4. Disconnect umbilical cable and re-zero system. Start a SVC test.
    a. If flow drift occurs, call Technical Support.
    b. If no drift occurs, proceed to step 5 (five).

5. Use a low-pressure gas line to flush the flow lines. Hold the gas line to the end of the disconnected flow line (not the umbilical clip) forcing any moisture out. Flush each line for 45 seconds. Reconnect umbilical to the CPFS/D USB and zero the flow. Start a SVC test.
    a. If flow drift occurs, call Technical Support.
    b. If flow drift does not occur, continue with calibration and testing.

If the CPFS/D USB is unable to successfully zero, exit BreezeSuite and unplug the CPFS/D USB from the computer. Leave disconnected for 1 minute. Restart the testing computer. Reconnect the CPFS/D USB cable and attempt to zero flow. If successful, continue calibration and testing. If unsuccessful, contact Technical Support.

7.0 CERTIFICATION

In order to become certified as an AsthmaNet pulmonary function technician, it is necessary to complete the following list of requirements:

1. Candidates shall view the MedGraphics training modules and successfully complete the ten (10) Self Checks.

2. Candidates shall perform five (5) spirometry test sessions and have them reviewed and approved by the overreader.

    The test subject should be spirometry naïve (ideally) and should not be asthmatic (preferably). Coordinators/technicians who will perform spirometry on participants in the 5-11 age range must perform three (3) of five (5) spirometry test sessions on their site's recruitment target population (i.e. 5-11 years of age). Otherwise, there is no age limit for the person being tested. Pediatric spirometry certification status will be tracked separately from adolescent/adult spirometry certification. Coordinators/technicians who possess adolescent/adult certification who now will be processing visits for children ages 5-11 years of age must complete three acceptable test sessions on children in this age range in order to secure pediatric certification. Please enter the ID # 99 for subjects used for certification.

During the course of the trials, spirometry sessions will be reviewed as they are downloaded to the overreader. Based on these test sessions, each technician will receive a separate grade for the performance and reproducibility of the FEV1 and FVC. In order for a technician to pass certification, the overreading scores must be perfect

(technician test evaluation = 4, best test = 5, and repeatability for FVC/FEV1 should = 2). If these quality grades are not satisfactory for two or more consecutive months, then the coordinator will need to repeat the certification process.

7.1 RECERTIFICATION

If a coordinator goes for more than one year without performing spirometry using the AsthmaNet MedGraphics spirometer, that coordinator will need to pass the certification once again. The five (5) spirometry sessions do not need to be performed using AsthmaNet subjects. If a coordinator is getting close to the one year absence, they should do one (1) spirometry session on a test subject to maintain their certification. Remember, any subject used for certification purposes needs to have an ID# that begins with 99.

8.0 OVERREADING

8.1. INTRODUCTION

When downloading participant files, copies are created, but the files are not deleted from the database. The files should be downloaded on a daily basis.

The overreader will grade the sessions that were downloaded, and send emails back to the sites with the scores of each session and comments if needed.

The sites will also receive monthly total scores for all of the sessions that were downloaded since the last report.

8.2. OVERREADING SPIROMETRY SCORE

Each spirometry session will get three (3) scores.

The first score will be the **Technician Test Evaluation**.
4 = Three (3) acceptable maneuvers
3 = Less than three (3) acceptable maneuvers with comment
2 = Less than three (3) acceptable maneuvers w/o comment
1 = Unacceptable maneuvers with comment
0 = Unacceptable maneuvers w/o comment

The second score will be the **Best Test Evaluation**
1= No delayed peak

1= High/Clearly determined peak
1= Acceptable back extrapolation
1= Free of cough within the first second
1= Appropriate end of test

The third score will be for **Repeatability**
(150ml for those >7 years old, and 10% for those 7 years old and younger)
1 = repeatable FVC
1 = repeatable FEV1

Each **Maximum Reversibility Testing Procedure** will receive three (3) overreading evaluations.

1= Three (3) acceptable baseline maneuvers performed (repeatable FVC and FEV1)
1= Procedure terminated at the appropriate stage
1= Results of the procedure acceptable

Best Test Review
If the overreader chooses another maneuver as best, the overreader will edit the data and send the information back to the site and to the DCC. The site will then need to delete what was first chosen as best so the new edited maneuver will be displayed.

8.3. PROCEDURE FOR UPLOADING AND DOWNLOADING PATIENT FILES

1. Click on DB Tools and then click “OK”.

2. Click on “Tools”, “Export”, “Patients”.

3. To list visits by date, click on the “Visit Date” tab.

4. Click on the browser box at the end of Directory. Highlight and open the “C” drive. Choose the AsthmaNet Folder and then click “OK”:

5. Click on the visits to be downloaded. Hold “shift” to select more than one visit.

6. Click on “Export”. An information screen will appear indicating “Export visits Completed”.

7. Click on “Done” and close DB Tools by clicking the red “X” in the upper right-hand corner. The files to be downloaded have now been uploaded into the AsthmaNet folder and are prepared to be downloaded.

8. Go to www.filestogo.com

9. Enter User ID and Password, click “login”
10. Click on the “Upload” tab.
11. Click on either “Upload A File Encrypted”, or “Upload Multiple Files Encrypted”.
12. Open the AsthmaNet folder under Desktop, and drag the visits to be downloaded to the Upload box on the right-hand side.
13. Click on “Start Upload”. A green check will appear as each visit is downloaded.
14. Click “Back”.
15. Close Explorer.
16. Delete files in the AsthmaNet folder on the Desktop.

9.0 PREDICTED VALUES

9.1. ADJUSTING FOR RACE AND ETHNICITY

The participant’s spirometry race/ethnicity (as appears on his/her AsthmaNet Registry report) should be entered into the MedGraphics system in the Race section. The pulmonary function technician or coordinator should refer to the participant’s Registry report to confirm this designation, or he/she may search the AsthmaNet Registry to retrieve this information.

The spirometry race corresponds to the participant’s self-reported primary racial identification given in Question #9 on the AsthmaNet Registry (REGISTRY) form. Individuals who report primary race category 1 (American Indian or Alaskan Native) or 6 (Other) will use Caucasian predicted value equations.

Based on the participant’s spirometry race/ethnicity designation, the following predicted lung function equations will be used.

Table 2: Predicted Lung Function Equations

Participant's Spirometry Race/Ethnicity Designation	Age 5	Age 6-12[1]	13 or older
Black	Female: Eigen[2]-Caucasian x .85 Male: Eigen-Caucasian x .91	Dockery[3]-African American	Hankinson[4]-African American
Caucasian	Eigen-Caucasian	Dockery-Caucasian	Hankinson-Caucasian
Asian	Eigen-Caucasian x .88	Dockery-Caucasian x .88	Hankinson-Caucasian x .88
Hispanic	Eigen-Caucasian	Hsu[5]-Hispanic	Hankinson-Mexican American

10.0 PROCEDURE FOR 3 LITER SYRINGE RECALIBRATION

Please note that there are stickers attached to the underneath of the calibration syringe. One sticker has the manufacturer's information: A-M Systems with the company's address. The second sticker has the calibration date printed on it and the recalibration due date. The third sticker contains the serial number, product number and manufacture date.

When the syringes become due for calibration you will contact A&M Systems for an RMA number and at this time you will provide the serial number of your particular calibration syringe that will be sent. The calibration fee is $55. You will also need to purchase an additional syringe through A&M's special program for $99, so you will always have a backup unit (regular price is $252). A&M will ship you the new syringe and once you receive it, you will box up the syringe that is due for servicing and send it into A&M.

Please indicate that you are part of the AsthmaNet study.

[1] Hankinson equations will be used for PEFR predicted values for all races in this age range.
[2] Eigen, Bieler, Grant, Christoph, Terrill, Heilman, Ambrosius, Tepper. Spirometric pulmonary function in healthy preschool children. Am J Respir Crit Care Med 2001;163:619-23.
[3] Wang, Dockery, Wypij, Fay, Ferris. Pulmonary function between 6 and 18 years of age. Pediatr Pulmonol. 1993;15:75-88.
[4] Hankinson, Odencrantz, Fedan. Spirometric reference values from a sample of the general US population. Am J Respir Crit Care Med 1999; 159:179-87.
[5] Hsu, Jenkins, Hsi, Bourhofer, Thompson, Tanakowa, Hsieh. Ventilatory functions of normal children and young adults – Mexican-American, white, and black. I. Spirometry. Pediatrics. 1979; 95(1):14-23.

Contact Person:

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Shipping Address (For Packages)

A&M Systems
131 Business Park Loop
Sequim, WA 98382
U.S.A.

11.0 CONTACTS

Procedural Questions:

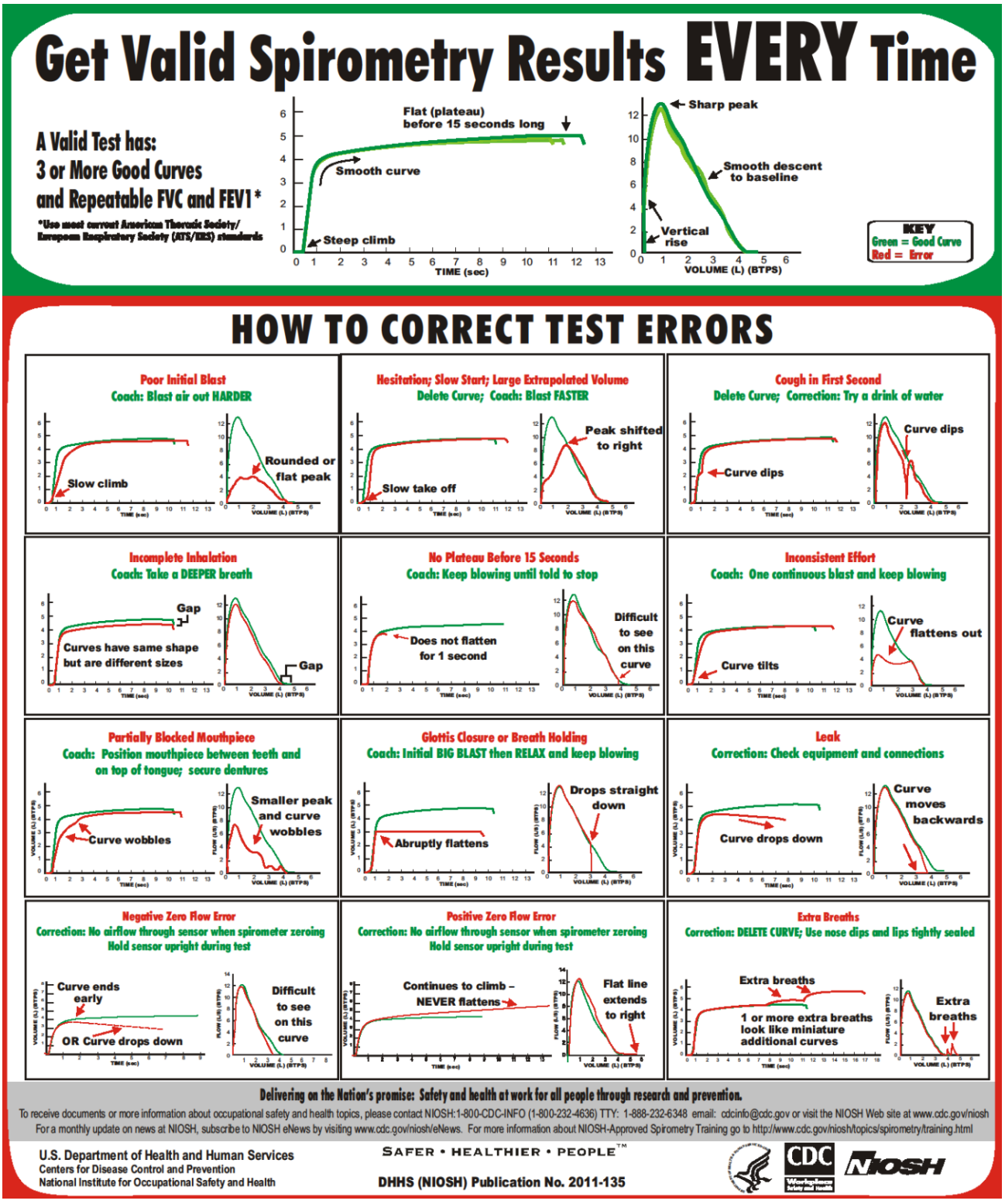
Rick Kelley
rlk@medicine.wisc.edu
1-215-594-1891 (cell)
1-715-282-7972 (landline)

Software and Hardware Questions:

Medical Graphics Tech Support
Primary: 1-800-333-4137
Secondary: Lisa Knepper, 1-800-950-5597, ext 1308

Sites are responsible for any additional supplies. Contact MedGraphics sales and service department (800-950-5597) for consumable orders and be sure to state you are participating in the AsthmaNet study. You will need a purchase order or credit card number when ordering supplies.

APPENDIX A: NIOSH POSTER



APPENDIX B: MAKING CORRECTIONS TO TEST DATA

The following steps should be implemented to make corrections to a spirometry or methacholine challenge test that has been uploaded to the MedGraphics database.

Note: Only the overreader will be authorized to make changes to the test data (in terms of the performance of the test) once it is in the MedGraphics database. DCC personnel may make changes only to identification (technician ID, participant ID) and demographic (date of birth, sex, race, etc.) data, as needed.

I. Overreader-initiated Corrections

Overreader Responsibilities (Rick Kelley)

1. Overread test and identify problem. Examples are: selection of unacceptable or otherwise incorrect maneuvers, selection of multiple maneuvers when only the best should have been selected (in the case of post-bronchodilator stages of maximum reversal test), incorrect reference FEV_1 used during methacholine challenge, etc.

2. Correct the test in the MedGraphics database. Include a comment in the database describing the problem and detailing what was changed.

3. Generate and send the final spirometry/methacholine challenge report from the MedGraphics database to the technician whose 4-digit ID is associated with the test. Carbon copy the protocol's data management team at the DCC using the established alias. For protocol number 01 (VIDA), the alias is AsthmaNet_VIDA_DM. When multiple studies are running concurrently, the alias for the protocol corresponding to the protocol number entered into the MedGraphics system for the test in question should be used.

   Steps for attaching a report in pdf format from the MedGraphics system to an e-mail message follow:
   a. Go to "Quick Print"
   b. Click on "Report Switchboard"
   c. Choose Report
   d. Click on "Print"
   e. Enter Comments (Note all changes made by the overreader)
   f. Click "OK"
   g. Save the XPS File to the desktop
   h. Copy file and save to overreader's desktop
   i. Click "Print"
   j. Click "Medgraphics PDF"
   k. Click "Print"

l. Enter file name as participant ID# <space> Date of test (exp. 1151099 05252011)
m. Save to desktop
n. Attach to e-mail message sent to the coordinator/tech and the DCC data management team

Coordinator/Technician Responsibilities (at performance site)

1. Review corrections made by overreader. To do this:
    a. Open and review the revised spirometry or methacholine challenge report and any comments made in the corresponding e-mail message.
    b. Contact the overreader if the reason for the change is unclear.
2. Print the corrected report and e-mail message.
3. Replace the original report in the participant's study folder with the corrected report and a copy of the e-mail message; discard the original report.
4. If the corresponding visit packet has not yet been submitted to the DCC:
    a. Review the data reported on spirometry (SPIRO, PALB2_SPIRO, PALB4_SPIRO, MAXREV, etc.) and methacholine challenge (METHA, METHA_ADD_TRT) forms for any corrections resulting from the change to the test data.
    b. Review protocol-specific eligibility checklists for changes resulting from the test data change, as applicable.
    c. Review procedure eligibility checklists for changes resulting from the test data change, as applicable. Examples: METHACHK_ADULT, METHACHK_PED, SKIN_TEST, SPUTUMCHK.
    d. Make necessary corrections on the data forms and in the AsthmaNet database before submitting data packets to the DCC.

Data Management Responsibilities (at DCC)

1. Check the status of the data packet containing the report that required corrections.
    a. If the packet has been received at the DCC (likely), attach a barcode label to the new report, discard the old report, and replace it with the new report. If the old report has already been scanned for electronic archival, submit a request for the old report image to be deleted and place the new report in the scanning folder.
    b. If the packet has not yet been received at the DCC, print a copy of the corrected report and place on hold in a folder. When the packet arrives, verify that the correct report has been submitted.
2. Once the correct report has been verified in the packet, check the data reported on the related case report forms for accuracy. Affected forms may include one or more of the following: SPIRO, MAXREV, PALB4_SPIRO, PALB2_SPIRO,

METHA, METHA_ADD_TRT, METHACHK_ADULT, METHACHK_PED, SPUTUMCHK, SPUTUM, SPUTUM_ADD_TRT, SKIN_TEST.

3. Document and send any necessary data corrections.
4. File MedGraphics report correction e-mails in the protocol-specific public site correspondence folders.

II. Coordinator/tech-initiated Corrections

If a test has been uploaded to the MedGraphics database and the technician or coordinator later discovers a problem with it, such as incorrect participant ID, visit number or demographics, the following procedures should be followed:

1. Correct the test on the local machine. DO NOT re-upload the test to MedGraphics. The corrected test will be flagged by MedGraphics as a duplicate (even if data are changed) and will not be incorporated in the AsthmaNet data files sent to the DCC.
2. Generate and print updated spirometry and/or methacholine challenge reports. Discard any previously-printed reports that contain incorrect information.
3. Update any data collection form data that changed due to the correction. Initial and date the changes. Submit the corrected forms to the DCC, or submit data corrections, as applicable.
4. E-mail the protocol's data management alias (e.g., AsthmaNet_VIDA_DM) details of the corrections that need to be made to the master MedGraphics database. The e-mail must include: participant ID, visit number, test type(s), and required change(s).
5. Print the e-mail. Include a copy in the participant's visit folder and submit a copy to the DCC with the visit packet (if it has not been shipped to the DCC).
6. If the visit packet has not yet been shipped to the DCC, note the changes required to the master database on the visit packet's cover sheet.

APPENDIX C: BUSINESS CREDIT INFORMATION

Business Credit information

Legal Business name: EIN#

Billing Address:

City: State: County: Zip:

Are purchase orders required: Years in business:

Will Purchase be tax exempt:

(If yes, please return a copy of your tax exemption certificate along with this form)

Purchasing agent: Phone:

Accounts Payable: Phone:

SPUTUM

MANUAL OF OPERATIONS

7. AsthmaNet Sputum

7.1 Purpose

Sputum induction is a relatively simple, repeatable and noninvasive method of collecting airway secretions. Cellular and biochemical analyses of induced sputum samples collected from asthmatic and non-asthmatic participants have revealed differences in markers of eosinophilic inflammation and bronchovascular permeability in an asthmatic population. Similarly, sputum induced samples have revealed the expected rise (following an antigen challenge) and fall (following a prednisone treatment) of markers of eosinophilic inflammation. Like any bronchoprovocation challenge, sputum induction can provoke bronchospasm and warrants close supervision during its performance.

Any deviation(s) from this manual will be study specific and described in the study protocol and sputum section of study specific manual of operation (MOP). Therefore, before inducing/processing/shipping study specific samples, it is important that everyone is familiar with the study to ensure there are no study specific requirements.

7.2 Set-Up

7.2.1 NOUVAG Ultrasonic Nebulizer

1. Fill the nebulizer chamber with distilled water to the fill line.

2. Properly seat the nebulizer chamber into the NOUVAG Ultrasonic Nebulizer.

3. Fill the medication cup with 40 mL 3% saline.

4. Attach the chamber lid to the medication cup and properly seat into the nebulizer chamber. If not using the adjustable arm with ring attachment, you will need to secure the cup to the chamber with a binder clip.

5. Connect the air output port of the NOUVAG Ultrasonic Nebulizer to the chamber lid with the aerosol hose. Cut a length of aerosol tubing appropriate for the height of the seated participant. With the NOUVAG Ultrasonic Nebulizer located on the lower shelf of the Nuaire Aerosol Containment Chamber we have found that a 6 or 7 cuff segment of aerosol tubing generally does the job. Connect one end to the chamber lid for the medication cup, using a plastic elbow, and feed the other

free end in through the rubber diaphragm on the side panel of the Nuaire Aerosol Containment Chamber.

6. Attach the plastic mouthpiece to the center port of the T adapter and a single cuff segment of aerosol tubing to one of the side ports.

7. Attach the 6 or 7 cuff segment of aerosol tubing to the remaining T adapter side port. Orient the T adapter so that the mouthpiece points upward to the participant's mouth and the single cuff segment of aerosol tubing points up and into the Nuaire Aerosol Containment Chamber.

8. Set the NOUVAG Ultrasonic Nebulizer for maximal air and maximal flow.

9. Do not use the timer feature.

10. Switch on the NOUVAG for a brief period of time to confirm adequate and proper nebulization. If there is poor or no nebulization, switch the NOUVAG off. For no nebulization, check the electrical plug and the fuse. For poor nebulization, verify that the medication cup and nebulizer chamber are properly seated, and that the nebulizer chamber and medication cup have adequate volumes. If this still does not correct the situation, contact the manufacturer.

7.2.2 Collection Containers

Label the sputum collection container with the participant's initials, ID number, date and visit number. Use a paper cup for saliva collection if it will be discarded.

7.2.3 Nuaire Aerosol Containment Chamber (for those using it)

1. Establish an unobstructed space of six to twelve inches behind the back of the Nuaire aerosol containment chamber before operating.

2. Switch on, and if your chamber comes with a magnehelic differential pressure gauge, check it for proper flow, otherwise visually inspect for an adequate flow. If there is poor flow, inspect the pre-filter and HEPA filter, and flow setting. Service as needed.

3. If the blower does not start up, switch off and check the plug and fuse. If this does not correct the problem, contact the manufacturer.

7.2.4 Electronic Timer / Intervalometer

1. Set the Electronic Timer's time setting for minutes, the display intensity to your preference, the time selector dials for 2 minutes (left knob to 0 and right knob to 2), the audio selection control for tone, and time cycle control to auto start.

2. Check that the timer is operating properly by activating the device. Once the operational status is verified, reset the timer by depressing the start/reset button again.

7.3 Sputum Induction Procedure

7.3.1 Active Participation

Active participation is extremely important to the success of this procedure. Vigorous and deep coughing is necessary to collect an adequate induced sputum sample.

7.3.2 NOUVAG Ultrasonic Nebulizer

Show the generation of the saline mist to the participant.

7.3.3 Nuaire Aerosol Containment Chamber

1. Inform the participant of the noise level of the Nuaire Aerosol Containment Chamber.

2. Determine if claustrophobia is an issue and if so, if continued participation with this procedure needs to be re-evaluated.

7.3.4 Symptoms That Might Occur

1. A salty aftertaste and a slight throat irritation are commonly noted.

2. Spontaneous cough and a sense of needing to swallow during sputum induction may occur.

3. Other symptoms that rarely occur include: shortness of breath, wheeze, chest tightness, lightheadedness, nausea and headache.

7.4 Induced Sputum Sample Acceptability Guidelines

1. Generally sputum will be induced for 12 minutes. However, if the first sputum induction of the study is terminated after 4 minutes and before the targeted 12 minutes, all subsequent sputum inductions performed in the same study for the same participant will end at that same time. Do not continue onward for the targeted 12 minutes. The minimal acceptable induced sputum sample is greater than or equal to 4 minutes.

2. There is no minimum volume required. A technician who will process the sample needs to determine if the volume is adequate for processing.

3. To ensure good quality, the sample should be processed within 1 hour after it is obtained. If the sample is not processed within 4 hours after it is obtained, the sample will not be acceptable. Protocol violation or deviation will be assigned if samples are not processed within acceptable time (see study specific protocol violations/deviations list for details).

4. An induced sputum sample cell differential has to be more than 20% non-squamous cells in order for the slides to be read.

7.5 Instructions

The following are instructions for a standard induced sputum procedure. As previously noted, any deviation(s) will be study specific and be described in the study protocol. **IMPORTANT: Please review sputum discussion in Section 2 of study specific MOP for any induced sputum procedural amendments.**

7.5.1 Prep

1. Instruct the participant to refrain from eating about an hour prior to this procedure.

2. In the unlikely event that there is a severe reaction to hypertonic saline inhalation during sputum induction, it is important to have a physician on-site and readily available to evaluate the participant and supervise treatment. The physician, however, does not need to be present in the room during the actual sputum induction. Record the physician's name and his/her pager on the sputum induction worksheet (SPUTUM_INDUCTION_WKS).

3. Have the *Emergency Drug Box* accessible in the same room where the sputum induction is performed. Drugs include: a metered-dose inhaler (metaproterenol or albuterol), ampoules of pre-measured albuterol solution to be nebulized, and injectable epinephrine.

4. If more than one set of equipment is available for sputum induction, record which equipment is used for the procedure on the sputum induction work sheet.

7.5.2 Spirometry/Peak Flow Monitoring

Prior to sputum induction:

Participant's FEV1 used for assessment of eligibility for sputum induction should be the one obtained after 4 or more puffs of albuterol (≥360µg). Participant's FEV1% predicted should be ≥ 50% predicted in order for a participant to proceed with the sputum induction.

Possible options where FEV1 will be obtained to assess eligibility:

1. After methacholine:

   A participant must be reversed initially with 4 puffs of albuterol. If a participant is not reversed to ≥ 90% of the baseline FEV1, additional albuterol treatment should be given as instructed on Methacholine Procedure (METHA) form. The last FEV1 obtained after methacholine (recorded on either METHA or METHA_ADD_TRT form) should be used to assess if a participant is eligible to proceed with the sputum induction. The Methacholine report does not give FEV1% predicted values so to calculate it for reporting on SPUTUMCHK form, use the final FEV1 (in liters) that will be used for assessing eligibility for sputum induction and FEV1 predicted value (in liters) that is given in the first row of the 'FEV1 absolute' column on the Methacholine report as follows:

   (final FEV1 after methacholine reversal/FEV1 predicted value)*100%

   Example:

   The final FEV1 after methacholine reversal=2.78
   FEV1 predicted value=3.06

   post albuterol FEV1% predicted=(2.78/3.06)*100%=91%

   Since the post albuterol FEV1% predicted used for assessing sputum eligibility is ≥ 50%, the participant is eligible to proceed with sputum induction.

2. After maximum reversibility or 4 puffs of albuterol reversal:

   To assess eligibility for sputum, use FEV1 after the final reversal. For maximum reversibility, that would be after either 6 or 8 puffs of albuterol. For standard 4 puffs reversal, that would be FEV1 obtained after 4 puffs of albuterol. Both MAXREV and PRE/POST reports show FEV1% predicted values.

In addition to FEV1, to monitor participant during sputum induction, peak flow values after the final albuterol reversal are necessary. Therefore, after participant's FEV1 for assessing sputum eligibility is obtained, perform 3 acceptable peak flow maneuvers. Post albuterol peak flow should be obtained using the spirotel® demo device that has PEF sessions available. The instructions for using demo PEF session are as follows:

1. When the device is turned on, select the #3 button to be taken to the PEF session. Note that this is different than the unscheduled peak flow session on the non-demo device.

2. The text 'EXHALE fast!' will appear on the top line and 'STOP' will appear above the 0 key on the second line.

3. After 6 seconds of forced expiration the device emits a long beep, helping the participant to understand the minimum expiratory time has been reached.

4. The measurement finishes automatically several seconds after the last volume variation (at zero flow), or by selecting STOP.

5. Spirotel® will display the peak flow for the completed blow. 'Peak Flow' will appear on the top line and the peak flow value will appear on the second line. The value will appear for 30 seconds.

6. Spirotel® will automatically turn itself off after 30 seconds. The device can also be shut off by holding the on/off button.

7. The data is NOT saved so the peak flow has to be recorded before the device turns off.

The process above has to be repeated until 3 acceptable peak flow values are obtained. The highest PEF will be used as a baseline for monitoring subject through sputum induction.

NOTE: If a subject blows three peak flows and the best one is very different from the other two, obtain additional PEF if you suspect that the outlier peak flow might have

been an error. For example, if a subject blows 175, 320, and 330 the first one might have been poor technique ("tooting" into the meter). In that case another blow should be done and if it is in the 320-330 range use those three to choose the best and discard the 175 value.

If FEV1 monitoring is necessary during sputum induction:

Spirometry monitoring during sputum induction should be done by using AsthmaNet spirometry system. New PRE/POST spirometry screen should be used. Since the requirements for spirometry monitoring during sputum induction differ from ATS requirements, the participant's AsthmaNet ID number (ABCDEFGH) should be constructed as follows:

- AB = 98
- CDE = site number
- FGH = participant's number in the study

The modified participant ID should be used for spirometry monitoring during sputum induction so that the AsthmaNet over-reader does not grade those tests and give low grade since the tests do not meet ATS criteria.

Spirometry AFTER sputum induction is completed:

Even if monitoring during sputum induction was done using spirometry system, to measure spirometry immediately after sputum induction (i.e. after the last saline inhalation), a new PRE/POST screen should be opened and the actual participant's study ID used. Under 'pre' stage, spirometry immediately after sputum induction should be performed. If albuterol reversal is necessary, 'post' stage should be used. If two albuterol reversals are required, both 'post' stages can be done under the same screen. Post-sputum spirometry report should be sent to the DCC with sputum related forms.

NOTE: If two reversals are performed, the best post test will be printed on the report first regardless of the order in which the tests were performed. Therefore, if second reversal is the better of the two, it will be printed first on the report. Transcription of the results has to be carefully done to ensure that SPUTUM_ADD_TRT form is filled correctly.

7.5.3 Collection of Induced Sputum

1. On the sputum induction worksheet (SPUTUM_INDUCTION_WKS):

a. Record the post-albuterol FEV_1 and corresponding FEV1% predicted used to assess participant's eligibility for sputum.

b. Record the best post-albuterol peak flow, of the 3 post-albuterol maneuvers measured.

c. Record the time the sputum induction procedure begins and ends.

2. Instruct the participant to put on the nose clip and to breathe at his/her usual rate and volume from the mouthpiece during the entire procedure except when actively providing a sputum sample or performing a peak flow or FEV_1.

3. If at any time during the induction, the need to cough or swallow should occur instruct the participant to:

    a. First, clear his/her mouth of saliva by spitting the saliva into the saliva container. If this is not possible simply proceed to 3b.

    b. Second, vigorously cough and spit everything into the sputum container.

    c. Resume breathing on the mouthpiece.

4. Encourage the participant to blow his/her nose quickly and as often as necessary.

5. At each 2 minute interval, (from the beginning of the sputum induction procedure) instruct the participant to:

    a. Remove the mouthpiece from his/her mouth and spit all saliva into the saliva container. Take the necessary time to clear his/her mouth of saliva.

    b. Return the mouthpiece to the mouth and breathe in deeply, and then exhale.

    c. Take in a second full deep breath, and while holding this breath, remove the mouthpiece from his/her mouth, bring the sputum collection container up to the mouth and cough (from the diaphragm) this full breath out along with any airway secretions and saliva into the collection container. Encourage the participant to spit everything into the sputum cup.

d. Perform peak flow (or spirometry if applicable).

e. Return the mouthpiece back to the mouth and resume regular breathing of the nebulized 3% saline solution as soon as possible.

7.5.4 Safety Monitoring During Sputum Induction

To assess for excessive bronchoconstriction during sputum induction, peak flow or FEV_1 measurements will be made every 2 minutes, and/or sooner if the participant becomes symptomatic. Spirotel® peak flow measurements will be done first to assess the participant's pulmonary function status because of the relative ease, and the minimal amount of time required to obtain this measurement. Perform spirometry once a confirmed > 20% fall in the peak flow from the post albuterol baseline PEF occurs. If you are performing spirometry because of a confirmed >20% fall in peak flow, you must continue monitoring with spirometry only for the remainder of the induction.

1. Measure a single expiratory peak flow every 2 minutes or sooner if the participant becomes symptomatic and record on the sputum induction worksheet. This measurement is done after the collection of the induced sputum sample. Repeat the peak flow measurement if the following occurs:

    a. The peak flow maneuver is technically unacceptable.

    b. To confirm a peak flow measurement of less than 80% from the post albuterol baseline peak flow value. If a confirmatory peak flow measurement is performed, record the best peak flow measured.

2. If the recorded **peak flow is greater than or equal to 80%** of the post albuterol baseline peak flow, continue the sputum induction, measuring peak flows every 2 minutes.

3. If the **peak flow is confirmed to be less than 80%** of the post albuterol baseline peak flow, **turn the nebulizer off (do NOT power down), stop the timer and perform a technically acceptable spirometry maneuver**. Turn the nebulizer and timer on when the sputum induction procedure resumes.

    a. If the recorded **FEV_1 is greater than or equal to 80%** of the post albuterol baseline FEV_1, continue with the sputum induction, performing 1 technically acceptable spirometry maneuver every 2 minutes. No additional peak flow measurements are done. Record the FEV_1 on the sputum induction worksheet.

b. If the recorded **FEV_1 is less than 80%** of the post albuterol baseline FEV_1, perform another spirometry maneuver. If the best FEV_1, which is the highest FEV1 value, is **greater than or equal to 80%** of the post albuterol baseline FEV_1, continue the sputum induction. If the best FEV_1 value is **less than 80%** of the post albuterol baseline FEV_1 measurement, stop the induced sputum procedure, administer 2 puffs of albuterol as described in the Spirometry MOP and proceed to "Monitoring After Sputum Induction".

7.5.5 Monitoring after Sputum Induction

If the sputum induction procedure was only PEF monitored, perform a technically acceptable spirometry maneuver after the last saline administration. It should be measured under the new PRE/POST protocol screen in PRE session using participant's study ID. Record this FEV_1 value on the sputum induction worksheet.

If the sputum induction procedure was monitored with spirometry, after the last saline administration, the spirometry needs to be measured under the new PRE/POST screen in PRE session using the participant's study ID. That value will represent the FEV1 immediately after completion of sputum induction. Record the FEV_1 value on the sputum induction worksheet.

1. If the post sputum induction **FEV_1 is greater than or equal to 90%** of the post albuterol baseline FEV_1, the sputum induction procedure is completed and the participant may be discharged from this test.

2. If the **FEV_1 is less than 90%** of the post albuterol baseline FEV_1, administer 2 puffs of albuterol as described in the Spirometry MOP. After 10-15 minutes, perform a technically acceptable spirometry maneuver in POST session.

3. If the post 180µg albuterol **FEV_1 is less than 90%** of the post albuterol baseline FEV_1 value, administer another 2 puffs of albuterol, and repeat another single technically acceptable spirometry maneuver 10-15 minutes later in POST session.

4. If the post 360µg albuterol **FEV_1 is less than 90%** of the post albuterol baseline FEV_1 value, consult the supervising physician. The participant should not be discharged from the clinic without the approval of the supervising physician.

7.5.6 Specimen Handling

Cover and transport the collected sputum sample for immediate processing (i.e., within 1 hour). The sample needs to be kept on ice or refrigerated until processing. It is very important that the sample is processed within 1 hour from collection to avoid cells degradation. Quality of cells will be monitored and reported to AsthmaNet Quality Control Committee.

7.6 Processing Induced Sputum

IMPORTANT: For study specific processing requirements, see sputum discussion in Section 2 of study specific manual of operation (MOP).

For each processed sample, Sputum Processing Worksheet (SPUTUM_PROCESS) needs to be completed and sent to San Francisco along with the associated slides.

7.6.1 Induced sputum processing

1. Determine the weight of the sputum sample collected. Tare the balance with an empty 50 mL conical polypropylene tube (or a 15 ml tube if a small volume is collected), transfer the collected sputum sample into this tube and record its weight.

2. To the sputum sample, add a volume (mL) of 10% Sputolysin** equal to the weight (gms) of the sputum sample. (SS: Sputum +10% Sputolysin)

   **10% Sputolysin (1 part Sputolysin and 9 parts phosphate buffered saline (PBS)). This solution should be discarded after 12 hours.

3. Mix the sputum and 10% Sputolysin sample (SS) with a serological pipette. To ensure complete homogenization, aspirate and dispense the sample slowly through the serological pipette several times. Do not vortex the sample.

4. Place the SS sample in a 37°C shaking water bath for 15 minutes.

5. Set shaker at 150 shakes/min.

6. At 5, 10 and 15 minute intervals briefly remove the SS sample from the shaking water bath, mix the sample well, but not vigorously, with the serological pipette (aspirate and dispense the sample several times) and return it to the shaking water bath.

7.6.2 Cell Count

1. Aliquot 0.5 to 1 ml (or more depending on the sample volume) cell count/cell differential designated SS sample and use this to do cell count and to make slides for differential count.

2. Mix 100 microliters of the SS sample with 100 microliters of Turks Solution*. (SST sample: SS sample +Turks solution)

   *Turks Solution:

   10 mg Crystal Violet
   3 mL Glacial Acetic Acid
   Bring total volume up to 100 mL with distilled water

3. Dispense 10 microliters of the SST sample into the well on one side of the cover slipped hemocytometer.

4. Count the number of cells bordered within the 4 large corner squares of the hemocytometer. You need to count at least 100 cells total from all 4 squares combined. If less than 100 cells are counted, see helpful hints on counted samples with very few cells.

Calculate the total cell count per ml:

Total cell count per ml = (total # of cells in 4 large squares / 4) X 10,000 X 2*X 2**
* Dilution with 10% Sputolysin
** Dilution with Turks solution

7.6.3 Slide Preparation for Cell Differential Count

1. Divide the total cell count per mL by 160,000 cells per mL to obtain an approximate dilution factor to prepare cell differential slides.

2. Dilute the cell count/cell differentiated designated SS aliquot (not the whole SS sample but the aliquot designated for cell count at step 1) according to the dilution factor obtained from Step 1 with normal saline if needed. Although a minimum volume of 1 mL is required for 4 slides, prepare at least a 1.5 mL aliquot.

3. On the part of the slide that has frosting, write with the pencil study name, participant ID and visit number. Assemble cytofunnels, filter papers, slides, and slide clips for 4 slides.

4. Place 150, 200, 200, 250 microliters of the sample obtained from step 2 in these four cytofunnels.

5. Centrifuge at 500 rpm for 5 minutes in the cytocentrifuge.

6. Check the cytospin slides to make sure that they are not too crowded or too sparse. If they are too crowded, further dilute the cell count/cell differentiated designated SS aliquot and make new slides. If they are too sparse, one may re concentrate, re suspend and prepare another set of slides. See helpful hints on preparing good slides.

7. Stain slides with KWIK-DIFF staining kit (Shandon). 5 seconds in green, 7 seconds in red, and 5 seconds in blue is highly suggested. Check the staining quality of the slides and re stain as necessary. See helpful hints on how to make good slides.

8. When the slides are dry, mount the slides with cover slips.

Helpful hints on processing small sputum samples:

SPUTOLYSIN DILUTION: Add an equal volume of sputolysin to the small sample. Dilution factor is 2. Do shaker water bath per protocol.

TURKS DILUTION: Standard protocol is to add 100 microliters of sample to 100 microliters of Turks solution. In the case of a small sample, add 10 microliters of sample to 10 microliters of Turks solution for the total volume of 20 microliters. Withdraw 10 microliters to fill the hemacytometer. Dilution factor is 2.

Perform the cell count on the hemacytometer as usual. Calculate dilution as usual.

SLIDE CALCULATIONS: Standard protocol is to prepare 1500 microliters of diluted sample and place 150, 200, 200, and 250 microliters in each cytofunnel to make slides. With the small sample, the volume can be reduced to 100 microliters/cytofunnel: 100 microliters/cytofunnel X 4 cytofunnels = 400 microliters total of diluted sample instead of 1500.

Perform the slide calculations on the sputum processing worksheet (SPUTUM_PROCESS) replacing 400 for every 1500 value. 400 microliters/B must be at least 50 microliters of sample. At least 50 microliters of sample MUST be added. Adjust fraction of sample to PBS until there is at least 50 microliters of sample.

EXAMPLE:
A=0.85 x 10^6 cells/ml
B=(0.85 x 10^6)/(0.16 x 10^6)=5.3125

400/5.3125=75.3 microliters of sample

400-75.3=324.7 microliters of PBS

Make slides using this ratio (75.3:324.7) adding 100 microliters of solution per funnel.

Spin residual sample for supernatant aliquots. NEVER add PBS to the supernatant to bring the volume up.

Helpful hints on counting samples with very few cells:

Standard protocol is to count the number of cells present in the 4 large corner squares of the hemacytometer. This is part of the formula to calculate 'A' on the sputum processing worksheet (SPUTUM_PROCESS). The total number of cells (adding all 4 squares together) MUST be at least 100 to obtain an accurate 'A'.

Rarely, this total is less than 100. If your total count is less than 100, you must count additional squares of the hemacytometer and adjust the formula used to calculate 'A'.

EXAMPLE:
Assume final dilution =4
[(18+19+22+20) x 4 x 10^4]/4= STOP. Only 79 cells counted on the hemacytometer.

Count center square of hemacytometer. Reassess formula.

[(18+19+22+20+23) x 4 x 10^4]/**FIVE**=A. This formula is accurate. It has at least 100 cells counted in **FIVE** squares of the hemacytometer so the denominator in the formula changed to 5. If you need to use 6 squares of the hemacytometer to count at least 100 cells, change the denominator to 6, etc.

Continue with the slide calculations.

Helpful hints on preparing slides:

- To avoid cells degradation, process the sample within one hour of collection.
- Change staining solutions as frequently as needed.
- Check cell density before staining. Remake slides if necessary.
- Check staining quality before mounting with a cover slip. Remake slides and stain as necessary.

When slides are stained too lightly or are monochromatic:

- It is difficult to see the cell morphology.
- Dip slides in the staining solutions longer to increase the intensity. If the staining is too pink, dip the slide in the blue solution (#3) a little longer. Do one slide at a time. Check the staining quality right away so that any necessary adjustments could be made for the next slide. If the slides are too blue, re-dipping in the pink solution (#2) does not help and new slides will need to be prepared and stained.

If the slide appears to be wet right after cytocentrifugation:

- The sample is likely too mucinous and the cells are likely to become compressed and will stain very dark. They do not show their normal morphology in this case. For example, neutrophils may look like lymphocytes, and it may be difficult to detect the granules in eosinophils.
- To solve this problem, homogenize the SS sample aliquot, dilute it further and make new slides.

After the 4 slides have been prepared:

1. Complete the Sputum Induction Lab Values (SPUTLAB) form.

2. The processor should assign to the slide with the best quality #1, second best #2, third best #3 and the slide with least quality #4. The quality is determined by the processor based on the staining.

3. When the slides are completely done, participant ID, initials, visit number and slide number (# __) should be completed on the Laser Cryo-Babies 1”x1” label generated through the Biological Sample Tracking (BST) module. Place the completed label over the frosted part of the slide. The label MUST NOT be placed over the stain. Label for each slide will have unique barcode.

4. Enter the participant's sputum slides information into the Biological Sample Tracking module. Information on the participant's sputum slides collection should be entered into the Biological Sample Tracking module on the day the slide is completed and label with the barcode is placed on the slide.

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.

7.6.4 Induced Sputum Supernatant Processing

1. Centrifuge the remaining SS sample at 4°C, at 2000 RPM for 10 minutes.

2. Aliquot – supernatant.

3. Prepare 2 mL O-ring microcentrifuge tubes (Fisher catalog # 02-681-374). The number of the tubes needed depends on the supernatant volume and should be determined using the rules in step # 4. For each tube, complete one Laser Cryo-Tags 1.50"x0.75" label generated through the Biological Sample Tracking (BST) module for SI Supernatant and place it vertically on a 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). On each label, the information that needs to be completed is: participant ID, participant initials and visit number. Also, using an alcohol-proof permanent Sharpie marker, write initials of the participant on the caps of the tubes.

Example label placement:

Top of Tube – (o-ring cap screwed on tightly)

Bottom of Tube

4. If the supernatant volume is:

    a. Less than or equal to 2.5 ml – transfer 250µl to each tube. If the volume is 2.5 ml, 10 tubes should be filled with 250µl in each. If the supernatant volume is less than 2.5 ml, fewer than 10 tubes should be used so that there is 250µl of sample in each tube (for example, if the volume is 2 ml, 8 tubes should be filled with 250µl in each).

    b. Greater than 2.5ml and less than 5ml - 10 tubes should be filled with 250µl in each. The leftover sample should be either discarded or stored and used in compliance with the rules and regulations of your local IRB. These leftover samples will NOT be tracked through the Biological Sample Tracking (BST) module.

    c. Greater than or equal to 5 ml – 10 tubes should be filled with 500µl in each. The leftover sample should be either discarded or stored and used in compliance with the rules and regulations of your local IRB. These leftover samples will NOT be tracked through the Biological Sample Tracking (BST) module.

5. Supernatant tubes should be placed in a freezer storage box, with 9x9 dividers, that is designated for the specific study and for the visit at which the supernatant

was collected. For example, if the supernatant was collected for VIDA study at visit 3, it must be stored in the box that is designated for VIDA samples collected at visit 3. The first box designated for a specific visit must be labeled as box # 1. When the first box is full, the next box designated for the same study and visit number must be labeled as box # 2. 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), visit number, box number and city and site name. For example, if the supernatant was collected at Boston at Brigham & Women's site at visit 3 for VIDA study and this is the first box for visit 3, the information on the box should be as follows: VIDA – SI-Supernatant, Visit 3, Boston- Brigham & Women's, Box # 1.

6. Place the aliquot tubes in the designated box. **Do not remove the box from the freezer while collecting and assembling new samples; instead, transport the new tubes to the freezer and insert them in the box.**

7. Place the box back in –80°C freezer.

8. Enter the participant's sputum supernatant collection information into the Biological Sample Tracking (BST) module. Information on the participant's sputum supernatant collection should be entered into the BST module on the day the collection process takes place.

7.6.5 Induced Sputum Pellet

1. The sputum pellet is the material left in the conical polypropylene tube after the supernatant has been aliquoted.

2. Add 1 mL of Saliva Protect Reagent directly onto the pellet. Vortex for 1 minute to resuspend the pellet.

3. Transfer the pellet from the conical tube to a 2 mL O-ring microcentrifuge tube (Fisher catalog # 02-681-374).

4. For each tube complete one Laser Cryo-Tag 1.50"x 0.75" label generated through the Biological Sample Tracking (BST) module for SI Pellet and place it vertically on a tube so that the barcode can be scanned. On each label, the information that needs to be completed is: participant ID, participant initials and visit number. Also, using an alcohol-proof permanent Sharpie marker, write the participant ID and visit number on the side of the tube.

5. Pellet tubes should be placed in a freezer storage box, with 9x9 dividers, that is designated for the specific study and for the visit at which the pellet was collected. The same rules for storing and labeling should be followed as for the supernatant storage except that for sample type SI-Pellet is written. For example, if the pellet is from collection at Boston at Brigham & Women's site at visit 3 for VIDA study and this is the first box for visit 3, the information on the box should be as follows: VIDA – SI-Pellet, Visit 3, Boston- Brigham & Women's, box # 1.

6. Store immediately in the -80°C freezer.

7. Enter the participant's sputum pellet collection information into the Biological Sample Tracking (BST) module. Information on the participant's sputum pellet collection should be entered into the BST module on the day the collection process takes place.

7.6.6 Shipment

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.

IMPORTANT: For study specific shipment requirements, see sputum discussion in Section 2 of study specific MOP. The general rules are to be followed unless there are study specific requirements which override the standard instructions below.

Standard sputum shipment instructions:

1. Ship only Monday through Wednesday with the next day receipt. If there is a holiday during the week, email Marrah Lachowicz-Scroggins at Marrah.Lachowicz-Scroggins@ucsf.edu in advance to confirm if it is fine to send a shipment that week.

2. On the day of shipment, clinical personnel should access the AsthmaNet Biological Sample Tracking module to mark the samples as shipped and to generate an inventory list that will be shipped with the sputum samples to the lab in San Francisco. Include a shipment comment detailing the contents of the shipment (i.e., sputum slides, sputum supernatants, sputum pellets). There will be three separate logs; one for sputum slides, one for supernatants and one for sputum pellets. All sample types might not be shipped on the same day so make sure that the correct inventory list is generated. Because this log is generated

directly from the tracking database, it is imperative that all sputum samples are entered into the system before generating the inventory list.

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 inventory lists.

The inventory list 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 log that will be sent with the shipment.

3. If the sample inventory list will not be saved electronically, make a copy for clinic records. Place the original log in a waterproof zip lock bag and include it at the top of the shipping box. Seal the box securely.

   Email a copy of the FedEx/Airborne Express airbill and the complete sputum specific sample inventory list, per instructions outlined in the Section 7 of the AsthmaNet General Manual of Operations, to confirm shipment and receipt dates for the lab. Email should be sent to Marrah Lachowicz-Scroggins at Marrah.Lachowicz-Scroggins@ucsf.edu. If the shipment bill and inventory list are faxed instead, fax to the attention of Marrah Lachowicz-Scroggins at 415-502-7814 and then email Marrah informing her that you faxed the shipment bill and inventory list and that samples have been shipped.

4. Ship to:

   Marrah Lachowicz-Scroggins
   University of California, San Francisco
   513 Parnassus Ave, HSE-1350
   San Francisco, CA 94143-0130

Slides

All the slides collected should be sent for reading in San Francisco. No backups will be stored at sites. Ship when you have about 10 collections (which will be 40 slides shipped if 4 were made per each collection). It is fine if fewer than 10 collections are sent but not more! Make sure not to ship any slides that are marked as excluded in the Biological Sample Tracking module.

Slides Packing/Shipping instructions:

1. Place the slides in slide storage container.
2. Wrap the slide storage container in the 'bubble' wrap.
3. Put the packed slides into the shipping box.
4. Place the original AsthmaNet sputum processing worksheets, associated with the slides that are being shipped, in a waterproof zip lock bag and place it into the shipping box.
5. Insert the inventory list.
6. Seal the shipping box.
7. Complete the federal express bill.

Sputum Supernatants and Pellets:

In general, all supernatant and pellet tubes collected as a part of a study will be shipped to San Francisco every six months. However, check Section 2 of the study specific MOP for any shipping amendments. At the start of each study, the DCC Scientific Coordinators will send dates when supernatants and pellets for that study are to be shipped to San Francisco. Make sure that only supernatants and sputum pellets for the specified study are shipped and not for other studies that are ongoing at the same time. Only the samples that are not marked as excluded in Biological Sample Tracking module should be shipped to San Francisco.

Supernatants and Pellets Packing/Shipping instructions:

1. Place about 10 lbs. dry ice in the Styrofoam container.
2. Place the specimen storage box in the zip bag, securely tape the bag closed with packaging tape, and pack in the Styrofoam container.
3. Place the inventory list in a Ziploc baggie and insert into container.
4. Seal the container.
5. Attach one "Exempt Human Specimen" sticker to the container.
6. Clearly write site name, site number and shipping date on the container.
7. Complete the federal express bill.

7.6.7 Sputum Samples With ≥ 80% Squamous Cells

Total number of squamous cells will be reported by sputum reader on the Sputum Induction Read (SPUTREAD) form. If a sample had ≥ 80% squamous cells, the DCC will send an e-mail to the coordinator listed as the study point of contact with instructions to exclude all relevant samples from the Biological Sample Tracking module. The following email will be sent to the lead coordinator for the performance site:

"PLEASE NOTE:

A SPUTREAD form was received for this participant/visit and Q1050 indicates there are >= 80% squamous cells present. Any supernatant and pellet samples collected at this visit should be marked as excluded in the Biological Sample Tracking module. The samples should be marked as excluded within the next 14 days or a query will be generated by the DCC to ensure this action is performed prior to the next supernatant or pellet shipment. Excluded samples should not be shipped to the Sputum Core Lab.

If the supernatant and cell pellet for this participant/visit have been shipped to the Sputum Lab, please forward this message to the Sputum Lab (AsthmaNet_993_bst@phs.psu.edu). Sputum Lab personnel should pull these samples, place them in the box for excluded samples, and mark them as unusable in the Biological Sample Tracking module.

Site: site name will appear here
Participant ID: participant study ID will appear here
Visit Number: visit number will appear here
Q1050: value in Q1050 will appear here
Number of supernatant samples to exclude: count of supernatant samples
Number of pellet samples to exclude: count of pellet samples

Thank you,
AsthmaNet DCC"

Site needs to ensure that excluded samples are not shipped, as discussed above in 'Shipment' section. Excluded supernatant tubes should be placed in a freezer storage box, with 9x9 dividers, which is designated for the specific study and marked "Excluded Samples". The first excluded sample box designated for a study should be labeled as box # 1. When the first excluded sample box is full, the next box designated for excluded samples for the same study should be labeled as box # 2. 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, SI-Supernatant Excluded Samples, and box number. For example, if the excluded supernatant sample was collected for the VIDA study and this is the first box, the information on the box should be as follows: VIDA – SI-Supernatant Excluded Samples, Box # 1. For excluded pellet samples, the same rules for storing and labeling should be followed as for the supernatant excluded sample storage except that for sample type, SI-Pellet is written. Excluded samples will be stored until study completion, and will be discarded with approval of the Steering Committee.

7.7 Problems or Questions

Following individuals should be contacted for problems or questions:

For sputum induction procedures and equipment that is used for induction issues:

Julian Silva
UCSF Medical Center
Airway Clinical Research Center
505 Parnassus Ave, M-1331
San Francisco, CA 94143-0130
Tel: (415) 476-5418
Fax: (415) 502-7814
Email: Julian.Silva@ucsf.edu

For Nouvag issues:

Mark Kuban
Nouvag USA
6201 Airport Freeway
Suite 200
Haltom City, TX 76117
Email: mark@nouvagusa.com

In addition to contacting Mark, include in the correspondence San Francisco and DCC contacts as well to keep everyone in the loop.

For processing of the sputum sample, slide preparation procedures, supernatant processing, equipment issues and shipping issues:

Marrah Lachowicz-Scroggins
UCSF Medical Center
Airway Clinical Research Center
513 Parnassus Ave, HSE-1350
San Francisco, CA 94143-0130
Phone: (415) 476-0752
Fax: (415) 502-7814
Email: Marrah.Lachowicz-Scroggins@ucsf.edu

For general problems and data issues:

Anne-Marie Dyer
Scientific Coordinator/Statistician
Data Coordinating Center
Tel: (717) 531-3663
Fax: (717) 531-4359
Email: adyer@phs.psu.edu

7.8 Clean Up

To ensure the safety of all participants in the sputum induction it is important that you adhere to your institution's guidelines. In general discard all single use items. Items to be sterilized are washed with a detergent, rinsed well and then sterilized per your institution guidelines. The recommendation is that plastic T-pieces and elbows are disinfected per your institution's guidelines and reused, while the medication cups, corroflex tubing and mouthpieces are discarded. If necessary due to low supply, medication cups can be washed with alconox and sterilized in Cidex OPA.

For NOUVAG Ultrasonic Nebulizer:

1. Discard all aerosol tubing.

2. Empty and thoroughly rinse the nebulizer chamber with distilled water.

3. Wipe down all surfaces with a disinfectant/decontaminant cleaner approved by your institution.

4. Allow to air dry.

7.9 Infectious Disease Issues

1. The aerosol containment chamber is utilized during sputum induction because it is a cough producing procedure. However its usefulness is dependent upon it being used correctly. Coughing outside of the chamber will not offer any protection to the staff.

2. Four criteria are necessary for a disease to be transmitted:

    a. Presence of a pathogen.
    b. Sufficient quantity of pathogen present.
    c. Susceptibility of an individual to the pathogen.

d. Presence of the correct entry site for disease to be transmitted.

3. Diseases of concern include colds and tuberculosis. Colds are transmitted by airborne, direct or indirect contact with body fluids. Tuberculosis is transmitted by airborne infectious aerosol droplets.

4. Although gloves are to be worn, it is good practice to wash your hands prior to and after each induction.

5. Do not eat in the designated area for sputum induction or processing.

6. Practice Universal Precautions when handling body fluids (saliva and sputum) and cleaning the equipment.

7.10 Maintenance

7.10.1 NOUVAG Ultrasonic Nebulizer

1. Clean and monitor as recommended by NOUVAG.

2. Bacteria filter should be replaced after every 10 hours of use. This is equivalent to roughly fifty 12-minute inductions. Quarterly nebulizer QCs should count towards the 10 hours of use.

7.10.2 Nuaire Aerosol Containment Chamber

1. Inspect the pre-filter quarterly or as usage dictates and replace as needed.

2. Inspect the HEPA filter biannually for adequate flow and face velocity and replace as needed.

7.11 NOUVAG Ultrasonic Nebulizer Output Assessment

1. Quality Control (QC) checks on the nebulizer should be done when set up for the first time, and quarterly thereafter. Set up the nebulizer following the instructions in section 7.2.1 of this MOP, filling the chamber with distilled water and the medication cup with 40 mL 3% saline as specified in section 7.2.1.

2. Dry the outside surface of the medication cup.

3. Weigh the filled medication cup. Record the pre-ultrasonic neb weight.

4. Seat the medication cup into the nebulizer chamber.

5. Set for maximal air and flow.

6. Set a timer for 12 minutes and switch the NOUVAG on.

7. After 12 minutes of nebulization, disconnect the medication cup with the chamber lid from the air output port and remove it from the nebulizer chamber.

8. Dry the outside surfaces of the medication cup.

9. Weigh the medication cup (post-ultrasonic neb weight). Record the post ultrasonic neb weight.

10. Be sure to change the water in the nebulizer chamber between each of the 5 trials, as the water tends to warm up and change the output.

11. For each QC trial, record the date when the trial was done, pre-weight and post-weight in the appropriate cells in the Excel spreadsheet provided by the DCC. The file can be found on the AsthmaNet site under General MOP > Appendices > Appendix 7 Sputum.

12. The Excel file is set up to calculate Ultrasonic nebulizer output for each of 5 trials using the following formula: Output/min (mL/min) =

    [pre-ultrasonic neb weight - post-ultrasonic neb weight] / 12 min

13. QC should be performed quarterly (January, April, July, October) and the Excel file e-mailed to Anne-Marie Dyer at adyer@phs.psu.edu.

7.12 Sputum Induction and Processing Supply and Equipment

For sputum induction:

Aerosol Containment Chamber OR negative pressurized or well ventilated room
Chamber: Nuaire Model #: NU-810-224
Tel: (800) 328-3352

NOUVAG Ultrasonic Nebulizer – *no substitutions allowed*
Model #: 3320
Tel: (800) 673-7427
Fax: (661) 724-1590
Website: www.nouvag.com

Medication cups
Nouvag Catalog # 3304
To order email, call or fax PO to:
Mark Kuban
Toll Free Tel: (800) 673-7427
Fax: (817) 887-9817
Email: mark@nouvagusa.com

Fisher Scientific Countdown Timer
Catalog #: 14-649-17
Tel: (800) 766-7000
Website: http://www.fishersci.com

Mouthpiece
Plastic Mouthtube
Catalog #: 1018-22
Vacu Med
Tel: (800) 235-3333
Website: http://www.vacumed.com

3% Saline – *no substitutions allowed*
Baxter
Catalog #: 2B1353Q
Website: www.baxter.com or order through the local pharmacy. Any bag size is fine.

Corr-A-Flex ® II 22 mm roll tubing (100 ft, 22 mm, cuff every 6 in.)
Model #: HUD 1680
Medical Supply Group
Tel: (800) 278-0227
Website: http://www.medicalsupplygroup.com

Connector Aerosol Tee (22 mm od, 15 mm ld)
Product #: 171083
Manufacturer #: 1648
Website: www.imed.com

Bacteria filter
Nouvag Catalog # 3213

Specimen cups (sterile)
Various brands acceptable (90mL is a good size)

Paper cups
Various brands acceptable. Used to collect saliva and will be discarded after induction.

For sputum processing:

Precision Reciprocating Shaker Bath and Rack
Bath: Catalog #: 15-455 or Catalog #: 15-455-5
Fisherbrand* Interlocking Four-Way Tube Racks: Catalog #: 03-448-11
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Microscope
Various makes and models acceptable. For additional information contact the individual listed above as the contact for the slide reading.
Micromaster
Catalog #: 12-561B
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Cytocentrifuge and cytofunnel assembly
Cytocentrifuge: Cytospin4 Catalog #: A-78300002
Cytofunnel: Catalog #: 10354

Cytology Funnel clips: Catalog #: 10357
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Phosphate Buffered Saline (PBS)
Catalog #: MT 21-031-CV
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Fisherbrand Sterile Microcentrifuge Tubes with Screw Caps – 2 mL; skirted bottom
Catalog #: 02-681-374
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

KWIK Diff. Staining Kit
Catalog #: 9990700
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

15 and 50 ml polypropylene tubes
Corning 15 ml: Catalog #: 05-538-59A; Corning #: 430790
Corning 50 ml: Catalog #: 05-526B; Corning #: 430828
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Refrigerated Centrifuge including 2000 rpm, without rotor, 120 V/60 Hz
Model #: Refrigerated: 5810R, order # 022625501
Swing bucket rotor A-4-81, order # 022638602
Lids, set of 2, order # 022638661 (need total of 4 lids)
Rotor key, order #022664174
Eppendorf North America
Tel: (800) 645-3050
Website: http://eppendorfna.com

10 µl, 200 µl, 1000 µl pipettes and tips
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Sputolysin reagent
Catalog #: 560000-1SET
EMD Merck
Website: http://www.emdmillipore.com

Balance
OHAUS Electronic Scale: Catalog #: 01-919-33; OHAUS #: CS200-001
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Cover slip mounting fluid (Cytoseal 60)
Catalog #: 18006
Electron Microscopy Sciences
Tel: (215) 412-8400
Website: http://www.emsdiasum.com/microscopy/default.aspx

Microscope slides and cover slips
Slides: Catalog #: 12-544-2
Cover slips: Catalog #: 12-544-10
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Storage boxes and shipping containers for slides
Boxes: Catalog #: 03-448-2
Shipping containers: Catalog #: 12-569-35
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Freezer storage boxes for supernatants and pellets
Boxes: Catalog #: 11-678-24A
Box divider for supernatants
Divider: Catalog #: 13-989-218
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Turks solution ingredients: Crystal Violet and Glacial Acetic Acid

5. Crystal Violet: Catalog # C0775
   Sigma-Aldrich
   Tel: (800) 325-3010
   Website: http://www.sigmaaldrich.com/united-states.html
6. Glacial Acetic Acid:
   Catalog #: A38-500
   Fisher Scientific
   Tel: (800) 766-7000
   Website: http://www.fishersci.com

Hemacytometer
Catalog #: 02-671-5 or 02-671-55A
Fisher Scientific
Tel: (800) 766-7000
Website: http://www.fishersci.com

Labels
For slides:
White Laser Cryo-Babies, 1”x1”
DiversifiedBiotech
Website: http://divbio.com/lasercryo-babies10x101600pk.aspx

For supernatants and pellets:
White Laser Cryo-Tags, 1.5” x 0.75”
DiversifiedBiotech
Website: http://divbio.com/lasercryo-tags150x0751200pk.aspx

7.13 AsthmaNet Sputum Certification

All AsthmaNet staff who plan to perform sputum induction and/or processing must be certified on a non-AsthmaNet participant before independently doing this on or in relation to AsthmaNet study participants. The sputum sample for certification does not have to come from an asthmatic; it can be from a healthy individual. The DCC will notify staff if and when certification becomes effective.

Before proceeding with a certification, the following tasks must be completed:

1. Review the Sputum Manual of Operations included as Appendix 7 of the AsthmaNet General Manual of Operations.
2. Watch the sputum induction and processing training video. The link for the video is http://cvri.ucsf.edu/~fahy/Resources.html. This link is also provided on the AsthmaNet secure website in the Certification/Sputum folder. In order to watch the video, Google Chrome or Internet Explorer version 8 or higher is required as well as QuickTime Player. Contact your local IT personnel if any issues arise.

7.13.1 Sputum Induction Certification

To become certified in sputum induction, complete the following:

1. Successfully perform the tasks indicated on the AsthmaNet Sputum Induction Certification checklist (SPUT_INDUCTION_CERT) form and be observed performing these tasks by certified technician. Checklist is located on the AsthmaNet secure website in the Certification/Sputum folder. It should be completed by the certified technician at the time of observation. (If certified in sputum induction for the NHLBI-sponsored SARP network, then this step is waived. Email the DCC at AsthmaNet-Certification@phs.psu.edu to notify them of SARP certification.)

2. Successfully pass the Sputum Induction written exam located on the AsthmaNet secure website in the Certification/Sputum folder.

3. Once the new technician has successfully completed the above steps, the certification checklist form and written exam should be emailed to the DCC at AsthmaNet-Certification@phs.psu.edu. In the subject of the email, include a brief description of the certification type and site ID (ex., Sputum Induction Certification Checklist and Written Exam, site 121).

7.13.2 Sputum Processing Certification

To become certified in sputum processing, complete the following:

1. Successfully perform the tasks indicated on the AsthmaNet Sputum Processing Certification checklist (SPUT_PROCESSING_CERT) form and be observed performing these tasks by a certified technician. Checklist is located on the AsthmaNet secure website in the Certification/Sputum folder. It should be completed by the certified technician at the time of observation. Once the new technician has completed all steps on the certification form successfully, the form should be emailed to the DCC at AsthmaNet-Certification@phs.psu.edu. In the subject of the email, include a brief description of the certification type and site ID (ex., Sputum Processing Certification Checklist, site 121). (If certified in sputum processing for the NHLBI-sponsored SARP network, then this step is waived. Email the DCC at AsthmaNet-Certification@phs.psu.edu to notify them of SARP certification.)

2. Complete an AsthmaNet Sputum Processing Worksheet (SPUTUM_PROCESS) for the sample processed for certification and email it to Marrah.Lachowicz-Scroggins@ucsf.edu or fax it to Marrah Lachowicz-Scroggins at 415-502-7814. If fax was sent, email Marrah as well to inform that the fax has been sent.

   Note to the site processor who is getting certified: Clearly indicate on the processing worksheet that it is for certification and include the full name and site ID. Since the sample used for certification should not be obtained from an AsthmaNet participant, participant ID, participant initials and visit number information will not be available and therefore should not be completed in the header of the SPUTUM_PROCESS form. Visit Date should be completed as the date when the sample was collected/processed.

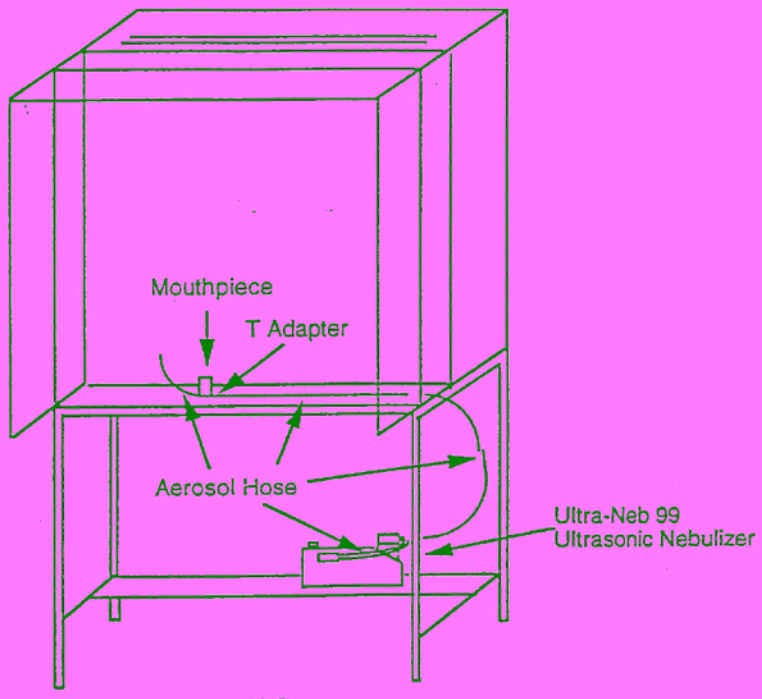
   If the completion of the processing worksheet was not satisfactory, the reviewer from San Francisco will contact you to review the necessary steps. If your first attempt was not satisfactory, process a new sample and email or fax the corresponding worksheet again as noted above.

   Note to the reviewer at San Francisco: If the worksheet was completed correctly, indicate on it that the completion was satisfactory, sign, date and email it to the DCC at AsthmaNet-Certification@phs.psu.edu. In the subject of the email, include a brief description of the certification type and site ID that the processor should have included on the worksheet (ex., Sputum Processing Certification

Worksheet, site 121). If it was not completed correctly, contact the processor and review the necessary steps.

Appendix I: Nuaire Aerosol Containment Chamber Figure

APPENDIX I: NUAIRE AEROSOL CONTAINMENT CHAMBER FIGURE



Nuaire Aerosol Containment Chamber

STANDARD FORMS

4 ASTHMANET STANDARD FORMS

4.1 Standard Forms and Instructions

This section provides specific instructions needed to complete the AsthmaNet standard data collection forms. Most of these forms are entered into the study database and submitted to the DCC. The instructions for each form are in alphabetical order based on the form name found in the header of the form.

The following information is provided for each form: the purpose of the form, who completes the form, when the form should be completed, and form instructions. Each field on every form is identified by a 4-digit annotation number such as 1000. Some forms have fields identified with a ‘D’ following the 4 digits such as 1020D. These fields represent description fields. The recorded text should be entered into the AsthmaNet clinical data management system allowing a maximum of 100 characters. Most forms have a comments section (Question 6000) at the bottom of the form. The coordinator can record additional comments or information related to the form in this section. This information (maximum length of 250 characters) is entered into the AsthmaNet clinical data management system. If you are unable to find the specific information needed to complete a form, please contact the protocol-specific Primary Data Manager at (717) 531-3663.

10.1.1 Acute Asthma Assessment Questionnaire (AAAQ)

Purpose: To measure severity of asthma symptoms related to asthma exacerbations.

Who: The participant completes this form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

The Acute Asthma Assessment Questionnaire is a 7-question tool for participants ages 12 and older to report a 72-hour review of the severity of their asthma and the possible cause of an asthma exacerbation. This tool is used primarily to measure the duration and severity of asthma exacerbations.

Participant completed forms that are completed at study visits should always be reviewed by the coordinator upon completion. If a correction is noted, the participant should make the correction and initial and date next to the change. Coordinators should not alter participant completed forms.

The participant must complete the source documentation box (using 2 or 3 initials) on page 2 (Q1070 – Q1090). Enter the Date field in the database in the format mm/dd/yyyy. Enter the time field based on a 24-hour clock.

10.1.2 Additional Treatment Post Methacholine Challenge Testing (METHA_ADD_TRT)

Purpose: This form should be completed following methacholine challenge testing if the participant did not reverse to 90% of baseline FEV_1 after the standard reversal treatment of 2 or 4 puffs of albuterol.

Who: The Pulmonary Function Technician administers the additional treatment, pulmonary function tests, and completes the form.

When: If the participant's FEV_1 is not greater than the reference value after standard reversal from methacholine challenge testing.

Form Instructions:

Complete this form ***only*** if the participant needed additional treatment after the Methacholine Challenge procedure. The METHA_ADD_TRT form is always entered as a single form at the same visit as the METHA packet form.

If the technician completing the procedure is not certified and this procedure is being used as an observation session for certification, please complete the Supervisor ID located in the header on the METHA_ADD_TRT form with the ID of the certified technician who supervised the procedure. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the Supervisor ID when applicable could result in a protocol deviation.

Question 1000. If Q1000 is answered 'Yes', record the types of additional treatment used within the first hour post-challenge in Q1010-Q1060. If no additional treatments were used during this time period, skip to Q1110.

Question 1070. Record the participant's FEV_1 after additional treatment within the first hour. The FEV_1 value should come from the Post II Composite row of the MedGraphics Methacholine Report or, if the participant performed more than one maneuver after additional treatment, the row within the Post II section corresponding to the time of the first manuever.

Question 1090. Record the time based on a 24-hour clock (military time).

Question 1100. Determine if the participant's FEV_1 at this time is greater than or equal to the methacholine reversal reference value (B) in the gray box at the top of the METHA form. If the participant's FEV_1 is greater than or equal to the reference value, ***stop*** completing this form and continue with the remaining visit procedures. If the participant's FEV_1 is not greater than the reference value, continue on to Q1110.

Question 1110. If Q1110 is answered 'Yes', record the types of additional treatment used after one hour in Questions 1120-1190. If no additional treatments were used, continue on to Question 1200.

Questions 1200. Record the participant's final FEV_1 after all reversal treatment following methacholine challenge. The FEV_1 value should come from the Post II Composite row of the MedGraphics Methacholine Report or, if the participant performed more than one maneuver after additional treatment, the row within the Post II section corresponding to the time of the final maneuver.

Question 1220. Record the time based on a 24-hour clock (military time).

Question 1230. Determine if the participant's FEV_1 at this time is greater than or equal to the methacholine reversal reference value (B) in the gray box at the top of the METHA form. If the participant's FEV_1 is not greater than or equal to the reference value, ***have the study physician complete the source documentation box at the end of this form (Q1240-Q1260).*** If the physician's signature was obtained, enter a 1 in the database. Otherwise, leave the field blank during data entry. Enter the Date field in the database in the format mm/dd/yyyy.

The corresponding report for the METHA_ADD_TRT form is the MedGraphics Methacholine Final Report, abbreviated METHA_RPT. Refer to the protocol-specific MOP for details on when this report is used.

10.1.3 Additional Treatment Post Sputum Induction (SPUTUM_ADD_TRT)

Purpose: This form should be completed if the participant experienced > 10% fall from reference FEV_1 immediately after completion of sputum induction.

Who: The Pulmonary Technician administers the additional treatment, pulmonary function tests, and completes the form.

When: If the participant experienced a > 10% fall from reference FEV_1 immediately after completion of the sputum induction procedure.

Form Instructions:

Complete this form ***only*** if the participant experienced a greater than 10% fall from reference FEV_1 immediately after completing the sputum induction procedures. The SPUTUM_ADD_TRT form is always entered as a single form at the same visit as the SPUTUM packet form.

The Sputum Induction Reversal Reference Value is the value recorded for Q1030 on the Sputum Induction Checklist (SPUTUMCHK) form multiplied by .90.

Questions 1000 and 1010. The FEV_1 and FEV_1 (% predicted) can be found in the Post Composite row of the MedGraphics Spirometry Report. Record Q1000 to the nearest hundredth of a decimal.

Question 1020. Record the time based on a 24-hour clock (military time).

Question 1030. If Q1000 is less than the calculated sputum induction reversal reference value (found in the Clinic Use Only gray box at the top of this form), administer 2 puffs of albuterol, perform spirometry, and continue to complete the rest of the SPUTUM_ADD_TRT form. Otherwise, stop and continue with the remaining visit procedures.

Questions 1040 and 1050. The FEV_1 and FEV_1 (% predicted) can be found in the Post Composite row of the MedGraphics Spirometry Report. Be careful to select the row with the later time stamp since the best post test will be listed first regardless of the order in which tests were performed. Record the value for Question 1040 to the nearest hundredths of a decimal place.

Question 1060. Record the time based on a 24-hour clock (military time).

Question 1070. If Q1040 is less than the calculated sputum induction reversal reference value, answer ‘No’ and have the attending physician complete the source documentation box (Q1080-Q1100).

The corresponding report for the SPUTUM_ADD_TRT form is the MedGraphics Spirometry Final Report, abbreviated SI_RPT. Refer to the protocol-specific MOP for

details on when this report is used. To clarify that this Spirometry Final Report is listing maneuvers performed post-sputum induction, please add a comment to the ‘Post Test Comments’ section of the report.

10.1.4 Adult Asthma and Allergy History (ASTHMA_HX_ADULT)

Purpose: To record an overview of an adult participant's asthma history including family history, symptoms, triggers, allergies, and smoking history.

Who: An AsthmaNet coordinator interviews the participant while completing the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Questions 1000 and 1070. Have the participant give a best estimate of his/her age. If the participant reports 20.5 years, round up to the nearest whole year. If the participant was younger than 1 year old, record the participant's age as 00 years.

Questions 1090 – 1110. If the participant is adopted and does not know his or her biological parents and/or siblings, answer 'Don't Know.'

Question 1110. Record 'N/A' if the participant does not have any biological siblings.

Question 1120. Record 'N/A' if the participant does not have any biological children.

Questions 1140, 1150, 1160, and 1170. If the participant's asthma symptoms vary by season (Q1130), multiple seasons may be answered as 'Yes'; however, not all 4 seasons should be answered 'Yes.'

Questions 1180 – 1220 and 1260. If the participant answered 'none' to any of these questions, record '0' for the response.

Question 1300. In the scenario where a female participant has had a hysterectomy and no longer has a monthly menstrual cycle, she would not be able to correlate fluctuations in her asthma symptoms with her cycle anymore. Q1300 should be answered "Don't Know."

Question 1400. If Q1400 is answered 'Yes', please record a description for 'Other' in Q1400D.

Question 1410. If Q1410 is answered 'Yes', please record a list of medications for Q1410D.

Question 1420. If Q1420 is answered 'Yes', please record a list of foods for Q1420D.

Question 1460. If Q1460 is answered 'Yes', please record a description for 'Other' in Q1460D.

Questions 1570 – 1590. If the participant is adopted and does not know his or her biological parents and/or siblings, answer ‘Don’t Know.’

Question 1590. Record ‘N/A’ if the participant does not have any biological siblings.

Question 1600. Record ‘N/A’ if the participant does not have any biological children.

Question 1740. If Q1740 is answered ‘Yes’, skip to Q1760. Otherwise complete Q1750.

Questions 1750 and 1770. 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.

Questions 1810 and 1820. Have the participant give a best estimate.

Questions 1830 and 1850. Have the participant give a best estimate. Month values should be rounded, as these fields only accept whole numbers. If the participant vaped or used a hookah for less than 6 months out of a year, round down to the nearest year. Six or more months of use should be rounded up to the next year. For example, if the participant has been vaping for 5 months, Q1830 should be answered ‘0’.

Questions 1860-1880. If the participant cannot remember the exact date, the month and year or the year only can be entered.

10.1.5 Adult Body Measurements (BODYMEAS_ADULT)

Purpose: To record the height, weight, and circumference measurements of an adult participant.

Who: An AsthmaNet coordinator completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Questions 1000 and 1010. The participant should remove shoes and heavy articles of clothing prior to taking these measurements. Calculate the participant body mass index (BMI) using the formula in the grey reference box and record the value. This value will not be entered into the study database.

Questions 1020 – 1040. The participant should be standing facing forward, with shoulders relaxed while taking these measurements using a plastic measuring tape.

For more information related to taking these measurements, please refer to Section 3 of the AsthmaNet General MOP.

10.1.6 Adult 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 the methacholine challenge testing.

Who: The Pulmonary Function Technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

If the technician completing the procedure is not certified in methacholine challenge testing, a supervisory technician who is certified should monitor the technician and record his or her number in the Supervisor ID field at the top of the form. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the Supervisor ID when applicable could result in a protocol deviation.

Complete this form only if the participant is eligible according to the protocol-specific Pulmonary Procedure Checklist and successfully completed baseline spirometry session(s).

Question 1000. If Q1000 is answered 'Yes', answer Q1010 and have a physician sign the form.

Question 1020. If the physician's signature was obtained, enter a 1 in the database. Otherwise, leave the field blank during data entry.

Question 1050. Refer to the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form and the Clinical Adverse Events (AECLIN) form.

Question 1060. The participant is ineligible to perform a methacholine challenge if the FEV_1 (% predicted) value recorded for Q1040 of the Spirometry Testing (SPIRO) form is less than 55% of predicted OR if the FEV_1 value recorded for Q1030 on the Spirometry Testing (SPIRO) form is less than 1.0 L.

Question 1070. Check 'N/A' if the participant is male or is a female that is post-menopausal, had a hysterectomy or a tubal ligation. A post-menopausal woman is defined as someone who has not had a regular period in over a year.

Question 1080. The participant is ineligible to proceed if his/her systolic blood pressure is > 200 mmHg or his/her diastolic blood pressure is > 100 mmHg.

Question 1100. If there is a reason the participant should not proceed with methacholine challenge testing that has not been captured on the form, explain in the space provided (Q1100D).

Question 1110. If the participant is deemed eligible, proceed to the Methacholine Challenge Testing using the Methacholine Challenge Testing (METHA) form. The participant is deemed ineligible for the diluent (solution #0) pulmonary function testing for the methacholine challenge if any of the shaded boxes are completed.

10.1.7 Allergy Skin Test Results (SKIN_TEST)

Purpose: To record whether or not the participant has a positive reaction to various allergens.

Who: An AsthmaNet coordinator certified to perform skin testing.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Question 1000. Respond 'Yes' to Q1000 if (1) prior skin testing was performed by an AsthmaNet certified coordinator or technician and (2) prior skin testing occurred within the protocol-specific time limit. Protocol-specific time limits for reusing the SKIN_TEST form can be found in the Manual of Operations for each protocol. If Q1000 is answered 'No', proceed to Q1030.

Questions 1010 and 1020. If Q1000 is answered 'Yes', provide the date of the previous skin test in Q1010 and the ID of the coordinator who performed the skin test in Q1020. Stop here and continue with remaining visit procedures. Attach a photocopy of the previous skin test form (pages 1 through 4) to this form. Update the participant ID and visit in the header of pages 2, 3 and 4 to reflect the current participant ID and visit. At the time of data entry, enter Q1000 - Q1020 from this form and enter the rest of the data from the photocopied form (original skin test).

Question 1030. If any exclusionary medications were taken within the washout period, the participant is ineligible to complete the allergy skin testing at this visit. See Appendix 5 of the AsthmaNet General MOP for more information regarding exclusionary drugs for skin testing.

Question 1040. If the participant's most recent FEV_1% predicted is < 60%, the supervising physician must give permission to proceed with the skin testing procedure. The most recent FEV_1% predicted is the last spirometry maneuver completed prior to skin testing. If the supervising physician gives permission to proceed, record 'Yes' for Q1050 and obtain the physician's signature on Q1055. If the physician's signature was obtained, enter a 1 in the database. Otherwise, leave the field blank during data entry. If the supervising physician does not give permission to continue, the participant is ineligible to complete the allergy skin testing at this visit.

Question 1060. If any of the shaded boxes are completed (Q1030 or Q1050), the participant is not eligible to proceed with allergy skin testing. Allergy skin testing may be rescheduled for the next visit (refer to protocol-specific MOP).

Question 1070. If the participant had a severe systemic reaction in the past to allergy skin testing, do not continue. Refer to the protocol-specific MOP for details on how to proceed.

Questions 1080 – 1100. Do not skin test a particular allergen if the participant has had a previous anaphylactic reaction to this allergen. Do not complete the 'reaction' questions (Q1430, Q1490, Q1550) for the corresponding allergen on the Allergy Skin Test Results (SKIN_TEST) form. Refer to the protocol-specific MOP for details on how to proceed.

Questions 1110 and 1120. Record the time based on a 24-hour clock (military time).There should be a 20-minute interval from the time the skin test sites were pricked (Q1110) and the time the participant's skin is evaluated (Q1120).

Questions 1130-1140. If the value of the positive control calculation (Q1130) is less than 3 mm (Q1140), the skin test is not valid. Do not complete the rest of the form. Refer to the protocol-specific MOP for details on how to proceed.

Questions 1150-1170. If the absolute value of the positive control calculation (Q1130) minus the negative control calculation (Q1150) is less than 3 mm (Q1170), the skin test is not valid. Do not complete the rest of the form. Refer to the protocol-specific MOP for details on how to proceed.

Questions 1190 – 1660. For each allergen, 1 – 16, indicate whether there was a positive reaction. A positive reaction is defined as a wheal size $\geq$ Q1180. Transfer the tracing of each wheal and record the longest diameter and the diameter at the perpendicular midpoint in mm. If the wheal is not measurable, record '0' for both diameters. The diameters should be recorded even if there was not a positive reaction. If a specific allergen will not be used (i.e. study participant has a known allergy to a specific food or other allergen), the Multi-Test arm should be broken off. The questions for that specific allergen should be left missing and any resulting errors pertaining to that allergen should be marked unresolvable with an appropriate comment.

For more information on skin testing procedures and definitions, see Appendix 5 of the AsthmaNet General MOP.

10.1.8 Asthma Bother Profile (ABP)

Purpose: To measure a participant's level of distress due to asthma.

Who: An AsthmaNet coordinator interviews the participant while completing the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

The coordinator should read to the participant the information contained in the four bulleted items at the top of the questionnaire.

AsthmaNet will define retired as no longer performing any work for monetary payment. For example, an individual retires from their primary job, however still works part-time or is paid for consulting work, the answer to question Q1000 should be 'No'.

Question 1000. If Q1000 is answered 'Yes', complete Q1010; otherwise, skip to Q1020.

Question 1020. If Q1020 is answered 'Yes', complete Q1030; otherwise, skip to Q1040.

Question 1040. If Q1040 is answered 'Yes', complete Q1050; otherwise, skip to Q1060.

Question 1090. If Q1090 is answered 'Yes', complete Q1100 and Q1110; otherwise, skip to Q1110.

Question 1170. If Q1170 is answered 'Yes', complete Q1180, otherwise, skip to Q1190.

10.1.9 Asthma Control Questionnaire© (ACQ)

Purpose: To measure control of asthma symptoms during the past week.

Who: The participant completes this form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

AsthmaNet negotiated a license agreement with Dr. Elizabeth Juniper to use the Asthma Control Questionnaire© form in AsthmaNet studies. However, the original format of the form cannot be changed in any way. The header information including the Participant ID, Participant Initials, Visit, Visit Date, and Coordinator ID will be completed in the upper right hand corner of the form using the fillable PDF feature when preparing the visit packet.

This form should be completed before the participant undergoes spirometry.

Review the form after the participant or participant's parent/guardian has completed the form to ensure he/she circled a response for each question. Participant completed forms should always be reviewed by the coordinator upon form completion. If a correction is noted, the participant should make the correction and initial and date next to the change. Coordinators should not alter participant completed forms.

During data entry of this form, Questions 1 through 6 will be designated as (1) through (6) on the entry screen.

10.1.10 Asthma Control Questionnaire® (7 Question Version) (ACQ7)

Purpose: To measure control of asthma symptoms during the past week.

Who: The participant or participant's parent/guardian completes Questions 1 – 6, and the coordinator completes Question 7.

When: Refer to Visit Procedure Checklists.

Form Instructions:

AsthmaNet negotiated a license agreement with Dr. Elizabeth Juniper to use the Asthma Control Questionnaire© form in AsthmaNet studies. However, the original format of the form cannot be changed in any way. The header information including the Participant ID, Participant Initials, Visit, Visit Date, and Coordinator ID will be completed in the upper right hand corner of the form using the fillable PDF feature when preparing the visit packet.

Questions 1 through 6 should be completed by the participant before he/she undergoes spirometry. Instruct the participant not to complete Question 7.

Review the form after the participant or participant's parent/guardian has completed the form to ensure he/she circled a response for Questions 1 - 6. Participant completed forms should always be reviewed by the coordinator upon form completion. If a correction is noted in Questions 1-6, the participant should make the correction and initial and date next to the change. Coordinators should not alter participant completed questions.

If a correction is noted in Question 7, the coordinator may make the correction and initial and date next to the change. The participant is not required to make any corrections to Question 7 since it is coordinator-completed.

Question 7. After the participant has undergone spirometry, the coordinator should record the pre-bronchodilator FEV_1, FEV_1 predicted, and FEV_1 % predicted on the dotted lines next to Q7. *These values are not entered.* The coordinator should then circle the response corresponding to the range in which the participant's FEV_1 % predicted falls, from 0 (> 95%) to 6 (<50%); this value is entered.

During data entry of this form, Questions 1 through 7 will be designated as (1) through (7) on the entry screen.

10.1.11 Asthma Control Test™ (ACT)

Purpose: To determine how well the participant's asthma is controlled.

Who: Participant or Participant's Parent/Legal Guardian completes this form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

The AsthmaNet negotiated a license agreement with Quality Metric Incorporated to use the Asthma Control Test™ form in AsthmaNet studies. However, the original format of the form cannot be changed in any way. The header information including the Participant ID, Participant Initials, Visit, Visit Date, and Coordinator ID will be completed in the upper right hand corner of the form using the fillable pdf feature when preparing the visit packet.

Review the form after the participant or participant's parent/guardian has completed the form to ensure he/she marked a response (within the designated box) for each question. Ignore the scoring instructions at the bottom of the screen. The Asthma Control Test (ACT) will be scored using the data entered into the AsthmaNet data management system.

During data entry of this form, Questions 1 through 5 will be designated as Q1 through Q5 on the entry screen.

10.1.12 Asthma Quality of Life Questionnaire (AQLQ_12)

Purpose: To evaluate the participant's quality of life as a result of asthma.

Who: The participant completes this form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

AsthmaNet negotiated a license agreement with Dr. Elizabeth Juniper to use the Asthma Quality of Life Questionnaire© (AQLQ) form in AsthmaNet studies. However, the original format of the form cannot be changed in any way. The header information including the Participant ID, Participant Initials, Visit, Visit Date, and Coordinator ID will be completed across the header of the form using the fillable pdf feature when preparing the visit packet.

This version of the AQLQ is validated for individuals ages 12 and older. Participants are asked how their asthma has affected their lives over the ***last two weeks***. Instruct the participant to read each question carefully and circle the number corresponding to his or her best answer for each question. Directions on the questionnaire itself should be followed exactly.

This form should be completed before the participant undergoes spirometry. Standard questionnaires that collect information on a participant's perception of his/her asthma are administered before study procedures begin in order to avoid bias. The order of procedures on the Visit Procedure Checklist should be followed exactly.

Review the form after the participant has completed it to ensure that he/she clearly circled only one response for each question. Participant completed forms should always be reviewed by the coordinator upon form completion. If a correction is noted, the participant should make the correction and initial and date next to the change. Coordinators should not alter participant completed forms.

During data entry of this form, Questions 1 through 32 will be designated as (Q1) through (Q32) on the entry screen. All pages of this form must be presented to the participant, including the cover sheet. However, the cover sheet does not need to be forwarded to the DCC.

10.1.13 Asthma-Specific Work Productivity and Activity Impairment Questionnaire (WPAI_ASTHMA)

Purpose: To measure the effect of asthma on the participant's ability to work, attend classes, and perform regular daily activities.

Who: The participant completes this form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

The participant should answer the questions based on the past seven days, not including the day the participant is completing the form.

Question 1000. If Q1000 is answered 'Yes', the participant should complete Q1010, Q1020 and Question #4 (Q1030); otherwise skip to Q1040.

Questions 1010 and 1020. Record to the nearest tenths of a decimal.

Question 1040. If Q1040 is answered 'Yes', the participant should complete Q1050, Q1060 and Question #8 (Q1070); otherwise skip to Q1080.

Review the form prior to the participant leaving the clinic to ensure that the participant completed the form correctly. The participant should clearly circle one number for Questions 4, 8, and 9.

After the participant has completed the form, the AsthmaNet coordinator completes Q1030, Q1070, and Q1080 by recording the number the participant circled in the corresponding grey boxes.

To verify that the information recorded on this form is correct, have the participant initial (using 2 or 3 initials), date, and record the time in the source documentation box provided (Questions 1090-1110). Enter the Date field in the database in the format mm/dd/yyyy.

10.1.14 Asthma Symptom Utility Index (ASUI)

Purpose: To record how often asthma symptoms bothered a participant in the past 2 weeks.

Who: An AsthmaNet coordinator interviews the participant while completing the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Participants are asked questions about the severity and frequency of their asthma symptoms over the ***past two weeks.***

The coordinator should encourage the participant to be as precise as possible when reporting the number of days that he or she was bothered by asthma symptoms.

Question 1080. If the participant was bothered by side effects of his/her medication for one or more days, describe the side effects the participant experienced in Q1080D.

To verify that the information recorded on this form is correct, have the participant initial (using 2 or 3 initials), date, and record the time in the source documentation box provided on page 2 (Q1100-Q1120). Enter the Date field in the database in the format mm/dd/yyyy.

10.1.15 Childhood Asthma Control Test For Children 4-11 Years Old™ (CACT)

Purpose: To determine how well a pediatric participant's asthma is controlled.

Who: Participant and Participant's Parent/Legal Guardian complete this form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

AsthmaNet negotiated a license agreement with GlaxoSmithKline to use the Childhood Asthma Control Test™ (CACT) in AsthmaNet studies. However, the original format of the form cannot be changed in any way. The header information including the Participant ID, Participant Initials, Visit, Visit Date, and Coordinator ID will be completed across the header of the form using the fillable pdf feature when preparing the visit packet.

The pediatric participant completes Questions #1 - #4. If the child cannot read, the parent/guardian can ask the child the first four questions while showing him/her the happy/sad face pictures. The parent or guardian completes Questions #5 - #7. It is usually not necessary for the parent or guardian to score the form; however, the protocol-specific MOP should be consulted.

The child and/or parent/guardian may circle a response and/or complete the response in the Score box to the right of the question. If the response is only circled or the response is only written in the Score box, it is not required that both be completed.

The coordinator should not make edits to or complete the individual Score box for each question. If there is a discrepancy between what is circled and what is indicated in the Score box, request that the parent/guardian make the necessary correction(s) to the response and initial and date the correction. The coordinator may total the score at the bottom of the form or correct this total if the parent/guardian completes it in error. Review the form after the participant and the participant's parent/guardian have completed it to ensure that a response has been provided for each question.

If there is a discrepancy between what is circled and what is indicated in the Score box for an individual question, and the parent/guardian is not available in the clinic to make the correction to the form, the coordinator should enter the circled response in the database during entry of CACT.

During data entry of this form, Questions 1 through 7 will be designated as (1) through (7) on the entry screen.

10.1.16 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: Refer to Visit Procedure Checklists.
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:

A clinical adverse event includes any new or worse than usual medical condition or inter-current illness the participant experiences during the course of the study. See Section 4 of the AsthmaNet General MOP for more information regarding adverse events.

At the first study visit, record all current events the participant experienced since signing the informed consent onto the Clinical Adverse Events (AECLIN) form. If a participant is not currently experiencing any adverse events, complete the information at the top right hand corner of the form and check the 'None' box.

At each subsequent visit, record all adverse events that have occurred since the previous visit. If a participant has not experienced any adverse events since the last visit, complete the information at the top right hand corner of the form and check the 'None' box. If any event is still ongoing at the current visit, leave the stop date and outcome blank and check the 'ONGOING at current visit' box (Q1040).

If the participant (or participant's guardian) contacts the clinic coordinator between visits, record the new adverse event on the AECLIN form completed at the last visit. This new adverse event should be updated in the Participant Data module within the data management application.

At each visit, review all ongoing events with the participant to acquire a stop date and outcome for each event. Ongoing events may be reviewed using the AECLIN form or the ongoing reports generated by the data management application. See Section 7 of the AsthmaNet General MOP for more information about Ongoing Adverse Events Reports.

At the participant's last study visit, review all ongoing events. If an event is ongoing at the final visit, leave the stop date and outcome blank and check the 'ONGOING at final visit' box.

A Clinical Adverse Events (AECLIN) form should be completed and entered for every study visit, even if the participant did not experience any adverse events at that visit.

Clinical Adverse Events forms should be data entered in the Participant Data module with the Entry Type of Concurrent (C) at the time the form is initiated. As the participant continues through the study and events are resolved, the updates should be recorded on the data collection form and then updated in the Participant Data module. Upon completion or early termination from the study, all Clinical Adverse Events forms should be sent to the DCC.

Question 1000. Describe the clinical adverse event. DO NOT complete the event number during form completion or prior to data entry. During data entry, record the number assigned by the module to each adverse event. This number will be a ***unique*** and ***chronological*** number assigned by the database. Number the form after all forms are completed to ensure related events are linked with the proper medication.

If an adverse event needs to be removed, for example, adverse event #07, simply cross that row off and initial and date the form. DO NOT RENUMBER the form.

Question 1010. Identify the appropriate ICD9 code to describe the underlying condition or disease. Procedure codes should not be recorded.

A list of all ICD9 Codes used for AsthmaNet is available in an Excel spreadsheet on the AsthmaNet secure web site under the Application link.

A link to the list of ICD9 Codes also can be found during data entry and editing in the Participant Data module of the data management application.

A searchable list is also available during entry and editing by selecting the "ICD" button next to the Q1010 entry. Type in the ICD9 code or health problem or parts of the health problem and a list of matching ICD9 codes and descriptions will display for you to select.

If the coordinator is still unable to identify an ICD9 code, please call the Primary Data Manager at (717) 531-3663 for assistance.

Questions 1020 & 1030. If the adverse event is still present at the time of the current visit, leave the 'Data Stopped' blank (Q1030) and check the 'ONGOING at current visit' box (Q1040).

Questions 1050 and 1060. Record the Type and Severity of each adverse event.

Question 1070. A serious adverse event is defined as an event resulting in hospitalization, extension of a hospital stay, or death. Outpatient procedures, including outpatient surgery, and visits to the Emergency Department (ED) without further hospitalization are NOT considered serious adverse events. If the adverse event is serious, complete the Serious Adverse Event Reporting (SERIOUS) single form and fax it to the DCC within 72 hours at 717-531-4359.

Question 1080. Study drug(s) should be interpreted as blinded or unblinded study inhalers, tablets, capsules, or pills.

Question 1090. Study drug(s) should be interpreted as blinded or unblinded study inhalers, tablets, capsules, or pills. Indicate “Unchanged” if the change in study drug was made according to the protocol. Indicate “Altered” only if the change in study drug was related to a non-protocol reason (i.e., principal investigator decides to take participant off of the inhaler(s)). If Q1090 is answered ‘Altered’, complete the appropriate Change in Medications form.

Question 1100. Do not complete this question if the ‘Date Stopped’ is missing. If the outcome is death, a Serious Adverse Event Reporting (SERIOUS) single form should be completed.

Question 1110. If the participant requires hospitalization, complete the Serious Adverse Event Reporting (SERIOUS) single form and fax it to the DCC within 72 hours. Hospitalization is defined as admittance to the hospital and does not include Emergency Department (ED) visits where the participant is not admitted to the hospital or outpatient surgery/procedures. If the participant requires medication, please record it on the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) concurrent form.

Question 1120. If at the final visit with the participant there are event(s) still ongoing (i.e., no ‘Date Stopped’ recorded and ‘ONGOING at current visit’ was checked), be sure that the ‘ONGOING at final visit’ box is checked.

If it is unknown whether an event was stopped or ongoing at the participant’s termination (for instance, in the case of a participant who has been lost to follow-up), check the ‘ONGOING at final visit’ box. This will indicate that the event was still ongoing as of the last contact with the participant.

The AECLIN forms should not be submitted to the DCC until the participant has completed the study or has been terminated from the study.

If there are ‘Date Stopped’ data missing (i.e., an event has not ended), or other changes to the form need to be made, the site can make changes as needed through the Participant Data module and then send the forms to the DCC all at one time after the participant completes or terminates the study.

10.1.17 Cold History (COLD_HX)

Purpose: To record a participant's cold history over the past 12 months and the effect on his/her asthma.

Who: An AsthmaNet coordinator completes the form while interviewing the participant or participant's parent or guardian.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Question 1000. If Q1000 is answered 'Other', record a description in Q1000D.

Question 1010. If the participant did not experience any respiratory tract infections/colds in the past 12 months, record '00'.

Question 1030. If Q1030 is answered 'No', do not complete the rest of the form.

10.1.18 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: Refer to Visit Procedure Checklists. **Note:** This form should be completed if the participant or participant's guardian 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:

At the first study visit, record all of the concomitant medications related to asthma/allergies and adverse events that the participant has taken since signing the informed consent. At each subsequent visit, record all of the concomitant medications related to asthma/allergies and adverse events that the participant has taken since the previous visit. Do not list routine use of study drugs or rescue medications. If a participant has not taken any asthma/allergy and adverse events related concomitant medications since the last visit, complete the information in the upper right hand corner of the form and check 'None'. If the participant is still taking a medication at the end of the current visit, leave the stop date blank and check the 'Ongoing at Current Visit' box.

At each visit, review all ongoing medications with the participant to acquire a stop date. Ongoing medications may be reviewed using the CMED form or ongoing records report generated by the data management application. See Section 7 of the AsthmaNet General MOP for more information on the Ongoing Medications Report.

At the participant's last study visit, review all ongoing medications. If the participant is still taking a medication at the final visit, leave the stop date blank and check the 'Ongoing at Final Visit' box.

A Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form should be completed for each participant in the study at each visit, even if the participant has not taken any concomitant medications since the last visit.
The Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) forms should be data entered in the Participant Data module with the Entry Type of Concurrent (C) at the time the form is completed. As the participant continues through the study and medications are discontinued, the updates should be recorded on the

data collection form and then updated in the Participant Data module. Upon completion or early termination from the study, all CMED forms should be sent to the DCC.

Question 1000. Name of Medication**.** DO NOT complete the event number during form completion or prior to data entry. During data entry, record the number assigned in the module to each medication. This number will be a ***unique*** and ***chronological*** number assigned by the database.

If a medication needs to be removed, for example, medication #05, simply cross that row off and initial and date the form. DO NOT RENUMBER the form. Duplicate numbers are not to be used; each medication must have its own ***unique*** medication number.

If the participant contacts the coordinator between visits, record the new medication 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.

If the medication is a combination drug, each component of the drug must be listed on the CMED form as a separate record. For example, Advair® would have two records entered on the CMED form; a record for fluticasone and a record for salmeterol.

Question 1010. Identify the appropriate drug code for the recorded medication. A list of drug codes can be found in an Excel spreadsheet on the AsthmaNet secure web site under the Application link. The drugs are listed as generic names. In addition, a Search Drug Codes link (that will display the same Excel spreadsheet) can be found during data entry and editing in the Participant Data module of the AsthmaNet data management application. A searchable list is also available during entry and editing by selecting the “D” button next to the Q1010 entry. Type in the medication name or a string of characters contained in the medication name and a list of matches will display with their corresponding drug codes. If the coordinator is still unable to find a corresponding drug code, please log into the AsthmaNet web site under Coordinator Resources: Forms and complete the Drug Code Request Form. Please complete your name, request date, the participant ID, and known information about the drug’s generic name, brand name, drug class or indication. Any questions/concerns should be directed to the asthmanet-drug_code_req@phs.psu.edu email alias.

Question 1020. If the medication is not related to an adverse clinical event, leave the event number missing, check the ‘N/A’ box and enter a zero into the database. If the medication is related to an adverse event, complete the event number from the AECLIN form after the AECLIN form has been entered and the Adverse Event number is assigned.

Question 1030. If the dose of medication is tapered across time, the dose that is listed should be the dose that is administered on the first day the medication is taken.

Questions 1040, 1050, and 1055. A list of codes for the units (Q1040), frequency (Q1050), and route (Q1055) are located on the AsthmaNet secure website under

Standard Forms/Reference/Units, Frequency, and Route Codes for Concomitant Medications (CMED_REF).

When recording the dose, units, and frequency of a medication, it is important to record the amount of the drug taken using weight or volume, if applicable. Do not record medication dosages in quantities (e.g., puffs).

Q1030 should always record the strength of the medication. If the participant or parent/guardian does not know the strength of the medication, the coordinator can go to the PDR (pdr.net) to get the information.

For example, a search on pdr.net for diphenhydramine, will return several results. The product label for Children's Benadryl Allergy Liquid will indicate 12.5 mg per 5 ml (5ml = 1 tsp). If the participant or parent/guardian indicates 1 tsp was taken, 12.5 would be recorded in Q1030 and '1' for milligrams would be recorded in Q1040.

If the participant was prescribed ceritizine, 1ml = 1mg (5 ml = 1 tsp) or 5mg per tsp. So if the participant or parent/guardian indicates 1 tsp was taken, 5 would be recorded in Q1030 and '1' for milligrams would be recorded in Q1040.

If the participant was prescribed 80 micrograms of beclomethasone, 80 would be recorded in Q1030 and '2' for micrograms would be recorded in Q1040.

Q1040 should always record the unit of measurement however there are specific responses that are required for the following medication categories.

Category	**Acceptable Response**
Antihistamine	Code 1 (mg) or 2 (mcg)
Corticosteroid	Code 1 (mg) or 2 (mcg)
Beta-2 Adrenergic Agonist	Code 1 (mg) or 2 (mcg)
Anticholinergic Agent	Code 2 (mcg)
Leukotriene Modifier	Code 1 (mg)
Xanthine Derivative	Code 1 (mg)

Indicate the correct codes for each medication listed on the form. If any code cannot be found, log into the AsthmaNet web site under Coordinator Resources: Forms and complete the Drug Code Request Form. Please complete your name, request date, the participant ID, and known information about the drugs generic name, brand name, drug class or indication. Any questions/concerns should be directed to the asthmanet-drug_code_req@phs.psu.edu email alias.

If a medication is a tapered dose, Q1050 should be recorded as ‘taper dose’ for the record.

At the time of entry, the medication number (Q1000) should be written on the form as it appears on the entry screen.

Question 1060. The ‘Start Date’ field (Q1060) must be completed. For any medications the participant started prior to signing the informed consent, record the informed consent date as the ‘Start Date’.

Questions 1070, 1080, and 1090. If the participant is still taking a medication at the time of the visit, leave the ‘Stop Date’ (Q1070) blank and check the ‘Ongoing at current visit’ box (Q1080).

Question 1090. If at the final visit with the participant there is a medication(s) still ongoing (i.e., no ‘Date Stopped’), check the ‘Ongoing at final visit’ to close out the record.

If it is unknown whether a medication was stopped or ongoing at the participant’s termination (for instance, in the case of a participant who has been lost to follow-up), check the ‘Ongoing at final visit’ box. This will indicate that the medication was still being taken as of the last contact with the participant.

Send all CMED forms to the DCC once a participant completes the study or terminates. When collating and mailing this form to the DCC, all Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) forms completed for a participant should be **paper clipped** together as one form. These forms should be arranged in visit number order. Once all forms are completed for a participant, complete the ‘Form Page __ of __ ’ field at the bottom center of each page. For example, if twenty-one Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) forms are completed, the first form should be numbered ‘Form Page 1 of 21’, the second as ‘Form Page 2 of 21’, and so on respectively regardless of visit number assignment.

For more information on recording concomitant medications, see the Concomitant Medications discussion in Section 7 of the AsthmaNet General MOP.

10.1.19 Effects of a Child's Asthma Flare-up on the Parents (PARENT_QOL)

Purpose: To measure the effects on the parent's quality of life when a child suffers an asthma flare-up.

Who: A child's parent or legal guardian completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Questions Q1000 – Q1210. Shade in one circle for each question.

Question 1220. If the parent/legal guardian did not miss any work or regular planned activities to take care of his/her child, record '00'.

Question 1230. If the parent/legal guardian wan not about to perform his/her work or regular planned activities at all, record '000'.

Question 1250. If Q1250 is answered 'Other', record a description in Q1250D.

10.1.20 Exhaled Nitric Oxide (ENO)

Purpose: To record the outcome measures from the participant's Exhaled Nitric Oxide collection.

Who: The ENO Technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

If the technician in charge of the procedure is not ENO certified, a supervising technician, who is certified, should monitor the technician and record his or her ID number in the Supervisor ID field at the top of the form. In addition, this field needs to be entered into the database in the (Sup ID) field.

Exhaled Nitric Oxide must be performed prior to spirometry testing. Refer to Appendix 8 of the AsthmaNet General MOP for more information on ENO collection.

Question 1030. If NO concentration indicated on report is "<5," enter this as "5" in the database. These fields on the data entry screen will not accept the "<" symbol.

Question 1040. Record time eNO started based on a 24-hour clock (military time).

10.1.21 GIS Consent Tracking Form (GIS)

Purpose: To record information on the participant's consent to the Ancillary Geographic Information System (GIS) study.

Who: An AsthmaNet coordinator completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Question 1000. Reference the participant's GIS consent document for the current study. Record the date the consent was signed in Q1000.

Question 1010. Reference the participant's GIS consent document for the current study. If the participant and/or his/her guardian consented to allow the use of his/her home address for GIS analysis, answer this question 'Yes.' Otherwise, answer this question 'No.'

Participant/Guardian Source Documentation. After all data is recorded on the GIS form, the participant or his/her guardian should be asked to review all information and initial and date the source documentation box to verify the accuracy of the information. *Source documentation is necessary even if consent for GIS participation was not granted.*

10.1.22 Home Environment Questionnaire (HEQ)

Purpose: To record the characteristics of the participant's home environment.

Who: An AsthmaNet coordinator interviews the participant or participant's parent/ legal guardian while completing the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

If the participant's parent or guardian is completing the form for a child, point out to him/her that the reference 'you' in the questions is the child who is the study participant.

'House' is defined as the place where the participant lives most of the time. If the participant does not have a bed or bedroom, he/she should answer questions related to characteristics of the bedroom based on the place where he/she sleeps.

Question 1000. Check only one box. If Q1000 is answered 'Other', Q1000D should be completed.

Questions 1010 and 1020. Record the years and months the participant has lived in the current house. For example, if the participant has lived in the current house for 5 years and 2 months, record 5 for the Q1010 and 2 for Q1020. If unsure of the number of months or years, estimate to the nearest number of years and months.

Question 1090. If Q1090 is answered 'No' or 'Don't Know', skip to Q1170.

Question 1160. If Q1060 is answered 'Yes', complete Q1160D.

Question 1170. If Q1170 is answered 'No', skip to Q1260.

Question 1250. If Q1250 is answered 'Yes', complete Q1250D.

Question 1260. If Q1260 is answered 'No', skip to Q1350.

Question 1340. If Q1340 is answered 'Yes', complete Q1340D.

Question 1410. If Q1410 is answered 'Other', complete Q1410D.

Question 1420. If Q1420 is answered 'Other', complete Q1420D. If Q1420 is answered 'None', skip to Q1440.

Question 1440. If Q1440 is answered 'No', skip to Q1460.

Question 1460. If Q1460 is answered ‘Other’, complete Q1460D. If Q1460 is answered ‘None’, skip to Q1480.

Question 1480. If Q1480 is answered ‘No’, skip to Q1570.

Questions 1490 – 1550. Enter ‘00’ if no pets of a particular type live in the household. If there is one or more pet of a particular type, check whether this (these) pet(s) are Indoor, Outdoor, or Both.

Question 1620. If Q1620 is answered ‘Yes’, complete Q1620D.

If the participant is 6 years of age or older, STOP HERE and complete the source documentation box. The source documentation must be completed even if Q1630 – Q1700 are skipped based on the age of the participant.

Questions 1630 – 1700. These questions should be completed only if the participant is 5 years of age or younger.

Question 1630. If Q1630 is answered ‘Yes’, complete Q1640.

Question 1650. If Q1650 is answered ‘No’, do not complete Q1660 – Q1700. The source documentation box must be completed even if Q1660 – Q1700 are not completed due to the skip pattern.

Review the form prior to the participant leaving the clinic to ensure that the participant completed the form correctly.

The participant or participant’s parent/legal guardian must complete the source documentation box (using 2 or 3 initials) on page 5 (Q1710 and Q1720). Enter the Date field in the database in the format mm/dd/yyyy.

10.1.23 Household Socio-economic Information (HOUSEHOLD_SEI)

Purpose: To record the socio-economic information of the participant's primary household.

Who: The participant or participant's parent/legal guardian completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

If the participant is a college student living away from home during the school year, the questions pertain to the parents' household.

For all other participants, 'Household' is defined as the place where the participant lives most of the time.

Question 1000. If Q1000 is answered 'Other', record a description in Q1000D.

Questions 1010 and 1020. The participant or participant's parent/legal guardian may refuse to answer these questions. Be sure to select the 'Decline to answer' option instead of leaving the question missing. Q1030 should be answered even if the participant or participant's parent/legal guardian declined to answer Q1020.

Review the form prior to the participant leaving the clinic to ensure that the participant completed the form correctly.

10.1.24 ImmunoCAP Results (IMMUNOCAP)

Purpose: To record a participant's ImmunoCAP results of one or more allergens.

Who: An AsthmaNet coordinator completes the form.

When: When allergy skin testing cannot be performed for one or more allergens.

Form Instructions:

ImmunoCAP Testing should be performed instead of allergy skin testing if the participant previously had a severe systemic reaction to allergy skin testing. Complete the testing for all allergens on the Allergy Skin Test Results (SKIN_TEST) form.

ImmunoCAP Testing should be performed if the participant has a history of or existing allergy to milk, egg, or peanut. The AsthmaNet coordinator certified in performing skin testing should not skin test for the food(s) that the participant is allergic to. Record the ImmunoCAP result(s) for the specific food(s) the participant had a reaction to.

ImmunoCAP Testing can be performed if the participant used any medications listed in the skin test section of the AsthmaNet General MOP within the exclusionary periods and an allergy skin test cannot be rescheduled.

Upon the discretion of the physician, based on interfering eczema, dematographisms, etc., ImmunoCAP Testing should be performed for both the food and aeroallergens on the Allergy Skin Test Results (SKIN_TEST) form.

Questions 1000 – 1130. If multiple values are received for a particular allergen, refer to the protocol-specific MOP as to how to record these values.

Upon receipt of the lab report, complete the ImmunoCAP Results (IMMUNOCAP) form as a single form at the same visit where the allergy skin testing was/should have been performed. Attach a Data Processing Cover Sheet (DPCS) to the form and send the single form to the DCC after data entry. Store the original lab report with a copy of the IMMUNOCAP form in the participant's study file at the clinical site.

The IMMUNOCAP form and original lab report will be reviewed at AsthmaNet site visits.

10.1.25 Pre-Bronchodilator IOS (IOS_PRE)

Purpose: To record the outcome measurements from the pre-bronchodilator IOS procedure.

Who: The Pulmonary Technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

If the technician in charge of the procedure is not certified in IOS testing, a supervising technician who is certified should monitor the technician and record his or her ID number in the Supervisor ID field at the top of the form. The supervisor ID number should be entered in the post test comments section of the test session. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the Supervisor ID when applicable could result in a protocol deviation.

The protocol-specific pulmonary procedure checklist (PULMONARYCHK) needs to be completed to determine eligibility before performing Pre-Bronchodilator Testing (IOS_PRE). Please refer to the protocol-specific MOP.

If a procedure needs to be rescheduled, complete a new Pre-bronchodilator IOS form with the current date. Do not enter the old Pre-bronchodilator IOS form into the study database and do not send it to the DCC. Only enter and submit (to the DCC) the most recent Pre-bronchodilator IOS form. However, all completed forms, the old original Pre-bronchodilator IOS form and the current photocopy of the Pre-bronchodilator IOS form should be filed in the subject's study file.

Question 1000. Record the time based on a 24-hour clock (military time).

Questions 1010-1080. These values will be obtained from the Act1 column on the Jaeger system IOS report.

Questions 1090-1160. These values will be obtained from the Act2 column on the Jaeger system IOS report.

Questions 1170-1240. These values will be obtained from the Act3 column on the Jaeger system IOS report.

Question 6000. This section is provided for the Clinic Coordinator to complete if the form requires addition comments or information. This question is data entered into the database.

Always print two copies of the Pre-bronchodilator IOS report from the Jaeger system.

If the form is a packet form, clip one report to the back of the visit packet and forward to the DCC (after data entry and resolution of entry errors). Place the report with the photocopy of the visit packet in the participant's study folder.

If the form is a single form, clip a report to the back of the single form and forward it to the DCC with a Data Processing Cover Sheet (DPCS) attached. Place the report with the copy of the single form in the participant's study folder.

During data entry for a visit packet or single form that contains the Pre-bronchodilator IOS procedure, you will need to acknowledge whether or not the Pre-bronchodilator IOS report is being submitted to the DCC. These reports are referred to as Tracking Forms in the AsthmaNet data entry application. When you reach the end of the visit packet data entry or single form data entry, possible reports related to that visit packet/single form will be listed with the default, 'Yes' (this report is being submitted) selected. Select 'No' if a particular report is not being forwarded to the DCC. If all other data entry procedures for this visit packet/single form are completed, select 'Save Data'.

See Section 2.21 in the AsthmaNet General Mop for more information regarding these procedures.

See Appendix 9 of the AsthmaNet General MOP for more information regarding Pre-bronchodilator IOS procedures.

10.1.26 Maximum Reversibility Testing (MAXREV)

Purpose: To record the maximal improvement in FEV_1 following albuterol treatment.

Who: The Pulmonary Function Technician completes the form.

When: Refer to Visit Procedure Checklists.

Complete this form ***only*** if the participant ***is eligible*** according to the protocol-specific Pulmonary Procedure Checklist (PULMONARYCHK) and ***successfully completed*** baseline spirometry sessions(s) (SPIRO).

Form Instructions:

If the technician in charge of the procedure is not certified in spirometry testing, a supervising technician who is certified should monitor the technician and record his or her ID number in the Supervisor ID field at the top of the form. The supervisor ID number should be entered in the post test comments section of the test session. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the Supervisor ID when applicable could result in a protocol deviation.

Administer 4 puffs of albuterol, wait 10 to 15 minutes, then perform spirometry and continue with Q1000.

Questions 1000, 1010, 1050, 1060, 1110, 1120. Record the time based on a 24-hour clock (military time).

Questions 1030 and 1040. These values should come from the Post 4 Composite row of the MedGraphics Max Reversibility Report. Record the value for Q1030 to the nearest hundredths of a decimal.

Administer 2 puffs of albuterol, wait 10 to 15 minutes, then perform spirometry and continue with Q1050.

Questions 1070 and 1080. These values should come from the Post 2 (6) Composite row of the MedGraphics Max Reversibility Report. Record the value for Q1070 to the nearest hundredths of a decimal.

Question 1090. This field will accept both positive and negative values. When applicable, note the negative sign in front of the value on the form. The calculated value will be rounded to the nearest tenth of a decimal.

Question 1100. Refer to the percent difference in Q1090. If the percent difference is $\leq$ 5.0%, the test should be stopped and Q1150 should be completed. If not, administer 2

puffs of albuterol, wait 10 to 15 minutes, then perform spirometry and continue with Q1110-Q1140.

Questions 1130 and 1140. These values should come from the Post 2 (8) Composite row of the MedGraphics Max Reversibility Report. Record the value for Q1130 to the nearest hundredths of a decimal.

The corresponding report for the MAXREV form is the MedGraphics Maximum Reversibility Final Report, abbreviated MAXREV_RPT. Refer to the protocol-specific MOP for details on when this report is used.

Photocopy the Max Reversibility Report. If the form is a packet form, clip the original report to the back of the packet and forward it to the DCC. Place the copy in the participant's study folder. If the form is a single form, clip the original to the back of the single form and forward it to the DCC with a Data Processing Cover Sheet (DPCS) attached. Place the copy in the participant's study folder.

10.1.27 MEMS®6 Monitor Quality Control (MEMSQC)

Purpose: To determine if the MEMS®6 monitor is functioning properly at each visit.

Who: A MEMS®6 monitor certified technician performs the procedure and completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Enter and send the MEMS®6 Monitor Quality Control (MEMSQC) form for both failed and successful MEMS®6 monitor trials to the DCC.

If the participant forgets his/her MEMS®6 monitor, do not complete a MEMS®6 Monitor Quality Control (MEMSQC) form. Indicate on the Visit Procedure Checklist that the participant forgot his or her MEMS®6 monitor. During data entry, register MEMS®6 Monitor Quality Control (MEMSQC) form as missing. If the participant brings in his or her MEMS®6 monitor at a later date (other than the visit date), use the later date as the visit date for the MEMSQC form. This form should then be entered as a single form.

When completing the MEMS®6 Monitor Quality Control (MEMSQC) form between visits, specify the last visit number completed and the current date in the upper right hand corner of the MEMS®6 Monitor Quality Control (MEMSQC) form. This form should be entered as a single form.

Question 1000. There should be a serial number recorded on the cap of each MEMS®6 monitor.

Question 1010. At each visit, record the date the MEMS®6 monitor information was read.

Question 1020. Respond ‘Yes’ to Q1020 if the MEMS®6 monitor being issued is new to the participant; then indicate the reason the ‘old’ MEMS®6 monitor is no longer being used by checking the appropriate box in Q1030. If the ‘old’ MEMS®6 monitor is being reissued, respond ‘No’.

Question 1030. Respond ‘First Issuing’ at the first study visit since this is the first time the participant is receiving the device. If at later visits the participant is given a new device, do not respond ‘First Issuing’, instead indicate the appropriate reason why a new device was given to the participant. If Q1030 is answered ‘Other’, complete Q1030D.

Questions Q1040-1060. Alerts regarding battery expiration, battery voltage, and used memory will appear when reading MEMS®6 cap for the first time. This should be done

prior to dispensing MEMS®6 cap for use. If no alerts appear, expiration, voltage, and memory are adequate and Q1040-Q1060 should be answered No.

Question 1070. If any of the gray boxes were checked in Q1040, Q1050 or Q1060, then the monitor did not pass quality control.

For more details on the quality control testing procedures for the MEMS®6 monitor, see the MEMS®6 Monitor Quality Control discussion in the MEMS®6 Monitor manual, Appendix 5 of the AsthmaNet General MOP.

When collating and mailing this form to the DCC, all MEMSQC forms completed for the same participant on the same day should be organized together in the order in which they were tested.

Example: Two MEMS®6 monitors were tested and both failed the quality control testing. As a result, a third MEMS®6 monitor was tested and it passed the quality control testing. The forms should be ordered as follows: The MEMSQC form for the 'old' MEMS®6 monitor tested should be the first page. The MEMSQC form for the 'second' MEMS®6 monitor should be the second page. The MEMSQC form for the 'third' MEMS®6 monitor tested should be the third page.

Complete the 'Form Page __ of __' fields at the bottom center of the form for each MEMS®6 monitor tested. For example, if three MEMS®6 monitors are tested, the first MEMSQC form 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'.

10.1.28 Methacholine Challenge Testing (METHA)

Purpose: To record outcome measurements from the methacholine challenge procedure.

Who: The Pulmonary Function Technician administers the methacholine and pulmonary function tests and completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Complete this form ***only*** if the participant has ***successfully completed*** either the Pediatric Methacholine Challenge Testing Checklist (METHACHK_PED) or the Adult Methacholine Challenge Testing Checklist (METHACHK_ADULT).

If the technician completing the procedure is not certified and this procedure is being used as an observation session for certification, please complete the Supervisor ID located in the header on the METHA form with the ID of the certified technician who supervised the procedure. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the Supervisor ID when applicable could result in a protocol deviation.

The Supervisor ID should be entered in the Post Test Comments area of the MedGraphics system. Refer to Appendix 4 of the AsthmaNet General MOP for detailed instructions on certification of technicians in methacholine challenge testing.

The Methacholine Reference Reversal Reference Value is the value recorded for Q1030 on the Spirometry Testing (SPIRO) form X .90.

Question 1000. This value should come from the Diluent or Diluent II (if necessary) Composite row of the Methacholine Report. Record the value for Q1000 to the nearest hundredths of a decimal.

Question 1010. If the participant's FEV_1 dropped $\geq$ 20% at the diluent stage(s), record 'Yes' for Q1010 and record '0' for Q1050. See Appendix 2 in the AsthmaNet General MOP for more information.

Question 1020. Record the concentration of the Challenge/Max stage of the MedGraphics Methacholine Report. Record the value for Q1020 to the nearest ten thousandths of a decimal.

Question 1030. Record the FEV_1 from the Composite Challenge/Max row of the MedGraphics Methacholine Report. Record the value for Q1030 to the nearest hundredths of a decimal.

Questions 1040 and 1050. If a PC_{20} was achieved, it will be printed on the last page of the MedGraphics Methacholine Report under the graph as 'PC='.

- If the participant's FEV_1 does not drop 20% or more after all concentrations of solution are administered during the methacholine challenge, answer Q1040 as 'No' and leave the PC_{20} value (Q1050) blank.
- If the challenge is stopped prematurely for any reason, answer Q1040 as 'No' and leave the PC_{20} value (Q1050) blank.
- Occasionally it may be necessary to recompute a PC_{20} value recorded by the methacholine software. When a problem occurs during a methacholine challenge, note the circumstances in the comments associated with the challenge so that the overreader may adjust the grade accordingly. Contact the protocol-specific scientific coordinator at the DCC and notify the overreader. The scientific coordinator will compute the PC_{20} and send the new value to the technician and the overreader via e-mail. The technician should submit a copy of the e-mail documentation along with the data collection form with the corrected value.

Question 1050. Record the value for Q1050 to the nearest hundredths of a decimal.

Question 1060. Record the time based on a 24-hour clock (military time).

Question 1070. Record the post-albuterol FEV_1 after standard reversal from methacholine. This value should come from the Post Composite row of the MedGraphics Methacholine Report. Record the value for Q1070 to the nearest hundredths of a decimal.

Question 1080. Record the time based on a 24-hour clock (military time).

Question 1090. To calculate the methacholine reversal reference value (B) in the gray box, round the calculation from Question A X 0.9 to the hundredths of a decimal. (Example: if the value is 1.2576, record the value in (B) as 1.26). The FEV_1 value used in Question A in the grey box should be obtained from Q1030 on the Spirometry Testing (SPIRO) form. If Question 1090 is answered 'Yes', STOP HERE and continue with the remaining visit procedures. Otherwise, proceed to the Additional Treatment for Methacholine Challenge Testing (METHA_ADD_TRT) form.

Photocopy the methacholine report from the MedGraphics system. Clip the original report to the visit packet that will be forwarded to the DCC (after data entry and resolution of entry errors). File the copy of the report in the participant's study file.

The corresponding report for the METHA form is the MedGraphics Methacholine Final Report, abbreviated METHA_RPT. Refer to the protocol-specific MOP for details on when this report is used.

10.1.29 Paediatric Asthma Quality of Life Questionnaire™ (PAQLQS)

Purpose: To measure a pediatric participant's quality of life living with asthma.

Who: An AsthmaNet coordinator completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

AsthmaNet negotiated a license agreement with Dr. Elizabeth Juniper to use the Paediatric Asthma Quality of Life Questionnaire© in AsthmaNet studies. However, the original format of the form cannot be changed in any way. The header information including the Participant ID, Participant Initials, Visit, Visit Date, and Coordinator ID will be completed across the header of the form using the fillable pdf feature when preparing the visit packet. **Note:** Do **NOT** complete the Name, Number, and Dates of Completion fields at the top of the Response Sheet. The participant's name is a HIPAA identifier and should never be listed on any documentation forwarded to the DCC.

PAQLQ(S) is a 23-question tool validated for ages 7-17 years. It has a 1-week recall. The interviewer-administered version will be used for AsthmaNet studies. Participants are asked to think about how their asthma has affected them during the previous week and to respond to each of the 23 questions on a 7-point scale. Scales are shown on blue and green response cards provided by Dr. Juniper. The coordinator should present the appropriate card for each question as it is administered. Directions for questionnaire administration on page 1 of the form must be followed exactly.

As stated in the directions, parents should not be present during the interview. The participant's own experiences that should be evaluated. Some parents may want to influence this evaluation and some children may want to look to the parent for guidance.

Show the blue and green response cards to the participant and explain the options. For children who can read, ask them to read aloud each of the response options. For younger children, read through each of the responses with them. Make sure the child understands the concept of grading from 1 (extremely bothered/all of the time) to 7 (not bothered/none of the time).

As the questions are administered, record the numeric answer to each in the 1st column on the response sheet (page 5 in the questionnaire packet with page number 4 on it).

This form should be completed before the participant undergoes spirometry. Standard questionnaires that collect information on a participant's perception of his/her asthma symptoms are administered before study procedures begin in order to avoid bias.

During data entry of this form, Questions 1 through 23 will be designated as (Q1) through (Q23) on the entry screen. All pages of the questionnaire must be present during administration of the questionnaire, but only the Response Sheet (Page 5 in the questionnaire packet) should be forwarded to the DCC with the visit packet. A new questionnaire should be administered at each study visit (i.e. the Response Sheet is not reused as its 4-column structure implies). Record all responses in the 1st Response column only.

10.1.30 Pediatric Long Physical Exam (LEXAM_PED)

Purpose: To record a pediatric participant's height, weight, parental height, evidence of oral candidiasis, and physical findings.

Who: An AsthmaNet coordinator completes Page 1 of this form. Page 2 is completed by a licensed medical practitioner.

When: Refer to Visit Procedure Checklists.

Form Instructions:

For more information related to performing a long physical exam, please refer to Section 3 of the AsthmaNet General MOP.

Questions 1010 – 1050. The biological parents' heights should be captured only once during a protocol. This should be attempted at the first visit and at every subsequent visit until the fields are completed. If the fields are all completed at the first study visit, the fields should be left blank for subsequent visits.

Question 1100. Plot this value on gender- and age-appropriate growth charts. See protocol-specific MOP for further details.

Question 1110. If Q1110 is answered 'No', please specify the reason why it was unacceptable in Q1120.

Question 1130. Plot this value on gender- and age-appropriate growth charts. See protocol-specific MOP for further details.

Question 1140. If Q1140 is answered 'Yes', complete the Clinical Adverse Events (AECLIN) form.

Only the responses from Page 1 and the Comments from Page 3 (Q6000) are entered into the study database.

The source documentation box is completed on Page 2 by a licensed medical practitioner verifying he/she completed Page 2 of the physical exam.

10.1.31 Pediatric Methacholine Challenge Testing Checklist (METHACHK_PED)

Purpose: To determine if a pediatric participant is eligible to proceed with the diluent (solution #0) pulmonary function testing for the methacholine testing.

Who: The Pulmonary Function Technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

If the technician completing the procedure is not certified in methacholine challenge testing, a supervisory technician who is certified should monitor the technician and record his or her number in the Supervisor ID field at the top of the form. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the supervisor ID when applicable could result in a protocol deviation.

Complete this form only if the participant is eligible according to the protocol-specific Pulmonary Procedure Checklist and successfully completed baseline spirometry session(s).

Question 1000. If Q1000 is answered 'Yes', Q1010 must be completed.

Question 1020. If the physician's signature was obtained, enter a 1 in the database. Otherwise, leave the field blank during data entry.

Question 1030. If Q1030 is answered 'Yes', Q1040 must be completed.

Question 1050. Refer to the Concomitant Medications for Asthma/Allergy and Adverse Events (CMED) form and the Clinical Adverse Events (AECLIN) form.

Question 1060. The participant is ineligible to perform a methacholine challenge if the FEV_1 (% predicted) value recorded on the Q1040 of the Spirometry Testing (SPIRO) form is less than 70% of predicted.

Question 1070. Check 'N/A' if the participant is male or is a female that has not started menses.

Question 1080 and 1090. A participant who is 12 years old or older is ineligible to proceed if his/her systolic blood pressure is > 200 or his/her diastolic blood pressure is > 100. If the participant is less than 12 years old, he/she is ineligible to proceed if his/her systolic blood pressure is > 180 or his/her diastolic blood pressure > 90.

Question 1100. If there is a reason the participant should not proceed with methacholine challenge testing that has not been captured on the form, explain in the space provided (Q1100D).

Question 1110. If the participant is deemed eligible, proceed to the Methacholine Challenge (METHA) form. The participant is deemed ineligible for the diluent (solution #0) pulmonary function testing for the methacholine challenge if any of the shaded boxes are completed.

10.1.32 Pediatric Respiratory Assessment Measure (PRAM)

Purpose: To measure the respiratory assessment of a pediatric participant.

Who: An AsthmaNet coordinator completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Questions 1000 and 1010. Record the time based on a 24-hour clock (military time).

10.1.33 Pediatric Short Physical Exam (SEXAM_PED)

Purpose: To record a pediatric participant's height, weight, evidence of oral candidiasis, and physical findings.

Who: An AsthmaNet coordinator completes this form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

For more information related to performing a short physical exam, please refer to Section 3 of the AsthmaNet General MOP.

Questions 1070, 1080, 1090 and 1100. Record to the nearest tenths of a decimal.

Question 1100. Plot this value on gender- and age-appropriate growth charts. See protocol-specific MOP for further details.

Question 1110. If 'No' is checked, please record the reason why it was unacceptable in Q1120.

Question 1130. Plot this value on gender- and age-appropriate growth charts. See protocol-specific MOP for further details. Record to the nearest tenth of a decimal.

Question 1140. If 'Yes' is recorded, complete the Clinical Adverse Events (AECLIN) form.

Only the responses from Page 1 and the Comments recorded on Page 2 (Q6000) are entered into the study database.

The coordinator completing the form should complete the source documentation box on Page 2.

10.1.34 Pediatric Asthma and Allergy History (ASTHMA_HX_PED)

Purpose: To record an overview of a pediatric participant's asthma history including family history, symptoms, triggers, allergies, and smoking history.

Who: An AsthmaNet coordinator interviews the participant or participant's parent/ legal guardian while completing the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Questions 1000 and 1010. Record the participant's age using both the years and months fields. For example, if the participant was 2 years, 3 months old when chest symptoms suggesting asthma first appeared, record '2' for Q1000 and '3' for Q1010. If the participant was 2 years old, record '2' for Q1000 and '0' for Q1010.

Questions 1070 and 1080. Record the participant's age using both the years and months fields. For example, if the participant was 2 years, 3 months old when asthma was first diagnosed, record '2' for Q1070 and '3' for Q1080. If the participant was 2 years old, record '2' for Q1070 and '0' for Q1080.

Questions 1090 – 1120. If the participant is adopted and does not know his or her biological parents and/or siblings, answer 'Don't Know.'

Question 1110. Record 'N/A' if the participant does not have any biological siblings.

Question 1120. Record 'N/A' if the participant does not have any biological children.

Questions 1140, 1150, 1160, and 1170. If the participant's asthma symptoms vary by season, multiple seasons may be answered as 'Yes'; however, not all 4 seasons should be answered 'Yes.' At least one 'Yes' should be checked.

Questions 1180 – 1240. If the participant or participant's guardian answered 'none' to any of these questions, record '00' for the response.

Question 1250. If Q1250 is answered 'No', skip to Q1290.

Question 1400. If Q1400 is answered 'Yes', record a description in Q1400D.

Question 1410. If Q1410 is answered 'Yes', list the medicines in Q1410D.

Question 1420. If Q1420 is answered 'Yes', list the food in Q1420D.

Question 1460. If Q1460 is answered 'Yes', record a description in Q1460D.

Question 1480. Record the participant's age using both the years and months fields. For example, if the participant was 2 years, 3 months old when the participant first had eczema, record '2' for Q1480 and '3' for Q1490. If the participant was 2 years old, record '2' for Q1480 and '0' for Q1490.

Question 1510. If Q1510 is answered 'None', skip to Q1570.

Questions 1570 – 1600. If the participant is adopted and does not know his or her biological parents and/or siblings, answer 'Don't Know.'

Question 1590. Record 'N/A' if the participant does not have any biological siblings.

Question 1600. Record 'N/A' if the participant does not have any biological children.

Question 1610. If Q1610 is answered 'No' or 'Don't Know', skip to Q1650.

Questions 1650 – 1680. These questions are related to a child's exposure to smokers in the household from birth to 5 years of age.

Questions 1690 – 1720. These questions are related to a child's exposure to smokers in the household at the present time.

10.1.35 Pediatric Quality of Life Inventory (5-7) (PEDSQL)

Purpose: To measure a pediatric participant's general quality of life.

Who: Participant's Parent/Legal Guardian completes this form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

AsthmaNet negotiated a license agreement with MAPI Research Trust in France to use the Pediatric Quality of Life Inventory™ (PedsQL) Parent Report for Young Children (Ages 5-7) in AsthmaNet studies. However, the original format of the form cannot be changed in any way. The header information including the Participant ID, Participant Initials, Visit, Visit Date, and Coordinator ID will be completed across the header of the form using the fillable pdf feature when preparing the visit packet.

This version of the PedsQL is a 23-question tool validated for ages 5-7. It has a 1-month recall. The participant's parent or guardian is asked to think about how the participant has been during the previous month and to respond to each of the 23 questions on a 5-point scale (0 = never to 4 = almost always). The form should be presented to the parent/guardian with all pages, including the instruction sheet. The parent/guardian should complete the form by circling clearly one answer to each question.

Review the form after the participant's parent/guardian has completed it to ensure he/she clearly circled only one response (within the designated box) for each question. Participant/guardian completed forms should always be reviewed by the coordinator upon form completion. If a correction is noted, the participant's parent/guardian should make the correction and initial and date next to the change. Coordinators should not alter participant completed forms.

During data entry of this form, the questions will be designated PHYSICAL_1 – PHYSICAL_8, EMOTIONAL_1 – EMOTIONAL_5, SOCIAL_1 – SOCIAL_5, and SCHOOL_1 – SCHOOL_5.

10.1.36 Perceived Stress Scale (PSS_10)

Purpose: To measure the participant's perceived level of stress during the last month.

Who: The participant completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Questions 1000 – 1090. The participant should only check one box for each question.

Review the form prior to the participant leaving the clinic to ensure that the participant completed the form correctly.

To verify that the information recorded on this form is correct, have the participant initial (using 2 or 3 initials), date, and record the time in the source documentation box provided (Q1100, Q1110 and Q1120). Enter the Date field in the database in the format mm/dd/yyyy.

10.1.37 Post-Advair® (2 puffs) Spirometry Testing (PADVAIR_SPIRO)

Purpose: To record the outcome measurements from the participant's pulmonary function testing after 2 puffs of Advair® were administered.

Who: The Pulmonary Function Technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

If the technician completing the procedure is not certified in spirometry testing, a supervisory technician who is certified should monitor the technician and record his or her number in the Supervisor ID field at the top of the form. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the Supervisor ID when applicable could result in a protocol deviation.

Administer 2 puffs of Advair®, wait 1 hour, then perform spirometry and continue with Q1010.

Questions 1000 and 1010. Record the time based on a 24-hour clock (military time).

Questions 1020-1060. These values should come from the MedGraphics Spirometry Final Report, Post Composite row. Record the values for Q1020 and Q1030 to the nearest hundredths of a decimal. Record the value to the nearest hundredths of a decimal.

The corresponding report for the PADVAIR_SPIRO form is the MedGraphics Spirometry Final Report, abbreviated PADVAIR_RPT. Refer to the protocol-specific MOP for details on when this report is used.

Photocopy the Spirometry Report from the MedGraphics system. Clip the original report to the back of the packet and forward it to the DCC. Place the copy in the participant's study folder.

See Appendix 1 of the AsthmaNet General MOP for more information regarding spirometry procedures.

10.1.38 Post-Albuterol (2 puffs) Spirometry Testing (PALB2_SPIRO)

Purpose: To record the outcome measurements from the participant's pulmonary function testing after 2 puffs of albuterol were administered.

Who: The Pulmonary Function Technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

If the technician completing the procedure is not certified in spirometry testing, a supervisory technician who is certified should monitor the technician and record his or her number in the Supervisor ID field at the top of the form. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the Supervisor ID when applicable could result in a protocol deviation.

Administer 2 puffs of albuterol, wait 10 to 15 minutes, then perform spirometry and continue with Q1000.

Questions 1000 and 1010. Record the time based on a 24-hour clock (military time).

Questions 1020-1060. These values should come from the MedGraphics Spirometry Report, Post Composite row. Record the values for Q1020 and Q1030 to the nearest hundredths of a decimal. Record the value to the nearest hundredths of a decimal.

The corresponding report for the PALB2_SPIRO form is the MedGraphics Spirometry Final Report, abbreviated SPIRO_RPT. Refer to the protocol-specific MOP for details on when this report is used.

Photocopy the Spirometry Report from the MedGraphics system. Clip the original report to the back of the packet and forward it to the DCC. Place the copy in the participant's study folder.

See Appendix 1 of the AsthmaNet General MOP for more information regarding spirometry procedures.

10.1.39 Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO)

Purpose: To record the outcome measurements from the participant's pulmonary function testing after 4 puffs of albuterol were administered.

Who: The Pulmonary Function Technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

If the technician completing the procedure is not certified in spirometry testing, a supervisory technician who is certified should monitor the technician and record his or her number in the Supervisor ID field at the top of the form. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the Supervisor ID when applicable could result in a protocol deviation.

Administer 4 puffs of albuterol, wait 10 to 15 minutes, then perform spirometry and continue with Q1000.

Questions 1000 and 1010. Record the time based on a 24-hour clock (military time).

Questions 1020-1060. These values should come from the MedGraphics Spirometry Final Report, Post Composite row. Record the values for Q1020 and Q1030 to the nearest hundredths of a decimal. Record the value to the nearest hundredths of a decimal.

The corresponding report for the PALB4_SPIRO form is the MedGraphics Spirometry Final Report, abbreviated SPIRO_RPT. Refer to the protocol-specific MOP for details on when this report is used.

Photocopy the Spirometry Report from the MedGraphics system. Clip the original report to the back of the packet and forward it to the DCC. Place the copy in the participant's study folder.

See Appendix 1 of the AsthmaNet General MOP for more information regarding spirometry procedures.

10.1.40 Post-Ipratropium (4 puffs) Spirometry Testing (PIPRA4_SPIRO)

Purpose: This form records outcome variables from the participant's post-ipratropium pulmonary function testing.

Who: The Pulmonary Function Technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

If the technician in charge of the procedure is not certified in spirometry testing, a supervisory technician who is certified should monitor the technician and record his or her ID number in the Supervisor ID field at the top of the form. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the Supervisor ID when applicable could result in a protocol deviation.

Administer 4 puffs of ipratropium, wait 10 to 15 minutes, then perform spirometry and continue with Q1000.

Question 1000 and 1010. Record the time based on a 24-hour clock (military time).

Questions 1020 - 1060. These values should come from the MedGraphics Spirometry Final Report, Post Composite row. Record the values for Q1020 and Q1030 to the nearest hundredths of a decimal.

The corresponding report for the PIPRA4_SPIRO form is the MedGraphics Spirometry Final Report, abbreviated PIPRA4_RPT. Refer to the protocol-specific MOP for details on when this report is used.

Photocopy the Spirometry Report from the MedGraphics system. Clip the original report to the back of the packet and forward it to the DCC. Place the copy in the participant's study folder.

See Appendix 1 of the AsthmaNet General MOP for more information regarding spirometry procedures.

10.1.41 Prior Asthma/Allergy Treatment (PRIOR_TRT)

Purpose: To record a participant's medications used to treat asthma or allergies over the last 12 months.

Who: An AsthmaNet coordinator completes the form while interviewing the participant or participant's parent/legal guardian.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Question 1000. If Q1000 is answered 'Other', record a description in Q1000D

Questions 1010 – 1910. Record 'Don't Know' if the participant does not know or is unsure if he or she has used the medication listed during the past 12 months. If the participant did use the medication during the past 12 months but does not know the exact date the medication was last taken, prompt the participant for the month and the year. If the participant cannot recall the day, leave the day blank; if the participant cannot recall the month, leave the month and day blank.

When entering these dates during data entry, the month, day, and year are each represented by a separate entry field. The first box represents the month. Only enter the month into this box. The leading zeros can be left out. The second box represents the day. Only enter the day of the month into this box. The leading zeros can be left out. The third box represents the year. Only enter the year into this box. The year must be entered as four digits. For example, when entering 01/10/2010, enter 01 or 1 into the first box, enter 10 into the second box, and enter 2010 into the third box.

Partial dates can also be entered during data entry for these fields by leaving the box for the unknown information blank. If the day is missing, the month and year can be entered. If both the month and day are missing, the year can be entered. If at least the year is not known, change the leading question to Don't Know.

Question 1050. If none was used, record '000'.

Question 1430. If Q1430 is answered 'Yes', complete Q1470, Q1480 and Q1490.

Question 1470. Refer to the Prior Asthma/Allergy Treatment Form (PRIOR_TRT_CARD) Reference Card to find the corresponding code for the inhaled steroid taken. If the inhaled steroid taken is not listed, record '999' and specify the name of the medication in Q1470D.

Question 1500. If Q1500 is answered 'Yes,' complete Q1535, Q1540, and Q1550.

Question 1535. Refer to the Prior Asthma/Allergy Treatment Form Reference Card (PRIOR_TRT_CARD) to find the corresponding code for the nebulized steroid taken. If the nebulized steroid taken is not listed, record '99' and specify the name of the medication in Q1500D.

Question 1560. If Q1560 is answered 'Yes,' complete Q1600, Q1610, and Q1620.

Question 1600. Refer to the Prior Asthma/Allergy Treatment Form (PRIOR_TRT_CARD) Reference Card to find the corresponding code for the inhaled steroid/long-acting beta-agonist taken. If the medication taken is not listed, record '9999' and specify the name of the medication in Q1600D and complete Q1610 and Q1620.

Question 1830. If Q1830 is answered 'Yes,' record the name(s) of the other medication(s) used during the past 12 months to treat asthma or allergies in Q1830D.

Question 1870. If Q1870 is answered 'Yes,' record the indication that oral steroids were used during the past 12 months for something other than asthma in Q1870D.

Question 1910. If Q1910 is answered 'Yes,' record the indication that injectable steroids were used during that past 12 months for something other than asthma in Q1910D.

10.1.42 Prior Conditions for Adult Participants (PRIOR_COND_ADULT)

Purpose: To record an adult participant's prior diseases, illnesses, conditions, and surgeries by bodily system.

Who: An AsthmaNet coordinator completes the form while interviewing the participant.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Questions 1000 – 1120. Record a 'Yes' or 'No' for each question. Do not leave a question missing. For each question where a 'Yes' is recorded, provide a description of the disease, illness, condition, or surgery in the corresponding row. The descriptions (Q1000D – Q1120D) will be entered into the study database.

10.1.43 Prior Conditions for All Participants (PRIOR_COND_ALL)

Purpose: To record a participant's prior diseases, illnesses, conditions, and surgeries related to skin, ears, nose, and throat, lungs, stomach or intestines, and sleep disorders.

Who: An AsthmaNet coordinator completes the form while interviewing the participant or participant's parent/legal guardian.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Question 1000. If Q1000 is answered 'Other', record a description in Q1000D.

Questions 1010, 1060, 1110, 1130, 1150, 1170, and 1180. If 'Yes' is recorded for these questions, provide a description of the disease, illness, condition, or surgery in the corresponding row. The descriptions (Q1010D, Q1060D, Q1110D, Q1130D, Q1150D, Q1170D and Q1180D) will be entered into the study database.

10.1.44 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: A clinical adverse event, laboratory adverse event, or significant asthma exacerbation is deemed serious.

Form Instructions:

The Serious Adverse Event Reporting Form (SERIOUS) is a single form only.

A serious adverse event is defined as an event resulting in hospitalization, extension of a hospital stay, or death.

A Serious Adverse Event Reporting Form (SERIOUS) should be completed and faxed to the DCC at (717) 531-4359 within 72 hours of notification of a serious adverse event. Relevant source documents (ER records, clinic notes, discharge summary, etc.) and related data collection forms (copies of the Clinical Adverse Events form [AECLIN] and the Concomitant Medications for Asthma/Allergy and Adverse Events [CMED]) should also be faxed to the DCC.

If the form is completed between visits, specify the number of the last visit completed and the date the form is completed.

Questions 1010 and 1010D. Describe the serious adverse event concisely (Q1010D) and identify the appropriate ICD9 code (Q1010). If the serious adverse event was related to a clinical adverse event, record the same description, ICD9 code, and date as on the Clinical Adverse Events (AECLIN) form. This will allow the DCC to link the serious adverse event to the respective clinical adverse event.

A list of all ICD9 Codes used for AsthmaNet is available in an Excel spreadsheet on the AsthmaNet secure web site under the Application link. A link to the list of ICD9 Codes also can be found during data entry and editing in the Participant Data module of the data management application. If the coordinator is still unable to identify an ICD9 code, please call the protocol-specific Primary Data Manager at (717) 531-3663 for assistance.

Question 1020. When answering this question, keep in mind that the term “study drug” refers to any medications given out as part of the study. Please refer to the protocol specific MOP for additional guidance.

Questions 1030 and 1040. Please refer to the protocol-specific MOP for guidance in determining the time interval between the last administration of the study drug and the adverse event.

Question 1050-1180. These questions outline the criteria for determining whether or not an event was serious. If the participant does not meet at least one of these criteria, do not complete this form.

Question 1070. If Q1070 is answered 'No', skip to Q1100. Otherwise, complete Q1080 and Q1090.

Question 1180. If Q1180 is answered 'Yes', describe the 'Other' in Q1180D.

Question 1190-1220. These questions outline the criteria for determining what may have caused the serious adverse event. At least one of the items must be answered 'Yes.'

Question 1210. If Q1210 is answered 'Yes', describe the concurrent medication in Q1210D.

Question 1220. If Q1220 is answered 'Yes', describe the other condition or event in Q1220D.

Questions 10 and 11. These questions do not get data entered.

REPORTING INVESTIGATOR section on page 3 should be completed for all serious adverse events. The AsthmaNet principal investigator should complete the Name, Signature and Date section below the comments.

See Section 4 of the AsthmaNet General MOP for more information regarding adverse events.

10.1.45 Sinonasal Questionnaire (SNQ)

Purpose: To record the frequency of sinonasal symptoms the participant has had over the last 3 months.

Who: The participant completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

Questions 1000 – 1040. The participant should only check one box for each symptom.

Review the form prior to the participant leaving the clinic to ensure that the participant completed the form correctly.

To verify that the information recorded on this form is correct, have the participant initial (using 2 or 3 initials), date, and record the time in the source documentation box provided (Q1050 – Q1070). Enter the Date field in the database in the format mm/dd/yyyy.

10.1.46 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: Refer to Visit Procedure Checklists.

Form Instructions:

The protocol-specific pulmonary procedure checklist (PULMONARYCHK) needs to be completed to determine eligibility before performing Spirometry Testing (SPIRO). Please refer to the protocol-specific MOP.

If the technician completing the procedure is not certified and this procedure is being used as an observation session for certification, please complete the Supervisor ID located in the header on the SPIRO form with the ID of the certified technician who supervised the procedure. The Supervisor ID ***is*** entered into the database during data entry. Failure to complete the Supervisor ID when applicable could result in a protocol deviation.

The Supervisor ID should be entered in the Post Test Comments area of the MedGraphics system. Refer to Appendix 1 of the AsthmaNet General MOP for detailed instructions on certification of technicians in spirometry testing.

If the participant is deemed ineligible to proceed with the pulmonary function testing, the participant's pulmonary function testing should be rescheduled within the visit window. On the day of the rescheduled procedure, complete a new Spirometry Testing (SPIRO) form with the **current date**. Do not enter the old form into the study database and do not send it to the DCC. Only enter and submit the most recent form. However, all completed forms, the old original form and the current photocopy of the form should be filed in the participant's study file.

Question 1010. Record the time based on a 24-hour clock (military time).

Questions 1020-1060. These values should come from the Spirometry Report, Pre Composite row. Record the values for Q1020 and Q1030 to the hundredths of a decimal.

Question 1060. Record the value for Q1060 to the hundredths of a decimal.

The corresponding report for the SPIRO form is the MedGraphics Spirometry Final Report, abbreviated SPIRO_RPT. Refer to the protocol-specific MOP for details on when this report is used.

Photocopy the Spirometry Report from the MedGraphics system. If the form is a packet form, clip the original report to the back of the packet and forward it to the DCC. Place the copy in the participant's study folder. If the form is a single form, clip the original report to the back of the single form and forward it to the DCC with a Data Processing Cover Sheet (DPCS) attached to the front. Place the copy in the participant's study folder.

If methacholine challenge is completed, the Spirometry Testing Report (SPIRO_RPT) should be marked 'No' in the database. The Methacholine Challenge Report (METHA_RPT) should be marked 'Yes' in the database. The spirometry session data is included on the Methacholine Challenge Report (METHA_RPT) and a separate Spirometry Testing Report (SPIRO_RPT) does not need to be printed.

If post-albuterol (4 puffs) spirometry testing is completed, the Spirometry Testing Report (SPIRO_RPT) should be marked 'No' in the database. The Post-Albuterol (4 puffs) Spirometry Testing Report (PALB4_RPT) should be marked 'Yes' in the database. The spirometry session data is included on the Post-Albuterol (4 puffs) Spirometry Report (PALB4_RPT) and a separate Spirometry Testing Report (SPIRO_RPT) does not need to be printed.

If post-ipratropium (4 puffs) spirometry testing is completed, the Spirometry Testing Report (SPIRO_RPT) should also be marked 'No' in the database. The Post-Ipratropium (4 puffs) Spirometry Testing Report (PIPRA4_RPT) should be marked 'Yes' in the database. The spirometry session data is included on the Post-Ipratropium (4 puffs) Spirometry Testing Report (PIPRA4_RPT) and a separate Spirometry Testing Report (SPIRO_RPT) does not need to be printed.

See Appendix 1 of the AsthmaNet General MOP for more information regarding spirometry procedures.

10.1.47 Spirotel® Quality Control (SPIROTELQC)

Purpose: To determine if the spirotel® device is functioning properly at each visit.

Who: A spirotel® certified AsthmaNet coordinator performs the procedure and prints the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

The Spirotel® Quality Control (SPIROTLQC) form will be generated automatically by the spirotel® software during the calibration testing procedure. See Appendix 6 of the AsthmaNet General MOP.

Only enter the SPIROTELQC form for the turbine and spirotel combination that passed calibration for the study visit and was sent home with the participant.

If the participant forgets his/her spirotel®, indicate on the Visit Procedure Checklist that the participant forgot his or her spirotel®. During data entry, register the Spirotel® Quality Control (SPIROTELQC) form as missing. If the participant brings in his or her spirotel® at a later date (other than the visit date) or between visits, the visit date on the SPIROTELQC report will pre-populate with the date the quality control testing was performed . This form should then be entered as a single form with the last visit number completed and the current date in the upper right hand corner.

Question 1000. Enter this date into the database in the format mm/dd/yyyy.

Questions 1040 – 1080. Record these values to the nearest hundredth of a decimal.

10.1.48 Sputum Induction (SPUTUM)

Purpose: To record information about the sputum induction procedure.

Who: A sputum induction technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

A technician must be certified in sputum induction in order to complete this form.

Question 1000. Do not complete Q1000 if this is the first time the participant is attempting sputum induction for a given study or if the sample was not adequate at a prior attempt. If the participant has previously attempted sputum induction at other study visits, and the sample was adequate, the duration at the current visit should not exceed the duration of the first adequate sample.

Questions 1010 and 1020. Record the time based on a 24-hour clock (military time).

Question 1030. Record duration of sputum induction at this visit. If monitoring with FEV_1 was necessary during sputum induction, the time spent on spirometry should not be counted in the duration. Subtract any time spent on spirometry from the total time of the sputum induction procedures to obtain the correct value for Q1030. The duration at the current visit (Q1030) should not exceed the duration of the first time the sample was adequate, as reported in Q1000.

Question 1040. If the duration of the induction did not last for at least 4 minutes at the visit, answer Q1040 ‘No’.

Question 1050. Record to the nearest tenth of a decimal.

Question 1060. The technician responsible for processing should determine if the volume is adequate for processing.

Question 1070. If either of the shaded boxes are completed (Q1040 or Q1060), the sample is not adequate and should not be processed. Answer Q1070 ‘No’ and **DO NOT** complete the Sputum Induction Lab (SPUTLAB) form.

If Q1070 is answered ‘Yes’, the sample should be processed.

Questions 1080 and 1090. The FEV_1 and FEV_1 (% predicted) and Time can be found in the Pre Composite row of the MedGraphics Spirometry Report. Record Q1080 to the nearest hundredth of a decimal.
Question 1100. Record the time based on a 24-hour clock (military time).

Question 1110. The percent difference field will accept both positive and negative values. When applicable, note the negative sign in front of the value on the form. The calculated value will be rounded to the nearest tenth of decimal.

Question 1120. Otherwise, continue with remaining visit procedures. If Question 1120 is answered 'Yes', proceed to the Additional Treatment for Sputum Induction (SPUTUM_ADD_TRT) form. Otherwise, continue with remaining visit procedures.

The corresponding report for the SPUTUM form is the MedGraphics Spirometry Final Report, abbreviated SI_RPT. Refer to the protocol-specific MOP for details on when this report is used. To clarify that this Spirometry Final Report is listing maneuvers performed post-sputum induction, please add a comment to the 'Post Test Comments' section of the report.

10.1.49 Sputum Induction Checklist (SPUTUMCHK)

Purpose: To determine whether the participant is eligible to proceed with sputum induction procedures.

Who: A sputum induction technician completes the form.

When: Refer to Visit Procedure Checklists.

Complete this form ***only*** if the participant ***is eligible*** according to the protocol-specific Pulmonary Procedure Checklist (PULMONARYCHK) and ***successfully completed*** baseline spirometry session(s) (SPIRO).

Form Instructions:

A technician must be certified in sputum induction in order to complete this form.

Question 1000. Only complete this question if the participant completed a methacholine challenge at this visit. Refer to Q1090 on the Methacholine Challenge Testing (METHA) form to answer this question OR Q1100 or Q1230 on the Additional Treatment Post Methacholine Challenge Testing (METHA_ADD_TRT) form.

Question 1010. If 'No', the participant is ineligible to proceed with sputum induction procedures. If 'Yes', the supervising physician must sign Q1020.

Question 1020. During data entry, enter a '1' for Q1020 if a signature is present, otherwise leave Q1020 blank.

Question 1030. If the participant completed a Methacholine Challenge at this visit, this value is Q1070 on the Methacholine Challenge Testing (METHA) form or Q1070 or Q1200 on the Additional Treatment Post Methacholine Challenge Testing (METHA_ADD_TRT) form. If the participant did not complete a Methacholine Challenge at this visit, this value comes from either Q1030 on the Post-Albuterol (4 puffs) Spirometry Testing (PALB4_SPIRO) form, Q1030 on the Post-Albuterol (2 puffs) Spirometry Testing (PALB2_SPIRO) form or Q1070 or Q1130 on the Maximum Reversibility Testing (MAXREV) form.

Question 1040. For post methacholine challenge procedures, refer to Appendix 7, Section IV, B in the AsthmaNet General MOP for instructions on how to calculate the FEV_1% predicted. Otherwise, refer to the FEV_1% predicted value recorded on the form related to Q1030 above.

Question 1060. If 'Yes', record an explanation in Q1060D.

Question 1070. If answered ‘Yes’, the participant is eligible, to proceed with sputum induction. If any of the shaded boxes are completed, the participant is not eligible to proceed with sputum induction procedures.

10.1.50 Sputum Induction Lab Values (SPUTLAB)

Purpose: To record information about slide processing.

Who: A sputum processing technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

A technician must be certified in sputum induction processing in order to complete this form.

Question 1000. Record the date the sample was processed. During data entry, please enter the date in the format mm/dd/yyyy.

Question 1010. Record the time based on a 24-hour clock (military time).

Question 1020. Record to the nearest tenth of a decimal.

Question 1030. Compare Q1020 on the Sputum Induction (SPUTUM) form to Q1010 on the Sputum Induction Lab Values (SPUTLAB) form. If the time difference is greater than 4 hours, record ‘No” and mark the samples as excluded in the Biological Sample Tracking module.

10.1.51 The RAND Impact of Asthma on Quality of Life Questionnaire SF-12 (RAND_IAQL_12)

Purpose: To evaluate the participant's quality of life as a result of asthma.

Who: The participant completes this form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

The RAND Impact of Asthma on Quality of Life Questionnaire (RAND_IAQL) was developed by Cathy Sherbourne and other researchers funded by NHLBI to be a freely available new system for measuring the impact of asthma on quality of life. The original item bank contains 65 items. AsthmaNet will employ the 12-item short form (RAND_IAQL_12). The authors of the questionnaire will use data collected in AsthmaNet studies to help validate their instrument against other well-known questionnaires such as the Asthma Quality of Life Questionnaire (AQLQ) developed by Liz Juniper and her colleagues. The questionnaire will be validated for participants who are ages 12 and older.

For the RAND_IAQL_12, participants are asked how their asthma has affected the quality of their lives in various areas over the ***last four weeks***. Instruct the participant to read each question carefully and mark the one box corresponding to his/her best answer for each question.

This form should be completed before the participant undergoes spirometry at the visit. Adhere to the procedure order on the Visit Procedure Checklist.

Review the form after the participant has completed it to ensure that he/she marked a response (within the designated box) for each question. Participant completed forms should always be reviewed by the coordinator upon form completion. If a correction is noted, the participant should make the correction and initial and date next to the change. Coordinators should not alter participant completed forms.

To verify that the information recorded on this form is correct, have the participant initial (using 2 or 3 initials), date, and record the time in the source documentation box provided (Questions Q1120 – Q1140). Enter the Date field in the database in the format mm/dd/yyyy. Enter the time field based on a 24-hour clock.

10.1.52 Urine Pregnancy Test (PREG_TEST)

Purpose: This form is completed for female participants ages 6 years and older. It assists in determining if a female participant is eligible to enter the study and perform methacholine challenges at the applicable visits.

Who: An AsthmaNet coordinator completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

This form must be completed for **all** female subjects ages 6 and older, regardless of whether they are able to bear children and regardless of whether they will perform a methacholine challenge during the study. Refer to previous PREG_TEST forms completed for the participant (if available) for the current protocol to verify reproductive status.

For more detailed discussion on pregnancy testing, refer to Section 3 of the General MOP.

Question 1000. If the participant is pre-menarche, skip Q1010-Q1040 and have the parent/guardian complete the source documentation box (Q1050-Q1060).

Question 1010. If it has been at least one year since the participant's last menses, or she had a complete hysterectomy, Q1010 should be answered Yes.

Question 1040. The answer to Q1040 is based on the results of an in-office urine pregnancy test. This test must be performed if the participant is able to bear children (i.e., none of the answers to Q1000-Q1030 is completed as 'Yes'). A history of infertility does not qualify the participant as 'Unable to bear children'. In these circumstances, a urine pregnancy test should be performed, assuming none of Q1000-Q1030 is completed 'Yes'.

If any of the shaded boxes in Q1000-Q1030 are checked, the participant is considered unable to bear children. In this case, no pregnancy test is required. The participant or participant's legal guardian still should complete the source documentation box to acknowledge the validity of the recorded data.

For more specific details pertaining to whether a coordinator should permit the participant to continue in the study, refer to the protocol-specific eligibility criteria.

To verify that the participant or participant's legal guardian was informed of the test results and present at the time this form was completed, have the participant or participant's legal guardian record her/his initials (using 2 or 3 initials) and

date (Q1050, Q1060) in the source documentation box at the bottom of the form. During data entry, please enter in the format of the date as mm/dd/yyyy.

10.1.53 Wisconsin Upper Respiratory Symptom Survey – 21 Daily Symptom Report (WURSS_21)

Purpose: To record a participant's daily upper respiratory symptoms when he or she is experiencing a cold.

Who: A participant or participant's parent/legal guardian completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

The AsthmaNet did not receive permission from the creator of this form to alter the format of the form. Therefore, the WURSS-21 form does not include the standard AsthmaNet header and footer information or 4-digit annotation for each question.

Participants in studies using the WURSS-21 form will be given several copies of the form. Pre-fill the participant's ID and initials prior to distributing the forms to the participant. The participants should follow the instruction sheet, Wisconsin Upper Respiratory Symptom Survey-21 (WURSS-21) Instructions (WURSSINST) when completing the WURSS-21 form. The participant should complete a form each day he/she has a cold and stop completing this form when he/she has answered the first question on the form, 'How sick do you feel **today**?" as "**Not sick**" for <u>two days in a row</u>. The participant must complete the 'Date' field on each form he or she completes. The participant should return all completed forms at the next study visit. Review the WURSS-21 forms completed by the participant to verify that the participant recorded a valid and unique date on each form. At each visit, determine if the participant should be given more WURSS-21 forms.

Ask the participant if he or she is still experiencing cold symptoms. It is possible that a participant will experience a cold that won't be resolved at the time of a study visit. The participant should return the completed WURSS-21 forms at the time of the current visit and get more to keep at home in case they are needed. The participant will continue to complete WURSS-21 forms until the cold has resolved. The participant should return the balance of the completed forms at the next study visit to complete the 'cold packet'. The cold packet will get entered with the next visit's data. Ex: If a cold starts between visits 5 and 6 and is ongoing at the time of visit 6, the participant will turn in any completed forms at visit 6. These will be held in the participant's folder. He/she will continue to complete WURSS-21 forms until the cold is resolved. The balance of the forms will be turned in at visit 7. The entire cold packet will get entered with visit 7 data.

Note: It is possible that a participant could have more than one cold between visits, so there may be multiple 'cold packets' with the same visit number on them (but with a number of days or weeks separating the two cold events).

When the cold packet is complete and ready to be entered, record the Coordinator ID and Visit at the top of each form. The data entry screen in the Participant Data module will contain the full or shortened text of each question for easy reference. Enter the code for each question response corresponding to the circle the participant filled in. For example, if the participant filled in the circle under 'Very mild' for 'Runny nose', then enter a '1' for this question.

The codes for the responses to the last question, '**Compared to yesterday**, I feel that my cold is …' are displayed on the data entry screen under the question text: 1 = Very much better; 2 = Somewhat better; 3 = A little better; 4 = The same; 5 = A little worse; 6 = Somewhat worse; and 7 = Very much worse.

The Participant Data module will not allow you to enter more than one form with the same 'Date' for a Visit.

When a cold is reported, add a record to the AECLIN form using a code such as 460. (acute nasopharyngitis [common cold]), 465.8 (acute upper respiratory infections of other multiples sites), or 465.9 (acute upper respiratory infections of unspecified site). The end date for the record should be the calendar date prior to the two "Not sick" records (where 'How sick do you feel today' = 0). If only one "Not sick" record is present, use the calendar date prior to that record. If no "Not sick" records are present, use the participant reported stop date.

4.2 STANDARD ADMIN FORMS AND INSTRUCTIONS

This section provides specific instructions needed to complete the AsthmaNet standard administrative forms. These forms are not entered into the study database and most are not submitted to the DCC. The instructions for each form are in alphabetical order based on the form name.

The following information is provided for each form: the purpose of the form, who completes the form, when the form should be completed, and form instructions. If you are unable to find the specific information needed to complete a form, please contact the protocol-specific Primary Data Manager at (717) 531-3663.

10.1.54 Adult Long Physical Exam (LEXAM_ADULT)

Purpose: To record the results of an adult participant's routine physical exam.

Who: An AsthmaNet coordinator completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

This form is an administrative form for clinical site use only. The data will not be entered into the study database.

Questions 1 – 3. Refer to Section 3 of the AsthmaNet General MOP for more information related to recording these measurements.

Question 4. If Q4 is answered 'Yes', complete the Clinical Adverse Events (AECLIN) form.

Questions 5 – 15. A licensed medical practitioner should determine whether the current findings are normal or abnormal. If abnormal, a comment should be provided.

To verify that the information collected on this form is correct, the attending physician should sign, print name, date and indicate the time of the form completion in the source documentation box provided on page 2.

For use only at the clinical site – DO NOT forward this form to the DCC.

This form will be reviewed during AsthmaNet site visits.

10.1.55 Adult Short Physical Exam (SEXAM_ADULT)

Purpose: To record the results of an adult participant's brief physical exam.

Who: An AsthmaNet coordinator completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

This form is an administrative form for clinical site use only. The data will not be entered into the study database.

Questions 1 – 4. Refer to Section 3 of the AsthmaNet General MOP for more information related to recording these measurements.

To verify that the information collected on this form is correct, the coordinator should sign, print name, date and indicate the time of the form completion in the source documentation box.

For use only at the clinical site – DO NOT forward this form to the DCC.

This form will be reviewed during AsthmaNet site visits.

10.1.56 Adult Participant Contact Information (CONTACT_ADULT)

Purpose: To record pertinent participant identification information for all adults so that the AsthmaNet coordinator will know how and when to easily contact the participant.

Who: The participant completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

This form ***must*** be completed at the first study visit. At subsequent study visits, update this form as indicated on the Visit Procedure Checklists.

For use only at the clinical site – DO NOT forward this form to the DCC.

This form will be reviewed during AsthmaNet site visits.

10.1.57 Asthma Control Questionnaire© Score (ACQ_SCORE)

Purpose: To determine the participant's average ACQ score.

Who: A coordinator completes this form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

From each question on the Asthma Control Questionnaire© form, transcribe the number directly into the appropriate field on the ACQ_SCORE form. Add the 6 scores and record this value in the 'Total' field. Divide the 'Total' by 6 to obtain the 'Average Score'.

10.1.58 AsthmaNet Research Drug Destruction Log (DRUG_DEST_LOG)

Purpose: Verify that all study drug at a clinical center has been destroyed.

Who: An AsthmaNet coordinator completes the form.

When: A new destruction log must be completed for each date that study medication is destroyed. Drug can be destroyed during the study or at study end.

Form Instructions:

The table information (columns) including the Participant ID number, Drug Description, Drug Identification Number/Lot #, Quantity Destroyed, Returned, and Expired should be completed for each date that study medication is destroyed.

Print and sign name, date and coordinator ID when form is completed. E-mail the log to: AsthmaNet-drugs-supplies@phs.psu.edu.

10.1.59 AsthmaNet Room Temperature Monitoring Record

Purpose: Monitor the room temperature where AsthmaNet study drug is stored.

Who: An AsthmaNet coordinator completes the form.

When: Daily.

Form Instructions:

Please complete all columns on a daily basis. If you do not have a thermometer that monitors humidty, leave columns blank.

Print and sign name, date and coordinator ID when form is completed. E-mail the log to: AsthmaNet-drugs-supplies@phs.psu.ed.

10.1.60 AsthmaNet Satisfaction Questionnaire (SATQX)

Purpose: This form gives a participant or participant's parent/legal guardian the opportunity to provide the AsthmaNet research staff with feedback related to his/her (or child's) participation in an AsthmaNet study.

Who: The participant or participant's parent/legal guardian completes the form.

When: The last study visit.

Form Instructions:

This form should be distributed to the participant during the termination visit or last visit of the study. If the participant does not return to the clinic site for a last visit, then the coordinator should mail the form to the participant with a self-addressed postage paid envelope for him/her to return it to the DCC.

Before distributing the form to the participant, the coordinator should record the protocol number and site number of the site distributing the form in the right-hand corner of the form and complete Question 1000 indicating the study status of the participant at the time of his/her termination.

Please remind the participant that no PHI (names, telephone numbers) should be written on the SATQXs.

This form is to be completed voluntarily, and all responses will be confidential. After the participant or participant's parent/legal guardian completes the form, he/she should seal it in the pre-addressed envelope and send it to the DCC. The site should not have access to the completed SATQX form, however if a completed SATQX is received at the site, the form should be placed immediately in a pre-addressed envelope and mailed directly to the DCC.

Pre-addressed and stamped envelopes will be provided to the clinical sites. Please contact the DCC if you run out of prepaid envelopes.

10.1.61 Concomitant Medications for Non-Asthma Drugs (CMED_NON)

Purpose: To record all concomitant medications not related to the treatment of asthma and allergy symptoms or adverse events that the participant takes after signing the informed consent.

Who: An AsthmaNet coordinator completes the form.

When: Refer to Visit Procedure Checklists. Note that this form should also be completed if the participant or participant's parent/legal guardian reports a non-asthma/allergy-related concomitant medication between visits. Questions on other forms may also prompt a coordinator to complete this form.

Form Instructions:

Medications for concurrent medical problems not related to asthma or allergies should be recorded here. Examples would include antihypertensives, thyroid medications, calcium supplements, vitamins, influenza vaccines, aspirin for cardioprotection, and lipid-lowering agents. Most of these medications will be those that participants are taking at the time of signing the informed consent/participant assent at study entry and continue through the study.

Note that some studies impose numerous restrictions on the use of non asthma-related medications for study entry. Many drugs have required washout periods prior to enrollment. Others are allowed throughout the study, but should be withheld prior to certain procedures (e.g., methacholine challenge testing and skin testing). See the Exclusionary Drugs reference card (EXCLDRUG) for each study for the appropriate study for a complete list. It is extremely important that drug washout periods be strictly enforced and that concomitant medications be monitored diligently throughout each study.

Use of herbal remedies (for asthma and non-asthma conditions) and other alternative therapies should be recorded on the CMED_NON form, as no drug codes are available for these treatments.

At the first visit, a CMED_NON form should be started for a participant by completing the Participant ID and the Participant Initials at the top of the form. For each medication, record the medication name, start date, stop date (if applicable), whether the participant is continuing on the medication at the end of the study (if applicable), and the reason the participant is taking the medication.

For any medications the participant started prior to signing the informed consent, record the informed consent date as the 'Start Date'.

At each subsequent visit, update the form. Indicate any new medications started and any old medications that were stopped since the last visit. If the participant is still taking a medication at the end of the study, leave the stop date blank and check the “Ongoing at end of study’ box. This should be the only time the stop date is not completely filled in. If the participant does not take any concomitant medications that are unrelated to asthma, allergies, or adverse events during the entire study, check the ‘None’ box.

In the event that an approved AHFS drug code cannot be entered into the database from the CMED form, it may be recommended by the DCC to record the drug on the CMED_NON form for record keeping purposes.

This form is not entered into the database and should not be sent to the DCC.

10.1.62 Data Processing Cover Sheet (DPCS)

Purpose: This form assists the clinical site and the DCC with the tracking of all data collection packets/forms from the time of collection at the clinical site to the final processing at the DCC.

Who: Clinical center and DCC personnel complete the form.

When: This form is completed each time a data processing activity is performed.

Form Instructions:

Complete the Participant ID number, Participant initials, Visit number, and packet/form code of the packet/form being processed. The person completing the data processing activities (reviewing and collating of the forms) should record the current date and his or her 4-digit interviewer/technician ID number for each activity.

Important:

When preparing copies of the Data Processing Cover Sheets (DPCS), please use the original document that can be printed from the AsthmaNet website. Photocopies should not be made from a copy of the DPCS. All forms are now scanned at the DCC and placed in an electronic archive. The quality of the print greatly diminishes when a photocopy of a photocopy is made, this may cause the form not to be scanned correctly.

When filling out the Data Processing Cover Sheets (DPCS) remember:

- ALL information must be handwritten or pre-filled on the DPCS
-Write in the Visit number in the Visit number space provided
-Write the form name in the form name space provided

******If the practice above is not followed, the DCC may send the DPCS form back to your site to be recreated correctly.***

Clip the Data Processing Cover Sheet (DPCS) to each visit packet and each single form before any data processing activity.

10.1.63 Diskus® Inhalation Technique Checklist (TECH_DISKUS)

Purpose: To record information related to the assessment of the participant's Diskus® inhalation technique.

Who: The AsthmaNet coordinator or Pulmonary Function Technician completes the form.

When: When a Diskus® is first dispensed to a participant and as indicated on the Visit Procedure Checklists.

Form Instructions:

When the participant demonstrates the use of the Diskus®, evaluate whether his or her performance on each step was wrong or correct. Calculate the participant's score by adding the total points. Record the participant's score on the form in the designated area.

If the score is less than 10, retrain the participant and complete a new form. Be sure to complete the page numbers at the bottom of the form: 'Page _ of _'. Store all completed forms in the participant's study folder.

For use only at the clinical site – DO NOT forward to the DCC.

This form will be reviewed during AsthmaNet site visits.

10.1.64 DOSER™ Tracking Log (DOSER_LOG)

Purpose: To track the Dosers™ used in AsthmaNet studies.

Who: An AsthmaNet coordinator completes the log.

When: The log is updated every time a Doser™ is dispensed or returned or a problem is experienced with a device.

Form Instructions:

In the Balance column of the first row of the log, record the total number of Dosers™ available for distribution at your site. When completing each subsequent row, subtract one from the Balance column. If a returned Doser™ is put back into the pool of Dosers™ for distribution to future participants, add one to the Balance column in the next available row.

A row must be completed in the Doser™ Tracking Log, every time a Doser™ is distributed to a participant in an AsthmaNet study. When a Doser™ is dispensed, record the Doser™ ID number, participant ID, the date dispended, and the dispenser's initials on the log. Unique identifying numbers should be assigned to and permanently marked on the Dosers™ sequentially from 001 to 999 at each site. You can record the Wake Up date of the Doser™ on the log to determine if you should dispense a returned Doser™ to another study participant. Dosers™ have a one year lifetime starting from the day they are "woken up" for the first time. Note that multiple participants can use a given Doser™ over the course of a study. However, in most studies, each device will be assigned to one participant during its lifetime. See Section 2 of the protocol-specific MOP for more information regarding the Doser™.

When a Doser™ is returned, record the date returned, the collector's initials, and comments if the participant experienced any problems with the device.

To organize multiple pages of the DOSER_LOG, complete and update the 'Page _ of _' at the bottom of each page.

This log will be reviewed during AsthmaNet site visits.

10.1.65 GIS Address Tracking Form (GIS_ADDRESS)

Purpose: To record the participant's home addresses during study participation for GIS tracking purposes.

Who: An AsthmaNet coordinator completes the form.

When: When GIS consent has been obtained and as indicated on the Visit Procedure Checklists.

Form Instructions:

When the participant has consented to allow the use of his/her home address for GIS analysis by researchers at the University of Pittsburgh, the GIS Consent Tracking Form (GIS) should be completed in conjunction with the GIS Address Tracking Form (GIS_ADDRESS).

Apt: Addresses should include Apartment Number, when applicable.

Zip Code: Zip Code should be recorded as the 9-digit zip code, if the last four digits are known. Otherwise, record the 5-digit zip code.

At Enrollment: Record the participant's current home address and dates of occupancy, if known, in the section titled "Address at Enrollment in Protocol." This address is entered in the GIS spreadsheet for the protocol to be submitted to the University of Pittsburgh.

At subsequent visits as indicated on the visit procedure checklists: the participant should be asked for updated home address information. Record new address information if the participant has resided there for at least 4 weeks or plans to remain there indefinitely. If the participant indicates that he/she is residing at an address temporarily (i.e., less than 4 weeks), do not record this address on the form.

Subsequent addresses are recorded in the next available spot in the section titled "Subsequent Address(es) during Study Participation."

If the participant has stopped residing at a previously recorded address, provide a stop date for the previous address's Dates of Occupancy.

Once recorded on GIS_ADDRESS, see the GIS MOP for more information on submitting address information to the University of Pittsburgh.

10.1.66 MDI Inhalation Technique Checklist (with face mask) (TECH_MDI_FACE)

Purpose: To record information related to the assessment of the participant's MDI inhalation technique using a face mask.

Who: The Pulmonary Function Technician completes the form.

When: When an MDI inhaler (with face mask) is first dispensed to a participant and as indicated on the Visit Procedure Checklists.

Form Instructions:

When the participant demonstrates the use of the MDI inhaler (with face mask), evaluate whether his or her performance on each step is wrong or correct. The participant is assigned 1 point for each correct step. Calculate the participant's score by adding the total points. Record the participant's score on the form in the designated area.

If the score is less than 6, retrain the participant and complete a new form. Be sure to complete the page numbers at the bottom of the form: 'Page _ of _'. Store all completed forms in the participant's study folder.

For use only at the clinical site – DO NOT forward to the DCC.

This form will be reviewed during AsthmaNet site visits.

10.1.67 MDI Inhalation Technique Checklist (with spacer) (TECH_MDI_SP)

Purpose: To record information related to the assessment of the participant's MDI inhalation technique using a spacer.

Who: The Pulmonary Function Technician completes the form.

When: When an MDI inhaler (with spacer) is first dispensed to a participant and as indicated on the Visit Procedure Checklists.

Form Instructions:

When the participant demonstrates the use of the MDI inhaler (with spacer), evaluate whether his or her performance on each step is wrong or correct. The participant is assigned 1 point for each correct step. Calculate the participant's score by adding the total points. Record the participant's score on the form in the designated area.

If the score is less than 12, retrain the participant and complete a new form. Be sure to complete the page numbers at the bottom of the form: 'Page _ of _'. Store all completed forms in the participant's study folder.

For use only at the clinical site – DO NOT forward to the DCC.

This form will be reviewed during AsthmaNet site visits.

10.1.68 MEMS®6 Monitor Log (MEMS_LOG)

Purpose: To track the MEMS®6 monitors used in AsthmaNet studies.

Who: An AsthmaNet coordinator completes the log.

When: The log is updated every time a MEMS®6 monitor is dispensed or returned.

Form Instructions:

In the Balance column of the first row of the log, record the total number of MEMS®6 monitors available for distribution at your site. When completing each subsequent row, subtract one from the Balance column. If a returned MEMS®6 monitor is put back into the pool of monitors for distribution to future participants, add one to the Balance column in the next available row. A previously distributed MEMS®6 monitor can be distributed to another participant if the validity date of the monitor is greater than the date the participant would be enrolled in the study, the monitor's battery voltage is $\geq$ 2.90 volts and the monitor's used memory is $\leq$ 90%. The validity date can be determined by selecting the "Read Monitor Info" in the PowerView software.

A row must be completed in the MEMS®6 Monitor Log, every time a monitor is distributed to a participant in an AsthmaNet study. When a MEMS®6 monitor is dispensed, record the serial number, validity date, participant ID, the date dispended, and the dispenser's initials on the log.

When a monitor is returned, record the date returned, collector's initials, and whether the MEMS®6 Monitor failed quality control testing. When a monitor fails quality control testing, indicate this on the MEMS®6 Monitor Log. Send the failed MEMS®6 Monitor along with a copy of the corresponding MEMS®6 Quality Control form (MEMSQC) to the DCC.

To organize multiple pages of the MEMS_LOG, complete and update the 'Page _ of _' at the bottom of each page.

This log will be reviewed during AsthmaNet site visits.

10.1.69 Pediatric Participant Contact Information (CONTACT_PED)

Purpose: To record pertinent identification information for all pediatric participants so that the AsthmaNet coordinator will know how and when to easily contact the participant.

Who: The participant or participant's parent/legal guardian completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

This form ***must*** be completed at the first study visit. At subsequent study visits, update this form as indicated on the Visit Procedure Checklists.

For use only at the clinical site – DO NOT forward this form to the DCC.

This form will be reviewed during AsthmaNet site visits.

10.1.70 Spirotel® Device Log (SPIROTEL_DEVICE)

Purpose: To track the spirotel® devices used in AsthmaNet studies.

Who: An AsthmaNet coordinator completes the log.

When: The log is updated every time a spirotel® device is dispensed or returned.

Form Instructions:

A row must be completed in the Spirotel® Device Log, every time a device is distributed to a participant in an AsthmaNet study. When a spirotel® is dispensed, record the serial number, participant ID, the date dispended, and the dispenser's initials on the log.

When a device is returned, record the date returned, collector's initials, and whether the spirotel® device failed quality control testing. When a spirotel® fails quality control testing, indicate this on the Spirotel® Device Log. Send the failed spirotel® to Respitech Medical, Inc. Record the Date Shipped to Respitech and any Comments on the log. Please refer to Appendix 6 of the AsthmaNet General MOP for details related to packaging and shipping the device to Respitech.

To organize multiple pages of the SPIROTEL_DEVICE log, complete and update the 'Page _ of _' at the bottom of each page.

This log will be reviewed during AsthmaNet site visits.

10.1.71 Spirotel® Performance Checklist (SPIROTEL_PERF)

Purpose: This form is a record of the participant's ability to use the spirotel® device for recording daily diary information and/or scheduled peak flow values.

Who: An AsthmaNet coordinator or technician certified in spirotel® procedures.

When: When spirotel® device is first dispensed to a participant and as indicated on the Visit Procedure Checklists.

Form Instructions:

When the participant demonstrates the use of the spirotel® device, evaluate whether his or her performance on each step is wrong or correct. The participant is assigned 1 point for each correct step. Calculate the participant's score by adding the total points. Record the participant's score on the form in the designated area.

If the score is less than 13, retrain the participant and complete a new form. Be sure to complete the page numbers at the bottom of the form: 'Page _ of _'. Store all completed forms in the participant's study folder.

See Appendix 6 of the AsthmaNet General MOP for detailed instructions on using the spirotel® device.

For use only at the Clinical Center – DO NOT forward to the DCC.
This form will be reviewed during AsthmaNet site visits.

10.1.72 Spirotel® Turbine Log (SPIROTEL_TURBINE)

Purpose: To track the spirotel® turbines used in AsthmaNet studies.

Who: An AsthmaNet coordinator completes the log.

When: The log is updated every time a spirotel® turbine is dispensed or returned.

Form Instructions:

A row must be completed in the Spirotel® Turbine Log, every time a turbine is distributed to a participant in an AsthmaNet study. When a spirotel® is dispensed, record the serial number (4-digit number etched into the turbine), participant ID, the date dispended, and the dispenser's initials on the log.

When a turbine is returned, record the date returned, collector's initials, and whether the spirotel® turbine failed quality control testing. When a turbine fails quality control testing, indicate this on the Spirotel® Turbine Log. After all steps have been taken to ensure that the turbine will not pass quality control testing (see Appendix 6 of the AsthmaNet General MOP), discard the turbine and contact Respitech Medical, Inc. to receive a replacement turbine. Record in the Comments section the date the turbine was discarded.

To organize multiple pages of the SPIROTEL_TURBINE log, complete and update the 'Page _ of _' at the bottom of each page.

This log will be reviewed during AsthmaNet site visits.

10.1.73 Sputum Induction Worksheet (SPUTUM_INDUCTION_WKS)

Purpose: To record information related to the participant's sputum induction procedure.

Who: The Sputum Induction Technician completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

For use only at the clinical sites. DO NOT forward this form to the DCC.

10.1.74 Sputum Processing Worksheet (SPUTUM_PROCESS)

Purpose: To record detailed information related to processing of the sample.

Who: The AsthmaNet sputum slide reader completes the form.

When: Refer to Visit Procedure Checklists.

Form Instructions:

One worksheet should be completed for each processed sample and sent to the AsthmaNet sputum slide reader when related slides are sent for reading.

For use only by the AsthmaNet sputum slide readers. DO NOT forward this form to the DCC.