MOSES SOPs for Clinical Centers

(SOP#7 includes the analyses of sputum samples by the sputum core lab)

(Original date June 22, 2012)

(Revised October 5, 2012)

Revised December 23, 2012

SOPs	SOP attachments	Authors Notes
Clinical Centers Common SOPS		
#1 Height and Weight (H&W) 30APR2012		MOSES Investigators
~~# 2 Vital signs(VS)/BP SCREEN AND TRAIN 30APR2012~~ # 2 Vital signs(VS)/BP SCREEN AND TRAIN V2 06OCT2012		MOSES Investigators Lowered lower BP limits to 90/50
~~# 3 Vital signs (VS)/BP PRE, EXP, POS 30APR2012~~ # 3 Vital signs (VS)/BP PRE, EXP, POS V2 06OCT2012		MOSES Investigators Lowered lower BP limits to 90/50
~~#4 Exer MV ECG BP (MINVEN) Training (TRAIN) 30APR2012~~ #4 Exer MV ECG BP (MINVEN) Training (TRAIN) V2 06OCT2012		MOSES Investigators Lowered lower BP limits to 90/50
~~#5 Exer MV ECG BP (MINVEN) Exposure Visits (EXP) 30APR2012~~ #5 Exer MV ECG BP (MINVEN) Exposure Visits (EXP) V2 06OCT2012		MOSES Investigators Lowered lower BP limits to 90/50
#6 Spirometry (SPIR) UNC 30APR2012 #6 Spirometry (SPIR) UCSF 30APR2012 #6 Spirometry (SPIR) UR 30APR2012		Hazucha Wong Frampton
#7 Sputum Induction Processing and Shipping (SPUTUM) 15JUN2012		Alexis
#11 Phlebotomy (PHLEB) SCREEN 05JUN2012		LabCorp
#12 Phlebotomy (PHLEB) EXP 30APR2012		MOSES Investigators
#13 Flow Cytometry (FLOW) 05JUN2012		Frampton
#21 12-Lead ECG (ECG) Screening 23MAR2012		MOSES investigatros
~~#22 Holter recording (HOLTER) 08JUN2012~~ #22 Holter recording (HOLTER) V2 05OCT2012		Zareba Costantini (modified to indicate that ADEPT will inform the core lab that a shipment was initiated)
~~#31 Brachial Artery Reactivity and Doppler (BAU) 24MAY2012~~ #31 Brachial Artery Reactivity and Doppler (BAU) V2 23DEC2012		Ganz Clarified the procedure, explained when procedure should be repeated,removed the NTG component
#32 Brachial Artery Ultrasound Analysys 27JAN2014	1. Brachial Artery Ultrasound Measurements (form.) 2.Phantom Protocol for Quality Assurance of Brachial Artery Ultrasound in HEI MOSES Study. 3. HEI MOSES Phantom Scan Form for Quality Assurance.	Ganz
#41 PES preparation, storage, and use (PES) 23MAR2012	Subject instruction for using the PES 23MAR2012	MOSES Investigators
#42 Tech Instructions for TAD (OZ DIARY) 23MAR2012	MOSES Activity Diary 06APR2012	MOSES investigators
~~#51 Adverse Event reporting process 11APR2012~~ ~~#51 Adverse Event reporting process V2 06SEP2012~~ #51 Adverse Event reporting process V3 21DEC2012	~~Adverse Event Report Form 11APR2012~~ Adverse Event Report Form 24DEC2012	Nash V2. Modified to clarify the reporting process V3. Modified definiton of Grade 2 events

Note: list does not include exposure chamber SOPs (see chmaber SOP folder for these SOPs)

MOSES Standard Operating Procedure (SOP)
SOP # 1 – H&W

Title: Measurements of subject's height and weight **Version:** 1.0

Directory/file[a]:

Author: MOSES Investigators Signature: Date: April 30, 2012

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

ANNUAL REVIEW

Signature	**Date**

Explanations: [a] Incorporate the date and the version of SOP into the file name

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. <u>If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!</u>

[c] Annual review does not change the version of the SOP.

MEASUREMENTS OF SUBJECT'S HEIGHT AND WEIGHT

1. SCOPE and APPLICATION

The purpose of this SOP is to provide guidelines for measuring height and weight of subjects in a uniform way.

This document should be reviewed annually. Any revisions should be properly documented in the cover form of this SOP.

2. PREREQUISITES

This SOP assumes familiarity with the installation and operation of the instrument(s) where applicable. For detailed instructions in the operation of the instrument(s) refer to a respective Operation and Service Manual. The following subsections provide a list of equipment and supplies needed to take the measurements, as well as required training needed to operate the instrument(s).

2.1. Equipment and Supplies

1. Height: Wall or scale mounted stadiometer
2. Weight: Electronic: Scale-Tronix 5005 Stand-On Scale (UR): Electronic Dial Scale Model HDR900 (UCSF) (UNC)

   Weight: Manual: Detecto mechanical scale (or other makes mechanical hospital grade scale) (UCSF, Model 758C)
3. Paper towels

2.2. Training Requirements

The operator is the most critical component of measuring subject's height and weight.

The operator conducting the measurements should be trained in performing such measurements in a safe and standardized manner.

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

The manual Detecto scale needs to be left in the weight position, not with the weights in the zero position.

4. PROCEDURES

It is assumed that the scale is setup according to manufacturer's instructions. The subsequent procedures may slightly differ depending on the make of the device(s).

4.1. Measuring height

1. Raise the flat reader/headpiece attached to the height rod higher than the anticipated subject's height.
2. Subject should remove shoes, bulky clothing, and hair ornaments, and unbraid hair that interferes with the measurement. A paper towel may be placed over the step on the scale if the subject is not wearing socks.

3. Assist the subject to standing on the scale until he/she is stable and secure on the step.
4. The subject should stand with feet flat, together, and directly under the headpiece, with heels against the wall (for wall mounted scale). Make sure legs are straight, arms are at sides, and shoulders are level.
5. Make sure the subject is looking straight ahead and that the line of sight is parallel with the floor and is directly under the reader/headpiece.
6. Lower the headpiece until it firmly rests level on the crown of the subject's head. Tighten the reader/headpiece down if needed. Ask the subject to step away from the wall (for wall-mounted scale).
7. Read the marking at the bottom of the mobile height rod (wall-mounted) or the reader' number (other scales) (at eye level) and record the subject's height (in cm).
8. Raise the reader/headpiece.

4.2. Measuring weight

a. Ask the subject to remove heavy objects from pocket, including keys, cell phones, etc.
b. Ask the subject to remove shoes. A paper towel may be placed over the step on the scale if the subject is not wearing socks.
c. Tap on the scale to power on –wait until it displays "0".
d. Ask the subject to stand on the dark boxes in the middle of the scale and ensure he/she is comfortable and stable. Subjects may hold onto the attached handrail for stability. Prepare to offer support until she/he is stable and secure on the scale.

4.2.1 Electronic scale

1. Wait until the weight is displayed
2. Record the weight (in kg).
3. Ask the subject to step off the scale. Stand next to the subject and prepare to offer support when he/she steps down from the scale.

4.2.2 Mechanical scale (as back up in the event the electronic scale malfunctions):

1. Adjust the 20 kilo weight to the right until the tip of the balance drops to bottom of the notch. At this point, move the 20 kilo weight back one notch. The tip of the balance should rise back to the top.
2. Move the 1 kilo weight slowly to the right until the tip of the balance begins to fall into the notch. Move the weight slowly until the tip is centered in the notch. Add the values on marked by both the 20 kilo and the 1 kilo weight. This is the subject's weight.
3. Record the weight

4. Ask the subject to step off of the scale. Stand next to the subject and prepare to offer support when she/he steps down from the scale.

4.3. Recordkeeping

Report height (in cm) to the nearest whole number (e.g., 167.4 cm would be reported as 176 cm; 176.5 cm would be reported as 177 cm), weight (in kg) to one decimal point (e.g., 76.65 kg would be reported as 76.7 kg) and enter in the Vital Signs and Blood Pressure -Screening Training Data Form.

BMI will be calculated by ADEPT using the following equation: BMI = [kg/(cm/100)^2]. BMI can also be obtained http://www.nhlbisupport.com/bmi/bmi-m.htm

BSA will be calculated by ADEPT using the Mosteller's equation found at www.globalrph.com/bsa2.htm:
BSA (m^2) = SQR RT ([Height(cm) x Weight(kg)]/ 3600)

4.4. Chain-of-Custody

UNC: The stadiometer, Electronic scale model HDR900, and Detecto Scale, Model 758C are located in room #527.

UCSF: The stadiometer, the Electronic Dial Scale, and the Detecto scale are located in Bldg 1, room #152

UR: The stadiometer and scale are located in the Clinical Research Center, room G-5035.

5. INFECTION CONTROL

1. Wipe exterior surface of stadiometer and weight scale with a mild soap detergent and water.
2. Wipe exterior surface of stadiometer and weight scale with a dry cloth.

6. QUALITY CONTROL

1. Calibrate the scale per recommended procedure by the manufacturer to make sure that the scale is accurate and performs according to specifications.
2. Perform routine maintenance of the instrument in accordance with manufacturer's recommendation.

7. QUALITY ASSURANCE

As part of the QA, NERI or HEI may request:

1. A copy of selected study forms with recorded subjects' data
2. A copy of a Certificate of Maintenance or other confirmation of periodic in-service maintenance of the instrument(s) as recommended by the manufacturer.

8. REFERENCES

None

MOSES Standard Operating Procedure (SOP)

SOP # 2 - VS/BP SCREEN AND TRAIN

Title: Vital signs/ Stand-alone Blood Pressure measurement during the screening and training visits (Version: 1.0)

Directory/file[a]:

Author: MOSES investigators **Signature:** **Date: April 30, 2012**

Revised by: MOSES investigators **New version[c]:** 2 **Date: October 6, 2012**
Revisions/additions: changed the lower acceptable limit for systolic and diastolic blood pressure from 100/60 to 90/50 (sections 4.2.1 and 4.2.2)

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

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[c] Annual review does not change the version of the SOP.

VITAL SIGNS/ STAND-ALONE BLOOD PRESSURE MEASUREMENT DURING THE SCREENING AND THE TRAINING VISITS

1. SCOPE and APPLICATION

The purpose of this SOP is to provide standardized guidelines for taking Vital Signs in a uniform way during the screening and training visits. Vital signs measurement includes temperature (optional), heart rate (pulse), breathing rate (optional), Oxygen saturation (optional), and blood pressure.

This document should be reviewed annually. Any revisions should be properly documented in the cover form of this SOP.

2. PREREQUISITES

This SOP assumes familiarity with the installation and operation of the instrument(s) where applicable. For detailed instructions in the operation of the instrument(s) refer to a respective Operation and Service Manual. The following subsections provide a list of equipment and supplies needed to take the measurements, as well as required training needed to operate the instrument(s).

2.1. Equipment and Supplies

1. Digital thermometer for oral or ear temperature measurement (UCSF Welch Allyn Braun ThermoScan Pro 4000 thermometer)
2. Thermometer probe covers
3. Automatic blood pressure/ vital signs device and various cuffs for blood automated blood pressure and heart rate (pulse) (UCSF: Critikon DINAMAP™ Vital Signs Monitor 8100.) (UNC: the WelchAllyn 420NTB-E1 SOPT vital signs monitor and Spacelabs Medical Mod.90467 NIBP SPO_2 module wall mounted.) (UR: Critikon DINAMAP 845XT and GE CARESCAPE DINAMAP V100.)
4. Sphygmomanometer and stethoscope for manual blood pressure.
5. Watch with a second hand or timer for breathing rate and manual heart rate

2.2. Training Requirements

The operator is the most critical component of accurate vital signs and blood pressure measurement.

The operator conducting the tests should be trained in performing the tests in a safe and standardized manner.

For accurate blood pressure measurement, the operator must: (1) be properly trained in the techniques of blood pressure measurement; (2) use an accurate and properly maintained device; (3) recognize subject factors, such as anxiety and recent nicotine use, that would adversely affect blood pressure measurements; (4) position the subject appropriately; (5) select the correct cuff and position it correctly; and (6) accurately record the values obtained (Pickering et al, 2005).

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

3.1 Pulse (Heart Rate)

1. Perform in a quiet area after the subject has rested for 5 minutes (except for the optional measurement during the training exercise).

3.2 Breathing rate

1. Perform in a quiet area after the subject has rested for ~~at least~~ 5 minutes
2. Perform unobserved by subject.

3.3 Blood pressure

1. Perform measurement in a quiet area
2. Allow the subject to sit quietly for 5 minutes (except for the optional measurement during the training exercise/rest periods)
3. Uncross arms, legs, and ankles.
4. Be able to recognize and trouble shoot unusual measurements.
5. Recognize external factors that can influence blood pressure measurement (e.g.: anxiety, excitement).

4. PROCEDURES

Set up the instrument(s) according to manufacturer's instructions. The subsequent procedures may slightly differ depending on the make of the device(s).

4.1 Measuring Vital Signs (in chronologic order)

1. Have the subject bare his/her right arm. Rolled-up sleeve should be loose as not to restrict blood flow.
2. Have the subject seated comfortably in a chair in a quiet place breathing quietly with the legs uncrossed, the back supported and the relaxed right arm resting on a firm surface at heart level for <u>5 minutes</u> prior to vital signs assessment (with the exception of the exercise period).

4.1.1. Taking body temperature

1. Slip on cover on the thermometer probe.
2. Instruct the subject to open their mouth and lift up their tongue. Place the end of the thermometer probe under the tongue and instruct the subject to close their mouth.
3. Once you hear a beeping sound to let you know that the measurement is completed read the temperature and record in appropriate data form (in Celsius).
4. Discard the cover and clean the probe.

4.1.2 Taking pulse (heart rate)

4.1.2.1 Automated pulse (default method)

1. Perform an automated blood pressure (BP) as described in section 4.1.4.
2. Record the automated displayed heart rate (HR) (beats per minutes) on the data form.

4.1.2.2 Manual Pulse (HR) (use this procedure in place of an automated measurement in case of device failure of if not measurable by the device)

1. Place your index, middle and/or ring finger tips on to the radial pulse of the subjects back of the hand or forearm.
2. Gently press down with your finger tips on to the subject's radial artery (alternatively the brachial or carotid artery may be used) until you feel a pulse.
3. While looking at the timer/watch, quietly count to yourself the number of pulse you feel for 30 seconds.
4. Calculate the HR (beats per minute) and record on the data form.

4.1.3 Taking breathing rate

1. Take breathing rate unobtrusively as you measure the subject's temperature or pretend to be performing other tasks. Try not to let the subject know you're counting their breaths since people tend to change their breathing pattern when observed.
2. Place your hands on the subject's radial pulse/wrist of the arm closest to you as describe in section 4.1.2.
3. With your hand positioned to take a heart rate, look at a timer/watch and the subject's chest and abdomen. Count the subject's number of breaths for 30 seconds.
4. Calculate the number of breaths per minute and record on data form.

4.1.4 Measuring blood pressure (as part of Vital Sign or as a stand-alone measurement)

4.1.4.1 Automatic blood pressure monitoring/recording device (default method)

1. Have the subject bare their right arm. Rolled-up sleeve should be loose as not to restrict blood flow.
2. Have the subjects seated comfortably in a chair in a quiet place breathing quietly with the legs uncrossed, the back supported and the relaxed right arm resting on a firm surface at heart level for 5 minutes.
3. Select a proper size of the cuff and wrap it snugly around the subject's arm.

The lower end of the cuff should be a minimum of 2 to 3 cm above the antecubital fossa and the cuff at the level of heart.

- Arm circumference 22 to 26 cm, 'small adult' cuff, 12 x 22 cm
- Arm circumference 27 to 34 cm, 'adult' cuff: 16 x 30 cm
- Arm circumference 35 to 44 cm, 'large adult' cuff: 16 x 36 cm
- Arm circumference 45 to 52 cm, 'adult thigh' cuff; 16 x 42

4. Neither the operator nor the subject should talk during the measurement.
5. Start the device. The peak inflation pressure should be ~30 mm Hg above the systolic pressure. If not, adjust the device.
6. Once the measuring cycle is completed record the systolic and diastolic pressure (mmHg) and heart rate (beats/min) on the data form.
7. Turn off the device

4.1.4.2 Manual (auscultatory) blood pressure measurements using a sphygmomanometer (use this procedure in place of an automated measurement in case of device failure of if not measurable by the device)

1. Repeat steps 1-4 of section 4.1.4.1
2. Position the bell or a diaphragm of the stethoscope at the elbow crease over the brachial artery.
3. Close the bulb and inflate the cuff while listening through the stethoscope. The inflation pressure should be 20 ~30 mm Hg above the pressure at which the radial pulse disappears (no audible sound is detected).
4. Listen to the Korotkoff sounds as you deflate the cuff at a rate of 2 to 3 mm Hg per second (or per pulse beat when the heart rate is very slow).
5. The pressure at the first audible sound (Korotkoff 1) should be taken as systolic pressure. The mercury column should be read to the nearest 2 mm Hg.
6. The pressure at which the sound of the pulse disappears (Korotkoff 5) should be taken as the diastolic pressure. In some individuals the sounds at this point may not totally disappear but will be barely audible (Pickering et al, 2005). If the Korotkoff sounds do not total y disappear as cuff pressure is lowered, one should use the pressure at which a marked sudden diminution of the loudness in the Korotkoff sounds first occurs (Korotkoff 4) as the diastolic pressure.
7. Once the measuring cycle is completed record the systolic and diastolic pressure (mmHg) and heart rate (beats/min) on the data form.

4.2 Evaluation and Safety Criteria of Blood Pressure (automatic/manual)

4.2.1 During the Screening Visit

1. If the first BP measurement is outside of study limits (upper limit 140/90, lower limit 90/50 ~~100/60~~) repeat up to two more times at 3 min intervals at rest

following steps 4.1.4.1.4 .

2. Record the individual BP measurements in the Vital Signs and Blood Pressure Screening Data Form. The first BP measurement that is within the study limits (and corresponding HR measurement) should be entered in ADEPT.

3. If none of the BP measurements are between 140/90 and 90/50 ~~100/60~~ inclusive, the subject is excluded. <u>However,</u> if the subject meets all other inclusion criteria, measure BP again after an hour or so and/or reschedule the subject for a limited screening visit to re-check the BP (refer to section 4.3).

4. If none of the follow-up BP measurements are between 140/90 and 90/50 ~~100/60~~ inclusive, discharge the subject from the study with a recommendation to see his/her primary care physician

4.2.2. During the Training visit (before exercise)

1. If the first measurement is above 155/95 or less than 90/50 ~~100/60~~, repeat the measurement up to two or more times at (3 min) intervals at rest.

2. Record the individual measurements on the Vital Signs and Blood Pressure Training Data Form. The first measurement within the study limit BP should be entered in ADEPT.

3. If none of the BP measurements are between 155/95 and 90/50 ~~100/60~~ inclusive, consult the physician on-call. The on-call physician will determine whether the subject should be excluded. The physician may opt to measure the BP again after an hour or so, and/or reschedule the subject's visit.

4. If none of the follow-up BP measurements are between 155/95 and 90/50 ~~100/60~~ inclusive, discharge the subject from the study with a recommendation to see his/her primary care physician.

4.2.3 During the Training Exercise - see SOP #4

4.3 Safety Criteria and Corrective Actions

The on-call physician should make the final determination on what corrective actions should be taken. .

4.4 Recordkeeping

The operator should enter the obtained data in the Vital Signs and Blood Pressure Screening Training Data Form. Refer to sections 4.2.1 and 4.2.2 for reporting BP into ADEPT during Screening and non-exercise Training. The measure of HR rate to be entered in the database should correspond to the measure of BP that is entered in the database. For reporting vital signs during the training exercise/rest periods refer to SOP #4.

4.5 Chain-of-Custody of instruments

UNC: The WelchAllyn 420NTB-E1 SPOT vital signs monitor resides in rm. #527. Spacelabs Medical Mod. 90467 NIBP SPO_2 module wall mounted resides in exposure chamber LA75 and LB77.

UR: Critikon DINAMAP 845XT is located in the Environmental Exposure Facility (B.11305). GE CARESCAPE DINAMAP V100 is located in the Clinical Research Center (G-5035).

UCSF: Critikon DINAMAP™ Vital Signs Monitor 8100, digital thermometer, and supplies are located in Bldg 1, room #152.

5. INFECTION CONTROL

5.1 Wash hands before and after each subject.

5.2 Practice universal precautions.

5.3 Discard single use device after single use.

5.4 Wipe external surface of equipment.

6. QUALITY CONTROL

6.1 Calibrate the monitor per recommended procedure by the manufacturer to make sure that the instrument is accurate and performs according to the specifications.

6.**3** Maintain a calibration log book. The log book should have the following entries: Date of calibration, the date of routine maintenance and maintenance by service department if any.

6.4 Perform routine maintenance of the instrument in accordance with manufacturer's recommendation if required.

7. QUALITY ASSURANCE

As part of the QA, NERI or HEI may request:

7.1 A copy of selected vital signs forms with recorded subjects' data

7.2 A copy of calibration log book pages recorded over a specified time period

7.3 A copy of a Certificate of Maintenance or other confirmation of periodic in-service maintenance of the instrument(s) as recommended by the manufacturer.

8. REFERENCES

Pickering TG, Hall JE; Appel LJ, Falkner BE, Graves J, Hill MN, Jones DW, Kurtz T, Sheps SG, Roccella EJ. 2005. Recommendations for Blood Pressure Measurement in Humans & Experimental Animals: Part 1: Blood Pressure Measurement in Humans: A Statement for Professionals from the Subcommittee of Professional & Public Education of the American Heart Association Council on High Blood Pressure Research. Circ, 111:697-716.

Critikon DINAMAP™ Vital Signs Monitor Operator Manual

Welch Allyn Braun ThermoScan Pro 400 Thermometer Manual.

9. APPENDICES

None

MOSES Standard Operating Procedure (SOP)

SOP # 3 - VS/BP PRE, EXP, POST

Title: Vital signs/ Stand-alone Blood Pressure measurement during the pre-exposure, exposure, and post-exposure visit (Version: 1.0)
Directory/file[a]:

Author: MOSES investigators **Signature:** **Date: April 30, 2012**

Revised by: MOSE investigators **New version[c]:** 2 **Date: October 6, 2012**
Revisions/additions: changed the lower acceptable limit for systolic and diastolic blood pressure from 100/60 to 90/50 (sections 4.2.1 and 4.2.3)

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
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Revised by: **New version[c]:** **Date:**
Revisions/additions:

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Explanations: [a] Incorporate the date and the version of SOP into the file name
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[c] Annual review does not change the version of the SOP.

VITAL SIGNS/ STAND-ALONE BLOOD PRESSURE MEASUREMENT DURING THE PRE- EXPOSURE, EXPOSRE, AND POST-EXPOSURE VISITS

1. SCOPE and APPLICATION

The purpose of this SOP is to provide standardized guidelines for taking Vital Signs in a uniform way during the pre- exposure, exposure, and post-exposure days. Vital signs measurement includes temperature (optional), heart rate (pulse), breathing rate (optional), Oxygen saturation (optional), and blood pressure.

This document should be reviewed annually. Any revisions should be properly documented in the cover form of this SOP.

2. PREREQUISITES

This SOP assumes familiarity with the installation and operation of the instrument(s) where applicable. For detailed instructions in the operation of the instrument(s) refer to a respective Operation and Service Manual. The following subsections provide a list of equipment and supplies needed to take the measurements, as well as required training needed to operate the instrument(s).

2.1. Equipment and Supplies

1. Digital thermometer for oral or ear temperature measurement (UCSF Welch Allyn Braun ThermoScan Pro 4000 thermometer)
2. Thermometer probe covers
3. Automatic blood pressure/ vital signs device and various cuffs for blood automated blood pressure and heart rate (pulse) (UCSF: Critikon DINAMAP™ Vital Signs Monitor 8100.) (UNC: the WelchAllyn 420NTB-E1 SOPT vital signs monitor and Spacelabs Medical Mod.90467 NIBP SPO_2 module wall mounted.) (UR: Critikon DINAMAP 845XT and GE CARESCAPE DINAMAP V100.)
4. Sphygmomanometer and stethoscope for manual blood pressure.
5. Watch with a second hand or timer for breathing rate and manual heart rate

2.2. Training Requirements

The operator is the most critical component of accurate blood pressure measurement.

The operator conducting the tests should be trained in performing the tests in a safe and standardized manner.

For accurate blood pressure measurement, the operator must: (1) be properly trained in the techniques of blood pressure measurement; (2) use an accurate and properly maintained device; (3) recognize subject factors, such as anxiety and recent nicotine use, that would adversely affect blood pressure measurements; (4) position the subject appropriately; (5) select the correct cuff and position it correctly; and (6) accurately record the values obtained (Pickering et al, 2005).

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

3.1 Pulse (Heart Rate)

1. Perform in a quiet area after the subject has rested for 5 minutes

3.2 Breathing rate

1. Perform in a quiet area after the subject has rested for 5 minutes.
2. Perform unobserved by subject.

3.3 Blood pressure

1. Perform measurement in a quiet area
2. Allow the subject to sit quietly for 5 minutes
3 Uncross arms, legs, and ankles.
4 Be able to recognize and trouble shoot unusual measurements.
5 Recognize external factors that can influence blood pressure measurement (e.g.: anxiety, excitement).

4. PROCEDURES

Set up the instrument(s) according to manufacturer's instructions. The subsequent procedures may slightly differ depending on the make of the device(s).

4.1 Measuring Vital Signs (in chronologic order)

1. Have the subject bare his/her right arm. Rolled-up sleeve should be loose as not to restrict blood flow.
2. Have the subject seated comfortably in a chair in a quiet place breathing quietly with the legs uncrossed, the back supported and the relaxed right arm resting on a firm surface at heart level for 5 minutes prior to vital signs assessment (with the exception of unscheduled measurement during the exercise period).

4.1.1 Taking body temperature

1. Slip on cover on the thermometer probe.
2. Instruct the subject to open their mouth and lift up their tongue. Place the end of the thermometer probe under the tongue and instruct the subject to close their mouth.
3. Once you hear a beeping sound to let you know that the measurement is completed read the temperature and record in appropriate data form (in Celsius).
4. Discard the cover and clean the probe.

4.1.2 Taking pulse (heart rate)

4.1.2.1 Automated pulse (default method)

1. Perform an automated blood pressure (BP) as described in section 4.1.4.

2. Record the automated displayed heart rate (HR) (beats per minute) in the data form The average of the second and third HR measurements should be entered in ADEPT. NOTE all three measurements have to be taken with the same device (see section 4.4). Note any abnormalities.

4.1.2.2 Manual pulse (HR) (use this procedure in place of an automated measurement in case of device failure or if not measured by the device)

1. Place your index, middle and/or ring finger tips on to the radial pulse of the subjects back of the hand or forearm.
2. Gently press down with your finger tips on to the subject's radial artery (alternatively the brachial or carotid artery may be used) until you feel a pulse.
3. While looking at the timer/watch, quietly count to yourself the number of pulse you feel for 30 seconds.
4. Calculate the number of pulse per minute on work sheet and record it on the data form as described in section 4.1.2.1.2 above

4.1.3 Taking breathing rate

1. Take breathing rate unobtrusively as you measure the subject's temperature or pretend performing other tasks. Try not to let the subject know you're counting their breaths since people tend to change their breathing pattern when observed.
2. Place your hands on the subject's radial pulse/wrist of the arm closest to you as describe in section 4.1.2.
3. With your hand positioned to take a heart rate, look at a timer/watch and the subject's chest and abdomen. Count the subject's number of breaths for 30 seconds.
4. Calculate the number of breaths per minute and record on the data form.

4.1.4 Measuring blood pressure (as part of Vital Signs or as a stand-alone measurement)

4.1.4.1 Automatic blood pressure monitoring/recording device (default method)

1. Have the subject bare his/her right arm. Rolled-up sleeve should be loose as not to restrict blood flow.
2. Have the subject seated comfortably in a chair in a quiet place breathing quietly with the legs uncrossed, the back supported and the relaxed right arm resting on a firm surface at heart level for 5 minutes (with the exception of unscheduled measurement during the exercise period).
3. Select a proper size of the cuff and wrap it snugly around the subject's arm. The lower end of the cuff should be a minimum of 2 to 3 cm above the antecubital fossa and the cuff at the level of heart.
    - Arm circumference 22 to 26 cm, 'small adult' cuff, 12 x 22 cm
    - Arm circumference 27 to 34 cm, 'adult' cuff: 16 x 30 cm
    - Arm circumference 35 to 44 cm, 'large adult' cuff: 16 x 36 cm

- Arm circumference 45 to 52 cm, 'adult thigh' cuff; 16 x 42

3. Neither the operator nor the subject should talk during the measurement.
4. Start the device. The peak inflation pressure should be ~30 mm Hg above the systolic pressure. If not, adjust the device.
5. Once the measuring cycle is completed record the systolic and diastolic pressure (mmHg) and heart rate (beats/min) (see section 4.1.2.6) on the data form.
6. Have the subject seat comfortably in a chair breathing quietly with the legs uncrossed, the back supported and the relaxed right arm resting on a firm surface at heart level for 1 minute.
7. Repeat steps 4-7 two more times for a total of 3 measurements (with the exception of BP measured during exposure rest periods 2 and 4 when only one measurement should be taken).
8. Turn off the device
9. The average of the second and third diastolic and systolic BP measurements should be entered in ADEPT. NOTE all three measurements have to be taken with the same device.

4.1.4.2 Manual (auscultatory) blood pressure measurements using a sphygmomanometer (use this procedure in place of an automated measurement in case of device failure of if not measurable by the device).

1. Repeat steps 1-3 of section 4.1.4.1
2. Position the bell or a diaphragm of the stethoscope at the elbow crease over the brachial artery.
3. Close the bulb and inflate the cuff while listening through the stethoscope. The inflation pressure should be 20 ~30 mm Hg above the pressure at which the radial pulse disappears (no audible sound is detected).
4. Listen to the Korotkoff sounds as you deflate the cuff at a rate of 2 to 3 mm Hg per second (or per pulse beat when the heart rate is very slow).
5. The pressure at the first audible sound (Korotkoff 1) should be taken as systolic pressure. The mercury column should be read to the nearest 2 mm Hg.
6. The pressure at which the sound of the pulse disappears (Korotkoff 5) should be taken as the diastolic pressure. In some individuals the sounds at this point may not totally disappear but will be barely audible (Pickering et al, 2005). If the Korotkoff sounds do not total y disappear as cuff pressure is lowered, one should use the pressure at which a marked sudden diminution of the loudness in the Korotkoff sounds first occurs (Korotkoff 4) as the diastolic pressure.

7. Once the measuring cycle is completed record the systolic and diastolic pressure (mmHg) and heart rate (beats/min) (see section 4.1.2.6) on the data form.
8. Have the subject seat comfortably in a chair breathing quietly with the legs uncrossed, the back supported and the relaxed right arm resting on a firm surface at heart level for 1 minute.
9. Repeat steps 2-8 two more times for a total of 3 measurements (with the exception of BP measured during exposure rest periods 2 and 4 when only one measurement should be taken).
9. The average of the second and third diastolic and systolic BP measurements should be entered in ADEPT. NOTE all three measurements have to be taken with the same device.

4.2 Evaluation and Safety Criteria of Blood Pressure

4.2.1. Pre-exposure day and exposure day baseline measurements

1. If the average of the second and third BP measurements is above 155/95 or less than 90/50 ~~100/60~~, consult the on-call physician. Continue with the visit if medically cleared, otherwise as appropriate measure the BP again after an hour or so, and/or reschedule the subject's visits.

2. If none of the follow-up BP measurements are between 155/95 and 90/50 ~~100/60~~ inclusive, discharge the subject from the study.

4.2.2 During the Exposure Period - see SOP #5

1. There are no scheduled BP measurements during exercise. At the discretion of the on-call physician unscheduled BP measurements might be made.
2. BP measurement is required only during Rest period 2 and 4. At the discretion of the on-call physician unscheduled BP measurements might be made.

4.2.3 Post-exposure measurements

1 If the average of the second and third BP measurements exceeds 155/95 or is less than 90/50 ~~100/60~~, consult the on-call physician. (Note: the first post-exposure measurement is taken immediately after the end of the exposure while the subject is still in the chamber).

4.3 Corrective Actions

The course of action after scheduled and unscheduled BP measurements will be determined by the on-call physician.

4.4 Recordkeeping

The operator should enter the obtained data in the Vital Signs and Blood Pressure Pre- and Post- Data Form and the Vital Signs and Blood Pressure Log - Exposure Day. Refer to section 4.1.4.1 for how BP should be calculated and reported. The measure of HR to be reported in ADEPT should correspond to the measure of BP that is reported. For measuring and reporting HR and BP during the exposure period refer to SOP#5.

4.5 Chain-of-Custody of instruments

UNC: The WelchAllyn 420NTB-E1 SPOT vital signs monitor resides in rm. #527. Spacelabs Medical Mod. 90467 NIBP SPO_2 module wall mounted resides in exposure chamber LA75 and LB77.

UR: Critikon DINAMAP 845XT is located in the Environmental Exposure Facility (B.11305). GE CARESCAPE DINAMAP V100 is located in the Clinical Research Center (G-5035).

UCSF: Critikon DINAMAP™ Vital Signs Monitor 8100, digital thermometer, and supplies are located in Bldg 1, room #152.

5. INFECTION CONTROL

5.1 Wash hands before and after each subject.

5.2 Practice universal precautions.

5.3 Discard single use device after single use.

5.4 Wipe external surface of equipment.

6. QUALITY CONTROL

6.1 Calibrate the monitor per recommended procedure by the manufacturer to make sure that the instrument is accurate and performs according to the specifications.

6.**3** Maintain a calibration log book. The log book should have the following entries: Date of calibration, the date of routine maintenance and maintenance by service department if any.

6.4 Perform routine maintenance of the instrument in accordance with manufacturer's recommendation if required.

7. QUALITY ASSURANCE

As part of the QA, NERI or HEI may request:

7.1 A copy of selected vital signs forms with recorded subjects' data

7.2 A copy of calibration log book pages recorded over a specified time period

7.3 A copy of a Certificate of Maintenance or other confirmation of periodic in-service maintenance of the instrument(s) as recommended by the manufacturer.

8. REFERENCES

Pickering, et al., Recommendations for Blood Pressure Measurement in Humans & Experimental Animals: Part 1: Blood Pressure Measurement in Humans: A Statement for Professionals from the Subcommittee of Professional & Public Education of the American Heart Association Council on High Blood Pressure, Res Circ, 111:697-716, 2005.
Critokon DINAMAP™ Vital Signs Monitor Operator Manual
Welch Allyn Braun ThermoScan Pro 400 Thermometer Manual.

9. APPENDICES

None

MOSES Standard Operating Procedure (SOP)

SOP # 4 - EXER/MINVEN/ECG/BP (MINVEN) – TRAIN

Title: Exercise with Minute Ventilation, ECG, Blood Pressure, and Heart Rate during training visit (Ver. 1.0)

Directory/file[a]:

Author: MOSES Investigators **Signature:** **Date: April 30, 2012**

Revised by: **New version[c]:** V2 **Date: October 6, 2012**
Revisions/additions: changed the safety criterion for the lower systolic and diastolic blood pressure limit during exercise from 90/60 to 90/50 (section 4.5).

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

ANNUAL REVIEW

Signature	Date

Explanations: [a] Incorporate the date and the version of SOP into the file name
[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!
[c] Annual review does not change the version of the SOP.

EXERCISE WITH ECG, MINUTE VENTILATION, BLOOD PRESSURE, AND HEART RATE (TRAINING VISIT)

1. SCOPE and APPLICATION

The purpose of this SOP is to provide guidelines for:

1) conducting treadmill or bicycle exercise at the subject's target expired minute ventilation (V_E) corrected by body surface area (BSA) of 15-17 L/min/m^2 (V_E BSA) and,

2) cardiac monitoring and measuring V_E, heart rate (HR), blood pressure (BP), and oxygen saturation (SpO_2-optional) at predetermined intervals, and determining exercise load, during the training visit.

This document should be reviewed annually. Any revisions should be properly documented in the cover form of this SOP.

2. PREREQUISITES

This SOP assumes familiarity with the installation and operation of the instrument(s) where applicable. For detailed instructions in the operation of the instrument(s) refer to a respective Operation and Service Manual. The following subsections provide a list of equipment and supplies needed to take the measurements, as well as required training needed to operate the instrument(s).

2.1. Equipment and Supplies

UCSF

1. Cybex 700T-01-4 Treadmill
2. Monark 818E Ergomedic Stationary Bicycle

UNC

1. Treadmill. Marquette 2000 series

UR

1. Cybex 530C Stationary Bicycle

Common equipment

1. Fleisch Pneumotachograph V_E assembly
2. Acquisition computer
3. ECG machine
4. Blood pressure monitor
5. Pulse oximeter (optional)
6. Electrodes, mouth pieces, nose clips

2.2. Training Requirements

The operator conducting the tests should be trained in operating the instruments and performing the tests in a safe and standardized manner.

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

3.1 Treadmill

1. While exercising on the treadmill subject's may grasp the hand grips intermittently but should not hold on to the hand grips during the entire exercise period.
2. To stop the treadmill in an emergency situation, instruct the subject to press the "STOP" red button or, if available, the alternate stop switch and step on to the frame/side panel.
3. To release the alternate stop switch, turn it ¼-turn in either direction or follow instrument-specific instructions.

3.2 Bicycle

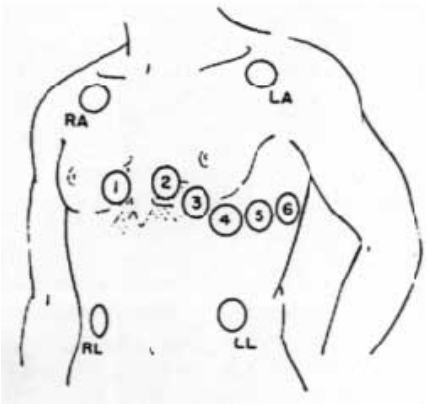
1. To stop in an emergency situation, instruct the subject to stop cycling and dismount from the bicycle when safe to do so.

4. PROCEDURES

It is assumed that the instrument(s) is (are) set up according to manufacturer's instructions. The subsequent procedures may differ depending on the make of the device(s). Either a 12-lead or a 3-lead ECG will be used for cardiac monitoring during the training visit.

4.1 Cardiac Monitoring: 12-Lead ECG

1. Apply 12-lead ECG electrodes to the chest for monitoring as shown below.



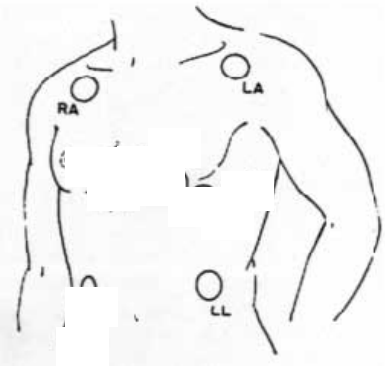
12-Lead Chest Placement

Lead wire	Position
V1	Fourth intercostal space at the right border of the sternum.
V2	Fourth intercostal space at the left border of the sternum.
V3	Midway between locations V2 and V4.
V4	At the mid-clavicular line in the fifth intercostal space.
V5	At the anterior axillary line on the same horizontal level as V4.
V6	At the mid-axillary line on the same horizontal level as V4 and V5.
RA and LA	Right and left arm just below the clavicle, or midway between the elbow and the shoulder of both limbs.
RL and LL	Right and left lower abdominal quadrant, below the rib cages or above ankles or upper leg as close to the torso as possible.

2. To locate the V1 position (fourth intercostal space):
   1. Place your finger on top of the jugular notch
   2. Move your finger slowly downward about 1.5 inches (3.8 centimeters) until you feel a slight horizontal ridge or elevation. This is the "Angle of Louis" where the manubrium joins the body of the sternum.
   3. Locate the second intercostal space on the right side, lateral to and just below the Angle of Louis.
   4. Move your finger down two more intercostal spaces to the fourth intercostal space which is the V1 position.
   5. For female subjects, place leads V3-V6 under the breast rather than on the breast.
3. Connect the wire harness to the recorder/transmitter and check that the recording is clear and free of artifacts.

4.2 Cardiac Monitoring: 3 –Lead ECG

1. Apply 3-lead electrodes to the chest for monitoring as shown below.



3-Lead Chest Placement

RA & LA	RA and LA just below the clavicle
LL	Left lower abdominal quadrant, below the rib cage

2. Connect the wire harness to the recorder/transmitter and check that the recording is clear and free of artifacts.

4.3 Blood Pressure and Heart Rate (Pulse) -automatic

1. Have the subject bare their right arm. Rolled-up sleeve should be loose as not to restrict blood flow.
2. Select a proper size of the cuff and wrap it snugly around the subject's arm. The lower end of the cuff should be a minimum of 2 to 3 cm above the antecubital fossa and the cuff at the level of heart.
    - Arm circumference 22 to 26 cm, 'small adult' cuff, 12 x 22 cm
    - Arm circumference 27 to 34 cm, 'adult' cuff: 16 x 30 cm
    - Arm circumference 35 to 44 cm, 'large adult' cuff: 16 x 36 cm
    - Arm circumference 45 to 52 cm, 'adult thigh' cuff; 16 x 42 cm
3. Start the device. The peak inflation pressure should be ~30 mm Hg above the systolic pressure. If not, adjust the device. (For more details see Vital Signs/BP SOP.)

4.4 Minute Ventilation (V_E) Measurement

UNC

1. Log on to the Acquisition computer. Follow the instructions as outlined in the SOP HSFRESOPR-SOP001R0 section 5.3 entitled "Acquisition Computer Operations." See Appendix.
2. Follow the instruction for minute ventilation data acquisition as outlined in section 5.3.1 entitled "Minute Ventilation." See Appendix.
3. Once the computer is ready, enter the file name.

UCSF

1. Approximately 10 - 15 seconds before each V_E measurement period, engage the UCSF LabView program.
2. Select START @ Pneumotach Ventilation Window Display. Follow UCSF Minute Ventilation Measurement SOP.

UR

1. Log on to the Acquisition computer. Engage the UR LabView program.
2. At the beginning of each exercise period, select RECORD at the top of program to collect the Validyne Integrated Signal. Follow instructions in Chamber SOP C-17 entitled "Exercise Minute Ventilation Measurement"
3. At the end of each exercise select STOP at the top of the program.

COMMON

1. Approximately 30 seconds before each V_E measurement period instruct the subject

to place the mouth piece of the ventilation apparatus into his/her mouth and the nose clip on his/her nose.

2. Approximately 10-15 seconds before each V_E measurement and subject is breathing through the mouth piece begin measuring V_E by pressing "START BREATH DATA COLLECTION" at Pneumotach Ventilation window display. Observe V_E being recorded. Note any anomalies.
3. Collect 1 minute of V_E
    1 Save data
    2. Record Count, Total Volume (L) and Average Tidal Volume (L) on the data work sheet (optional)
    3. Print screen display
    4. Exit
4. Collect a 2nd minute of V_E
    1. Save data
    2. Record Count, Total Volume (L), and Average Tidal Volume (L) on the work sheet (optional)
    3. Print screen display
    5. Press "Stop breath data collection" and close the file
5. Instruct the subject to remove the mouth piece and nose clip
6. Average the 2nd one minute V_E measurements and record on the Training Exercise Data Form.

   The target V_E L/min is calculated by multiplying 15-17 L/min/m^2 BSA (taken from the Vital Signs and Blood Pressure Screening Training Data Form)

7. Evaluate the V_E and adjust the speed and/or % grade on the treadmill or load setting on the bicycle as needed to reach the target V_E.

4.5 Exercise and Measurement of V_E

4.5.1 Pre-exercise instructions

The subject should wear comfortable clothing and shoes that are suitable for exercise.

1. Familiarize the subject with the exercise equipment and demonstrate if necessary. *Treadmill:* Show the subject how to stand on the frame/side panels of the treadmill so that the feet straddle but are not on the treadmill belt and then walk on the treadmill.

   Stationary Bicycle: Have the subject take a seat on the bicycle. Adjust the handle bar and seat's height for the subject and record the settings on the Training Exercise Data Form.

2. Ask the subject to attach the mouth piece to the ventilation assembly and assist if necessary.

3. Instruct the subject that during exercise, he/she will be asked from time to time to breathe through the ventilation assembly by placing a nose clip on his/her nose and a mouthpiece in his/her mouth, with the lips sealed tightly around the mouthpiece (like a big straw) and the teeth resting on the rubber blocks of the mouthpiece.
4. Show the subject how to properly apply the nose clip – squeeze the handles together and place the soft tabs on the nose – the handles should point up. The subject should ensure that he/she cannot breathe through the nose. Allow the subject to remove the nose clip and come off the mouth piece between V_E measurements.
5. Familiarize subjects with the exercise monitoring equipment
   - Cardiac monitor
   - Automatic Blood Pressure and Pulse
   - Oximeter (optional)
6. Ask the subject to place water bottle/electrolytes (e.g., Gatorade), towel, V_E assembly and SpO_2 sensor (optional) within easy reach.
7. Connect subject to the various exercise monitoring equipment (heart monitor, blood pressure, oximeter-optional)
8. Ask the subject to let you know if any respiratory symptoms develop

4.5.2 Determination of exercise load, V_E, cardiac function (ECG), and blood pressure

The total time is 45 minutes: 15 minute of exercise, 15-minute of rest, and 15 minute of exercise, and at least 5 minutes of rest. An overview of the timeline of the measurements can be found in Table 1 (page 9).

1. Before starting the exercise, print baseline rhythm strip (for at least 6 seconds) and evaluate. Measure HR and BP and record on the Training Exercise Data Form.
2. Ask the subject to take a position on the treadmill or bicycle and to put oximeter on one finger and begin to monitor oxygen saturation (optional). Have liquids and towel nearby. Remind subject periodically to drink water or electrolytes.
3. *Treadmill:* Ask subject to place his/her feet on the two 6 in wide side platforms located on the top of the chassis to each side of the running belt. He/she may gentle hold onto the handrail for initial support. Do not stand directly on the running belt.
   Bicycle: Ask the subject to seat on the bicycle seat; adjust seat height as needed
4. Press “ON/REZERO” button on the control console to start the treadmill.
5. Ask the subject to step on the belt while holding onto the railings.
6. Set the starting speed and elevation to initial stage value (enter in the Training exercise data form)
7. When the subject is comfortably walking on the moving treadmill belt with hands

on their sides, slowly increase/decrease the speed using respective UP/DOWN arrow key on the control console and/or the % grade (press on the up pointing "Δ" to increase the % grade) so that he/she will be comfortable to adjust the exercise load during exposure and to a point when he/she is moderately working. .

8. At 5 minute into the exercise print rhythm strip (for at least 6 seconds) and evaluate for normality. Record heart rate and SPO_2 (optional) on Training Exercise Data Form
9. At 7 minute into the exercise measure BP (optional) and record on Training Exercise Data Form
10. At 7.5 min into exercise ask the subject to put on the mouth piece (ventilation assembly) and nose clip.
11. At 8 minute into the exercise measure minute ventilation for 2 min (follow section 4.4).
12. At 10 min into the exercise print rhythm strip (for at least 6 seconds) and evaluate for normality. Ask the subject to take off the mouthpiece. Measure HR and record the on a Training Exercise Data Form. Report average of minute ventilation during last minute and treadmill/bicycle speed on Training Exercise Data Form.
13. Adjust the speed and/or % grade on the treadmill, or resistance on the bicycle, so that the subject is exercising at the target V_E.
14. At 12.5 min into the exercise ask the subject to put on the mouthpiece and nose clip
15. At 13 minute into the exercise measure minute ventilation for 2 min (follow section 4.4).
16. At 15:00 min print rhythm strip (for at least 6 seconds) and evaluate for normality. Ask the subject to take off mouthpiece. Measure HR and end exercise. Report HR and average of minute ventilation during last minute and treadmill/bicycle speed on Training Exercise Data Form.
17. Adjust the speed and/or % grade on the treadmill or resistance on the bicycle so that the subject is exercising at the target V_E BSA (15-17 L/min/m^2).
18. <u>Upon completion of the initial 15 min exercise</u> ask subject to turn OFF the treadmill/bicycle on the console. Remove pulse oximeter.
19. Subject should step off the belt/bicycle. He/she may hold the hand rail for support.
20. Have the subject sit in a wheel-less chair and rest. Remind subject to drink water or Gatorade.
21. At 15.5 min (30 seconds into the rest period) print a rhythm strip (for at least 6 seconds) and evaluate for normality. Measure HR and record on the Training Exercise Data Form.
22. After each session use a damp cloth to wipe any remaining perspiration from the handrails, handgrips, and painted surfaces.
23. At 20 min (5 minutes into the rest period) print rhythm strip (for at least 6

seconds) and evaluate for normality. Measure HR and record on Training Exercise Data Form.

24. At 25 min (10 minutes into the rest period) measure BP. Record on Training Exercise Data form

25. At 30 min (at the end of the 15 min rest period) repeat steps 2-24.

Table 1. Timeline of measurements of V_E, BP, ECG, HR, during training visit

V2 Training Measurements	**V_E** (take average of last minute)	**BP**	**ECG** print outs, **HR and *SpO_2**
Rest baseline		*Before exercise starts*	*Before exercise starts*
Exercise #1	**@ 8 – 10 min** **@ 13 – 15 min**	**7 min Optional**	**@ 5 min** **@ 10 min** **@ 15 min**
Rest #1		***@10 min***	*@ 30 sec.* *@ 5 min.*
Exercise #2	**@ 8 – 10 min** **@13 – 15 min**	**7 min Optional**	**@ 5 min** **@ 10 min** **@ 15 min**
Rest #2		***@10 min***	*@ 30 sec.* *@ 5 min.*

*SpO_2 is optional

4.5.3 Evaluation and safety criteria during exercise

Heart Rate

1. Calculate 80% of predicted maximum HR using the following equation: [208 – 0.7 x age (yrs)] x 0.8. For example, a 70-year old subject would have a maximum predicted heart rate of 159 beats/min and the exercise required to achieve a minute ventilation of at least 14.5 L/min/m^2 BSA in that subject must not cause the heart rate to exceed 127 beats/min.

2. If, in order to achieve a minute ventilation of at least 14.5 L/min/m^2 BSA, it is necessary to have an exercise intensity that causes HR to exceed 80% of predicted maximum for a subject, that subject should be excluded from the study. (The figure of 14.5 L/min/m^2 BSA, is set to allow for rounding up to the agreed upon lower limit of 15 L/min/m^2 BSA.)

3. If the HR exceeds 80% of predicted maximum during any exercise lower the exercise load. Ask subject about undue dyspnea or fatigues, chest pain, leg cramps, faintness, dizziness and general well-being. If subject complains of any of the above symptoms, **STOP** exercise and consult the physician on duty to evaluate, measure BP and record rhythm strip. If the subject feels well continue at lower load.

4. If at any time subject complains of dyspnea, chest pain, fatigue, leg cramps, weakness, faintness, dizziness or wheezing, **STOP** exercise and call physician on duty to evaluate.

5. If there is a sudden increase in HR query the subject about his/her well-being and decide about the continuation of exercise.
6. If the heart monitor tracing shows an abnormality, **STOP** exercise and call a physician on duty to evaluate.

Blood Pressure During Exercise

The measurement of BP during the exercise is optional. If BP is measured, evaluate the measurements as follows;

1. If the BP reaches or exceeds 230/120 or drops below 90/50 ~~90/60~~, **STOP** the exercise and call the on-call physician.

Blood Pressure During Rest

1. If after 10 minutes of rest the BP exceeds 160/100 or is below 90/50 ~~90/60~~ notify the on-call physician.

4.5.4 Corrective Action

The supervising physician should make the final determination of whether or not the exercise should continue or what corrective action should be taken.

4.6 Recordkeeping

The operator should enter each stage treadmill/bicycle parameters, related minute ventilation, BP and the HR into Training Exercise Data Form as described in section 4.4.6 for minute ventilation and 4.5.2 for BP and HR.

4.7 Location of Instruments

UNC

Marquette 2000 treadmill, Ventilation assembly, Blood pressure monitor and the Acquisition computer reside in rm # 75-77 (LA75/LB77) for exercise and ventilation measurements to be done during exposure.

UCSF

Cybex 700T-01-4 Treadmill, Monark 818 E Ergomedic Stationary Bicycle Fleisch Pneumotach, Critikon Dinamap™ Vital Signs Monitor (blood pressure, pulse, cardiac and SpO_2) and the Mac (acquisition) computer reside at SFGH, Bldg. 1, Room 152.

UR

Cybex 530C Stationary Bicycle resides in Exposure Room at URMC (Bldg. MRBX B.11305B). Fleisch Pneumotach and PC acquisition computer resides in the Ante Room at URMC (Bldg. MRBX B.11305A).

5. QUALITY CONTROL

All UNC calibrations and maintenance logs for the pneumotachograph ventilation assembly and the treadmill are done and kept by TRC. If there is any question regarding the

calibrations or the maintenance contact TRC

6. QUALITY ASSURANCE

HEI may request copies of the data forms and review the cardiac monitoring print out and the maintenance and calibration records.

7. INFECTION CONTROL

7.1 A single use, disposable filter or sterilized mouth piece will be used for each subject on each study day.

7.2 The filter may be reused on the same day, but the subjects name must clearly be written on the filter.

7.3 Any filter which will be used repeatedly during the day should be disconnected from the pneumotachograph to prevent excessive moisture buildup.

7.4 Re useable mouth pieces and nose clips should be sterilized/disinfected prior to use.

7.5 The external surface of the pneumotach should be wiped weekly (more if needed) with a disinfectant solution.

7.6 Hand washing is mandatory between subjects, and after handling nose clips, filters, mouth piece or the pneumotach. Use universal precautions with body fluids.

8. REFERENCES

Tanaka H, Monahan KD, Seals DR. 2001. Age-predicted maximal heart rate revisited. J Am Coll Cardiol 37(1):153-156

UNC. Series 2000 Treadmill service manual. Marquette Electronics Medical Systems. PN 409110-001, August 1995.

UCSF. Cybex 700T-01-4 Treadmill Owner's Manual. Monark 818E Ergomedic Stationary Bicycle, Critikon Dinamap™ Portable Adult/Pediatric and Neonatal Vital Signs Monitor, Model 81000 Operation Manual.

UR. Cybex 530C Bicycle Owner's Manual.

9. APPENDICES (UNC)

TRC Environmental Corporation. Operating the Chamber Consoles HSFRESOPR-SOP001R0, March 22, 2010.

MOSES Standard Operating Procedure (SOP)

SOP # 5 - EXER/MINVEN/ECG/BP (MINVEN) –EXP

Title: Exercise with Minute Ventilation, ECG, Blood Pressure, and Heart Rate during exposure visit (Ver. 1.0)

Directory/file[a]:

Author: MOSES investigators **Signature:** **Date: April 30, 2012**

Revised by: **New version[c]:** V2 **Date: October 6, 2012**
Revisions/additions: changed the safety criterion for the lower systolic and diastolic blood pressure limit during the exposure period from 90/60 to 90/50 (section 4.4.4).

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

ANNUAL REVIEW

Signature	**Date**

Explanations: [a] Incorporate the date and the version of SOP into the file name

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!

[c] Annual review does not change the version of the SOP.

EXERCISE WITH MINUTE VENTILATION, ECG, BLOOD PRESSURE,

AND HEART RATE (EXPOSURE VISIT)

1. SCOPE and APPLICATION

The purpose of this SOP is to provide guidelines for:

1) conducting treadmill or bicycle exercise at the subject's target expired minute ventilation (V_E) corrected by body surface area (BSA) of 15-17 L/min/m^2 (V_E BSA) and,

2) cardiac monitoring, measuring V_E, heart rate (HR), blood pressure (BP), and oxygen saturation (SpO_2 - optional) at predetermined intervals and exercise load during the exposure to ozone or clean air.

This document should be reviewed annually. Any revisions should be properly documented in the cover form of this SOP.

2. PREREQUISITES

This SOP assumes familiarity with the installation and operation of the instrument(s) where applicable. For detailed instructions in the operation of the instrument(s) refer to a respective Operation and Service Manual. The following subsections provide a list of equipment and supplies needed to take the measurements, as well as required training needed to operate the instrument(s).

2.1. Equipment and Supplies

UCSF

1. Cybex 700T-01-4 Treadmill
2. Monark 818E Ergomedic Stationary Bicycle

UNC

1. Treadmill. Marquette 2000 series

UR

1. Cybex 720 Stationary Bicycle

Common equipment

1. Fleisch Pneumotachograph V_E assembly
2. Acquisition computer
3. Heart monitor
4. Blood pressure monitor
5. Pulse oximeter (optional)
6. Electrodes, mouth pieces, nose clips

2.2. Training Requirements

The operator conducting the tests should be trained in operating the instruments and performing the tests in a safe and standardized manner.

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

3.1 Treadmill

1. While exercising on the treadmill subject's may grasp the hand grips intermittently but should not hold on to the hand grips during the entire exercise period.
2. To stop the treadmill in an emergency situation, instruct the subject to press the "STOP" red button or, if available, the alternate stop switch and step on to the frame/side panel.
3. To release the alternate stop switch, turn it ¼-turn in either direction or follow instrument-specific instructions.

3.2 Bicycle

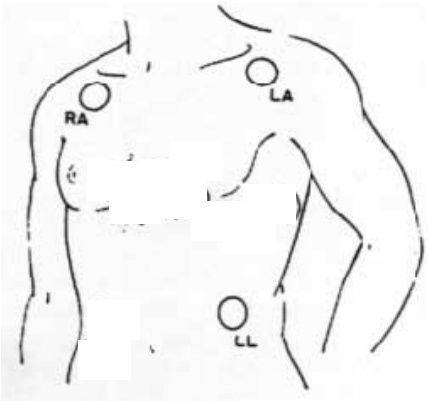
1. To stop in an emergency situation, instruct the subject to stop cycling and dismount from the bicycle when safe to do so.

4. PROCEDURES

It is assumed that the instrument(s) is (are) set up according to manufacturer's instructions. The subsequent procedures may differ depending on the make of the device(s).

4.1 Cardiac Monitoring by 3-Lead ECG

1. Apply 3-lead electrodes to the chest for monitoring as shown below.



3-Lead Chest Placement

RA & LA	RA and LA just below the clavicle
LL	Left lower abdominal quadrant, below the rib cage

2. Connect the wire harness to the recorder/transmitter and check that the recording is clear and free of artifacts.

4.2 Blood Pressure and Heart Rate (Pulse) -automatic

1. Have the subject bare their right arm. Rolled-up sleeve should be loose as not to restrict blood flow.

2. Select a proper size of the cuff and wrap it snugly around the subject's arm. The lower end of the cuff should be a minimum of 2 to 3 cm above the antecubital fossa and the cuff at the level of heart.
   - Arm circumference 22 to 26 cm, 'small adult' cuff, 12 x 22 cm
   - Arm circumference 27 to 34 cm, 'adult' cuff: 16 x 30 cm
   - Arm circumference 35 to 44 cm, 'large adult' cuff: 16 x 36 cm
   - Arm circumference 45 to 52 cm, 'adult thigh' cuff; 16 x 42 cm
3. Start the device. The peak inflation pressure should be ~30 mm Hg above the systolic pressure. If not, adjust the device. (For more details see Vital Signs/BP SOP.)

4.3 Minute ventilation (V_E) measurement

UNC

1. Log on to the Acquisition computer. Follow the instructions as outlined in the SOP HSFRESOPR-SOP001R0 section 5.3 entitled "Acquisition Computer Operations." See Appendix.
2. Follow the instruction for minute ventilation data acquisition as outlined in section 5.3.1 entitled "Minute Ventilation." See Appendix.
3. Once the computer is ready, enter the file name

UCSF

1. Approximately 10 - 15 seconds before each V_E measurement period, engage the UCSF LabView program.
2. Select ventilation MEASURE. Select START @ Pneumotach Ventilation Window Display. Follow UCSF Minute Ventilation Measurement SOP.

UR

1. Log on to the Acquisition computer. Engage the UR LabView program.
2. At the beginning of each exercise period, select RECORD at the top of program to collect the Validyne Integrated Signal. Follow instructions in Chamber SOP C-17 entitled "Exercise Minute Ventilation Measurement".
3. At the end of each exercise select STOP at the top of the program.

COMMON

1. Approximately 30 seconds before each V_E measurement period instruct the subject to place the mouth piece of the ventilation apparatus into his/her mouth and the nose clip on his/her nose.
2. Approximately 10-15 seconds before each V_E measurement and while the subject is breathing through the mouth piece begin measuring V_E by pressing "START BREATH DATA COLLECTION" at Pneumotach Ventilation window display. Observe V_E being recorded. Note any anomalies.
3. Collect 1 minute of V_E

    1 Save data

2. Record Count, Total Volume (L) and Average Tidal Volume (L) on the data work sheet (optional)
3. Print screen display
4. Exit

4. Collect a 2nd minute of V_E
    1. As soon as the program allows start measuring V_E by pressing "Start breath data collection". Observe V_E being recorded. Note any anomalies.
    2. Save data
    3. Record Count, Total Volume (L), and Average Tidal Volume (L) on the work sheet (optional)
    4. Print screen display
    5. Press "Stop breath data collection" and close the file
5. Instruct the subject to remove the mouth piece and nose clip
6. Average the 2nd one minute V_E measurements and record on the Exposure Day Exercise Data Form.

   The target V_E is calculated by multiplying 15-17 L/min/m^2 by the BSA (taken from the Vital Signs and Blood Pressure Screening form)
7. Evaluate the V_E and adjust the speed and/or % grade on the treadmill or load setting on the bicycle as needed to reach the target V_E.

4.4 Exercise and measurement of V_E during exposure

The total time of the exposure is 180 minutes. The session starts with 15 minutes of exercise followed by 15minutes of rest, which are repeated 6 times. An overview of the timeline of the measurements can be found in Table 1 (page 7).

1. Ask the subject to have water bottle/electrolytes, towel and pulse oximeter sensor (optional) nearby. Remind subject periodically to drink liquids.
2. Ask the subject to enter the exposure chamber.
3. Ask the subject to put the pulse oximeter on a finger (optional) and press "ON/REZERO" button on the control console to start the treadmill holding onto the railings.
4. a) UNC and UR: Instruct the subject to set the treadmill speed and elevation or the bicycle work and load as determined during the training session (recorded on Training Exercise Data Form) or as adjusted during a preceding exercise period. Once his/her gait becomes steady he/she should walk with hands to their sides.

   b) UCSF: Set the treadmill speed and elevation or the bicycle work and load as determined during the training session (recorded on Training Exercise Data Form) or as adjusted during a preceding exercise period.

- Treadmill: Instruct the subject to step on the moving treadmill while holding on to the railing. Once his/her gait becomes steady he/she should keep their hands at their sides.
- Bicycle: Instruct the subject to cycle at comfortable steady 60-80 revolutions per minute (RPM).

5. <u>During the first exercise period only</u>
    1. At 7.5 min into the exercise ask the subject to put on the mouthpiece and nose clip. Subject should not hold onto the railings.
    2. At 8 minute into the exercise measure minute ventilation for 2 min (see section 4.3). Once completed ask the subject to come off the mouth piece. Record V_E and treadmill/bicycle settings on Exposure Day Exercise Data Form.
    3. At 9 minute into the exercise measure HR and SpO_2 (optional) and record on the Exposure Day Exercise Data Form.
    4. At 10 min, after the subject has removed the mouthpiece, adjust the speed and/or % grade on the treadmill/bicycle so that the subject is exercising at the target V_E.
6. <u>During exercise #1, #2, #4, and # 6</u>
    1. At 12.5 min into the exercise ask the subject to put on the ventilation assembly and the nose clip. Subject should not hold onto the railings.
    2. At 13 minute into the exercise measure minute ventilation for 2 min (see section 4.3). Once completed ask the subject to take off the mouthpiece. Record V_E and treadmill/bicycle settings on Exposure Day Exercise Data Form.
    3. Adjust the speed and/or % grade on the bicycle/treadmill, if needed, so that the subject is exercising at the target V_E .
    4. Once the exercise period is completed ask the subject to turn the treadmill OFF and step off the treadmill to rest.
    5. Repeat steps 1-4 as required by the protocol.
7. <u>During all exercise periods</u>
    1. At 14 minute into the exercise measure HR and SpO_2 (optional) and record on the Exposure Day Exercise Data Form.
8. <u>During rest period #2 and #4</u>
    1. At 10 minutes into the rest period measure BP according to SOP #3 with the exception that only one measurement should be taken. Record on Exposure Day Exercise Data Form.
9. <u>During all exercise and rest periods</u>
    1. Monitor cardiac function and obtain and an ECG printout when an aberrant pattern is observed or if symptoms occur

10. <u>At the end of the exposure</u>

    1. Measure BP according to SOP #3 (take three measurements one minute apart and record on the Vital Signs and Blood Pressure Log - Exposure Day).

Table 1. Timeline of measurements of V_E, ECG, BP, HR, and BP during exposure period (in the chambers)

V4, V7, & V10 leads Exposure/Exercise Measurements	**V_E** Initial time indicates when measurement of V_E should start (Take average of last minute)	**Cardiac Monitor;** (print when symptoms or aberrant pattern)	**BP Record as indicated**	**HR and *SpO_2; Record as indicated**
Rest pre-exposure			***@ 3 min. before EX (w/ other VS)***	*@ 3 min. before EX1*
Exercise #1	**@ 8 – 10 min.** **@ 13 – 15 min.**			**@ 9 min.** **@ 14 min.**
Rest #1				
Exercise #2	**@ 13 – 15 min.**			**@ 14 min.**
Rest #2			***@ 10 min.***	
Exercise #3				**@ 14 min.**
Rest #3				
Exercise #4	**@ 13 – 15 min.**			**@ 14 min.**
Rest #4			***@ 10 min.***	
Exercise #5				**@ 14 min.**
Rest #5				
Exercise #6	**@ 13 – 15 min.**			**@ 14 min.**
Rest #6				
End of Exposure			***+@00 min. (w/ other VS)***	

*SpO_2 is optional

4.4.4 Evaluation and safety criteria during exposure exercise and rest periods

Heart rate

1. Calculate 80% of predicted maximum HR using the following equation: [208 – 0.7 x age (yrs)] x 0.8 (Tanaka et al, 2001). For example, a 70-year old subject would have a maximum predicted heart rate of 159 beats/min and the exercise required to achieve a minute ventilation of at least 14.5 L/min/m2 BSA in that subject must not cause the heart rate to exceed 127 beats/min.

2. Note that, in order to have qualified for the Exposure session (ie, included in the study) a subject must have not been excluded on the basis of SOP #4. However, if HR exceeds the predicted maximum (using the exercise load determined during the training visit) reduce (or ask the subject to reduce) the exercise load as needed in order to lower the HR to the safety standard of ≤ 80% predicted maximum HR). Do not communicate a sense of panic to the subject if he/she has such an HR event, but no distress, and if the heart monitor does not reveal anything aberrant. Record and accept the V_E measured even if it is less than 14.5 L/min/m^2 BSA.
3. If the HR exceeds 80% of predicted maximum during any exercise period lower the exercise load. Ask subject about undue dyspnea or fatigue, chest pain, leg cramps, faintness, dizziness and general well-being. If subject complains of any of the above symptoms **STOP** exercise and call the physician on duty to evaluate. If the subject feels well continue at lower load.
4. If there is a sudden increase in HR query the subject about his/her well-being and decide about the continuation of exercise.
5. If the ECG tracing shows an abnormality **STOP** exercise and notify the on-call physician to evaluate.

Blood Pressure

1. If BP is >230/120 or <90/50 ~~90/60~~ during an exposure period, **STOP** the exposure and notify the on-call physician.
2. If BP is >160/100 or <90/50 ~~90/60~~ during a rest period notify the on-call physician.

4.4.5 Corrective Actions

The supervising physician should make the final determination of whether or not the exercise should continue or what corrective action should be taken.

4.5 Recordkeeping

The operator should enter each stage treadmill/bicycle parameters, related minute ventilation, BP and the HR into Exposure Day Exercise Data Form. The pre-exposure and the end-of-exposure VS measurements should be entered in the Vital Signs and Blood Pressure Log - Exposure Day.

4.6 Location of Instruments

UNC

Marquette 2000 treadmill, Ventilation assembly, Blood pressure monitor and the Acquisition computer reside in rm # 75-77 (LA75/LB77) for exercise and ventilation measurements to be done during exposure.

UCSF

Cybex 700T-01-4 Treadmill, Monark 818 E Ergomedic Stationary Bicycle Fleisch Pneumotach, Critikon Dinamap™ Vital Signs Monitor (blood Pressure, pulse, cardiac and SpO_2) and the Mac (acquisition) computer reside at SFGH, Bldg. 1, rm. 152.

UR

Cybex 530C Stationary Bicycle resides in Exposure Room at URMC (Bldg. MRBX B.11305B). Fleisch Pneumotach and PC acquisition computer resides in the Ante Room at URMC (Bldg. MRBX B.11305A).

5. QUALITY CONTROL

All UNC calibrations and maintenance logs for the pneumotachograph ventilation assembly and the treadmill are done and kept by TRC. If there is any question regarding the calibrations or the maintenance contact TRC.

6. QUALITY ASSURANCE

HEI may request copies of the data forms and review the cardiac monitoring print out and the maintenance and calibration records.

7. INFECTION CONTROL

7.1 A single use, disposable filter or sterilized mouth piece will be used for each subject on each study day.

7.2 The filter may be reused on the same day, but the subjects name must clearly be written on the filter.

7.3 Any filter which will be used repeatedly during the day should be disconnected from the pneumotachograph to prevent excessive moisture buildup.

7.4 Re useable mouth pieces and nose clips should be sterilized/disinfected prior to use.

7.5 The external surface of the pneumotach should be wiped weekly (more if needed) with a disinfectant solution.

7.6 Hand washing is mandatory between subjects, and after handling nose clips, filters, mouth piece or the pneumotach. Use universal precautions with body fluids.

7. REFERENCES

Tanaka H, Monahan KD, Seals DR. 2001. Age-predicted maximal heart rate revisited. J Am Coll Cardiol 37(1):153-156

UNC. Series 2000 Treadmill service manual. Marquette Electronics Medical Systems. PN 409110-001, August 1995.

UCSF. Cybex 700T-01-4 Treadmill Owner's Manual. Monark 818E Ergomedic Stationary Bicycle. Critikon Dinamap™ Portable Adult/Pediatric and Neonatal Vital Signs Monitor Model 8100 Operation Manual.

UR Cybex 530C Bicycle Owner's Manual.

9. APPENDICES (UNC)

TRC Environmental Corporation. Operating the Chamber Consoles HSFRESOPR-SOP001R0, March 22, 2010

MOSES Standard Operating Procedure (SOP)
SOP # 5 –SPIR (UCSF)

TITLE: Spirometry Testing (UCSF)

Version: 1.0 **Directory/file[a]:**

Author: MOSES Investigators HW Signature: **Date: April 30, 2012**

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

ANNUAL REVIEW[d]

Signature	**Date**

SOP UPDATED TO VERSION [e]

1. **Explanations:** [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.
2. [b] If available.
3. [b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. <u>If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!</u>
4. [c] Annual review does not change the version of the SOP.
5. [d] Change the version # as necessary.

SPIROMETRIC TESTING

1. SCOPE AND APPLICATION

The purpose of this SOP is to provide guidelines to perform spirometry, a test to measure lung function. Spirometry for the MOSES Study includes the measurement of forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), forced expiratory flow measurements between 25% and 75% of forced vital capacity ($FEF_{25-75\%}$), peak expiratory flow, and FEV_1/FVC. A graphic representation (spirogram) of the procedure should be part of the results. Either a volume-time or flow-volume display is acceptable.

This document should be reviewed annually. Any revisions should be properly documented in the cover form of this SOP.

2. PREREQUISITES

2.1 Equipment and supplies

1. Vertical dry rolling seal spirometer (S&M Instruments, pds Instrumentation 908 Main St., Louisville, CA 80027 303/666-8100)
2. Computer: Dell Optiplex GX240 Computer with PDS Spirometer (PDS Instrumentation/Ferraris CardioRespiratory, nSpire Health, Inc., 1830 Lefthand Circle, Longmont, CO 80501 303/666-5555).
3. Printer: HP Printer Deskjet 810C
4. 3 Liter Calibration Syringe: Model 5530 Series, Hans Rudolph, Inc. 8325 Cole Parkway, Shawnee, KS 66227 (800/456-6695) hri@rudolph.com
5. 1-L Vitalograph Precision Syringe: Model 20408, Vitalograph, Inc., 13310 West 99th St., Lenexa, KS 66215 (800/255-6626) vitcs@vitalograph.com
6. Single use bacterial/viral filter
7. Mouth piece (optional)
8. Nose clips
9. Thermometer (optional)

2.2 Training requirements

1. The operator performing the spirometry test should be trained to calibrate, demonstrate, and perform the test according to the most recent ATS/ERS standards.
2. The operator must be proficient in coaching the subject for accurate, reproducible results, as well as assessing the spirograms for validity.
3. The operator is a critical component of performing spirometry.

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

3.1 Multi-center studies require attention to perform tests in a standardized manner by all centers so that data can be collectively analyzed.

3.2 Do not refer back to previous tests before testing. Each test session should stand on its own.

3.3 The operator needs to get the subject to “buy in” and do his/her very best, and

enthusiastically function as a cheer leading coach.

3.4 Each spirometry test counts as spirometry involves considerable effort from the subject; he/she may experience some discomfort, soreness or dizziness during the test.

3.5 A syncope episode may develop in an older subject following a dizzy episode due to prolonged interruption of venous return to the thorax.

3.6 Forced expiratory maneuvers can precipitate bronchospasm; rescue medication (albuterol) should be available when performing spirometry.

3.7 The operator should observe for spirometry-induced bronchospasm and be prepared to treat with a bronchodilator (albuterol).

3.8 Do not exceed 8 spirometry maneuvers per test session.

3.9 Treat all subjects with respect, providing gentle, warm enthusiasm and patience.

3.10 Comment in writing on the subject's report about his/her inability to achieve satisfactory, reproducible efforts and protocol deviation(s).

3.11 The PI or supervising physician should be notified if the best initial FEV_1, FVC, or FEV_1/FVC ratio are outside the acceptable limits for the study.

4. PROCEDURES

Set up equipment according to manufacturer's instructions.

4.1 Powering the system and accessing the software

1. Computer
    1. Power up CPU.
    2. Power switch is located at the lower right side of the computer monitor.
2. Spirometer
    1. Power switch located at the right sided base of the Spirometer.
    2. Click on PDS icon to bring up nSpire PFT Software (nSpire Health, Inc., 1830 LefthandCircle, Longmont, CO 80501 303/666-5555)
3. Power up the printer.

4.2 Calibrating the spirometer

4.2.1 Daily

1. Leak test
    1. Open the QUALITY CONTROL menu.
    2. Choose the "CHECK SPIROMETER LEAKAGE" option.
    3. Complete Study Log Input; Enter USER and PASSWORD information, tab to "OK" and depress the "ENTER" key.
    4. Immediately flush the spirometer (Empty to 0 Liters and re-fill to 10 Liters twice, and then empty to 2-3 Liters) to initiate the program. The "Spirometer Leakage Test" window should display.
    5. Filled a 3 Liter calibration syringe to full and attach it to the open end of the breathing tube.
    6. Attach 2-3 rubber bands between the chrome plated volume hand gauge of the spirometer and the top of the housing case of the spirometer, and gently push up once on the spirometer volume gauge.
    7. Depress the "SPACE BAR" or select the "START" option. Do not move the breathing tube.

8. The leak test program will run for 30 seconds.
9. After the leak test program is done, remove the rubber bands.
10. Depress the “SPACE BAR” to return to the menu.
11. Enter comments in the “Quality Control Checks” display, select “OK.”
12. If a leak is present, repeat the leak test to confirm the leak. If confirmed, replace the breathing tube and repeat the leak test (section 5.2.1.1.).
13. Promptly report any serious leaks to the supervisor.

2. Calibration test
    1. Open the QUALITY CONTROL menu.
    2. Choose the “CHECK CALIBRATION OF THE SPIROMETER” (CALIBRATION) option.
    3. Complete Study Log Input: Enter USER and PASSWORD information, tab to “OK” and depress the “ENTER” key.
    4. Immediately flush the spirometer (Empty to 0 Liters and re-fill to 10 Liters twice, and empty to 3 -5 Liters) to initiate the program.
    5. The “Instrument Calibration Check” Window should display.
    6. Attach a completely air filled 3 Liter calibration syringe to the open end of the breathing tube and exchange the air from the syringe to the spirometer and back again two - three times to ensure a uniform air temperature within the spirometer and syringe. Stop with the syringe completely full of air and depress the “SPACE BAR”.
    7. Empty the 3 Liter volume of air from the syringe into the spirometer and press the “SPACE BAR”. Check the measured volume (acceptable ± 0.5% of 3 L calibrating syringe volume: (2.985 – 3.015 L) and volume error (acceptable error < 1%). Respond to the “Adjustment Complete” question by pressing the “SPACE BAR”.
    8. Enter comments in the “Quality Control Checks” display and select “OK.”
    9. If the volume, volume error or calibration is out of range repeat the Calibration Test.
    10. Promptly report any problems to the supervisor.
    11. Run adjust (ADJ) if two subsequent calibrations are excessively out of range (> 1%).

4.2.2 Weekly

1. Linearity test
    1. Open the QUALITY CONTROL menu.
    2. Choose the “VERIFY THE LINEARITY OF THE SPIROMETER” (LINEARITY) option;
    3. Fill the spirometer to 10 Liters, and then complete the Study Log Input: Enter USER and PASSWORD information, tab to “OK” and depress the “ENTER” key and immediately empty the spirometer to 0 Liters, refill to 0.5 Liter, pause for a moment and finally empty to 0 Liters.
    4. If the “Linearity Check” window does not appear, flush the spirometer (fill to 10 Liters, empty to 0 Liters) and wait for a few seconds. The “Linearity Check” window should display 13 empty

rows. Depress the "ESC" key and return to section 5.2.2.1 if less than 13 empty rows are displayed.

5. Connect the 1 Liter calibration syringe to the open end of the breathing tube, make sure the calibrating syringe is filled with 1 Liter of air, and depress the "SPACE BAR".
6. Follow the computer prompts
    1. "Please Do Not Move Syringe or Hose".
    2. Empty 1 Liter of air into the spirometry, and do not move the syringe or hose.
    3. Refill the 1 Liter syringe with air, and press the "SPACE BAR".
    4. Empty another 1 Liter of air into the spirometer when prompted to, and do not move the syringe or hose.
    5. Repeat section 5.2.2.1.6.2 – 5.2.2.1.6.4 until ten 1 Liter incremental volume data points has been collected.
7. Review the "Linearity Results" when done.
    1. Today's date and time should be displayed just beneath the "Linearity Results" heading.
    2. The acceptable slope of the line is 1.0.
    3. The acceptable "Worst Case Linearity" < 1 %.
8. Depress the "SPACE BAR". Enter comments in the "Quality Control Checks" display and select "OK."
9. Repeat Linearity Test (section 5.2.2.1) if calibration data is out of range.
10. Promptly report any problems to the supervisor.

2. Biological check
    1. Perform spirometry tests on a normal healthy staff.
    2. Review actual data (FEV_1, FVC, $FEF_{25-75\%}$, and PEF) with previous tests looking for significant differences/trends.
    3. Promptly report any problems to the supervisor.

4.3 Entering subject demographic data

1. Open the PRE or POST Tests menu.
2. Select the PATIENT INFORMATION (INF) option.
3. Complete the Study Log Input: Enter USER and PASSWORD information, tab to "OK" and depress the "ENTER" key.
4. Complete Subject Demographics input
    1. Enter subject ID. Previously entered ID's will retrieve the corresponding subject demographics and will require one to update the physician, height (cm) and weight (kg). Additional edits may be done, and will require a justification note.
    2. Height (cm) and Weight (kg) (kg = measured weight in lbs X 2.204 kg/lbs) (from Vital Signs and Blood Pressure - Screening Training Data Form)
    3. FEV_1 Predicted values: age, gender, African American, Caucasian (default), Mexican American (NHANES III Reference Values, which can be found at http://www.cdc.gov/niosh/topics/spirometry/nhanes.html).
    4. Comments (Study acronym, visit number, time point……)
    5. Technician

5. When done, select “OK”. The “Environment” window should display. Enter room temp (°C), BP (Millibar) and RH %. Select “OK”. Information is automatically stored.

4.4 Subject Preparation

1. Test Description (the subject should be seated in a non-rolling chair)

    1. Explain the test to the subject stating that this breathing test is a routine test to measure how much air his/her lungs can hold and how quickly he/she can empty his/her lungs.
    2. The test is performed at least three times (and up to eight times) with a brief rest break between each test.
    3. The test requires the subject’s active participation.
    4. Explain that you will describe the test before the he/she does the test and again while he/she is doing the test. Therefore he/she does not need to memorize the test.
    5. Assure the subject that if he/she gives his/her best effort and follows your instruction there is a good chance the test will be done in three to four attempts.
    6. Show him/her the tubing on the dry rolling seal spirometer or pneumotach, and the filter (use a new one for each subject on each test day), rubber mouthpiece and the noseclips.
    7. Instruct the subject that during the maneuver, he/she will place the mouthpiece in his/her mouth, with the lips sealed tightly around the mouthpiece (like a big straw) and his/her upper teeth on the upper part of the mouth piece, his/her lower teeth on the underside of the mouth piece, and his/her tongue under the mouth piece.
    8. Show the subject how to properly apply the noseclips – squeeze the handles together and place the soft tabs on the nose – the handles should point up. The subject should ensure that no air come from the nose, and then allow the subject to take them on and off between the trials.
    9. Explain to the subject that he/she will be asked to breathe in and out normally for 2 or 3 breaths before taking the “test breath”.
    10. Explain that when it is time to take the test breath, he/she will be asked to “take a big breath in all the way full”. Tell the subject that he/she will need to “feel completely full” and that he/she should “pause briefly” before they exhale. Instruct the subject that at this point he/she will have to “BLAST” the breath out as hard and as fast as he/she can. Advise the subject that when he/she is “empty”, he/she should inhale.

2. Subject Set-up

    1. Have the subject sit straight up in the chair with both feet on the floor.
    2. Loosen any tight fitting clothing.
    3. Remove any loose fitting dentures, and any object in the subject’s mouth.
    4. Open the mouth piece package and without touching the mouth end of the mouth piece, attach the mouth piece to the open end of the breathing tube.
    5. Demonstrate the placement of a mouth piece and nose clip on yourself to the

subject (optional).

6. Point out that you will ask the subject to place his/her upper teeth on the upper part of the mouth piece, his/her lower teeth on the underside of the mouth piece, and his/her tongue under the mouth piece.

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4.5 Performing the spirometry

1. Instructions

   Explain to the subject in words that he/she will understand.
   "After I place the mouth piece in your mouth and the nose clip on your nose, I want you to breathe regularly through your mouth. After a few regular breaths, I will ask you to take the biggest and the quickest breath in you can until you are full. When I see that you are full, I will ask you to BLAST that air out as hard and as quickly as you can till you are empty. When I see that you are empty I will ask that you take a breath in and then come off the mouth piece."

2. Performing the spirometry

   1. Have the subject place the noseclips, and put the mouthpiece into his/her mouth. Make sure that the lips are sealed around the mouthpiece.
   2. Ask the subject to "breathe in and out normally" for 2 to 3 breaths. Ensure that the tracing is accurate, and that the subject is following instructions.
   3. When ready to collect the spirogram, at the end of a usual tidal volume expiration (FRC) enthusiastically coach the subject to "take a big breath all the way in!" When inhalation is complete (observe both the subject and the computer display) and that there is a cessation of flow for $\leq$ 1 second, prompt the subject to "BLAST the air" out of his/her lungs for at least 6 seconds.
   4. Continue to coach the subject to exhale by saying "keep going" until end of test criteria are achieved. Once the exhalation is complete, have the subject inhale. The subject should not be allowed excessive body movement during the maneuver. At end expiration the subject should inhale fully. This concludes one trial.
   5. Each trial should be reviewed for acceptability, and either saved or discarded. **Perform a minimum of 3, but no more than 8 trials.**

4.6 "Flow Volume Loop" menu program

1. Select "FLOW VOLUME LOOP".
2. Complete Study Log Input: Enter USER and PASSWORD information, tab to "OK" and depress the "ENTER" key.
3. Flush the spirometer (empty to 0 Liters and fill to 10 Liters a few times), and leave filled to about 5 Liters.
4. Note the "Flow Volume Loop" window display.
5. After satisfactory instructing/demonstrating as necessary(section 4.4) and the spirometry test procedure to the subject have the subject properly place the mouth piece into his/her mouth and nose clip on his/her nose (as described in section 4.5.
6. Depress the "SPACE BAR" to start data collection.
7. Data collection is to include a regular inspiratory and expiratory breath (tidal breath),

a rapid deep breath in to full (Total Lung Capacity) and the forceful expiratory breath out to empty and then a breath in to regular breathing.

8. Depress the "SPACE BAR" to end data collection or the "ESC" key to exit the program.
9. Flush the spirometer and set at 10 Liters.

4.7 Acceptability and reproducibility

1. For a trial to be acceptable there should be

1. A maximal inspiration followed by explosive exhalation with no cough and maximal subject effort.
2. Continuous linear expiratory flow with no interruption, cough or abrupt stop (cough in the first second affects the FEV_1; cough at other times may interfere with other measurements.)
3. There should not be any leaks in the system
4. There should be a 1 second plateau (no change in volume for 1 second), and preferably a 6 second exhalation.
5. There should be no glottis closure (this causes a cessation of airflow) and no mechanical obstruction (tongue in mouthpiece, loose dentures, etc). Also, observe for leaks around the mouthpiece.
6. The extrapolated volume should not be greater than 5% of the FVC or 150 ml; the software should assess and prompt for inappropriate extrapolated volumes.

2. Review trials for acceptability status and reference legend as needed.

1. Respond to "Is this Acceptable Patient response?" (Y/N or ESC)
    1. "Y" to accept. A yes response will prompt another question, "Is this acceptable?"
    2. Review the graphic, numerical data, ranking and the software assessment of the test.
    3. To accept the test, select Y.

2. Depress "space bar" to do another test, continuing from section 5.7.6., or depress the "ESC" key to exit.
3. To reject test, select "N" or "ESC".
4. To print test, print screen.
5. When two or more acceptable tests are done, review the numerical data of all tests by selecting the "Expand" option, review the software accessed Acceptability Status and Reproducibility/Repeatability status at the bottom of the table.

3. For a trial to be reproducible/repeatable

1. The largest FVC should match within 150 ml of the second largest FVC; and the 2 largest FEV_1's should also match by 150 ml.
2. Peak Flow should within 10% of the second largest peak flow
3. A minimum of 3 trials should be collected, and these trials should be acceptable and repeatable. If a subject cannot reach repeatability after 8 trials, this subject should not be included in the study.

4. **Review trials for reproducibility/repeatability status**

    1. If the trials are reproducible, but do not meet all ATS criteria, the physician/investigator will make the final decision on acceptance or rejection of the trials.

5. RECORDKEEPING

Results of all spirometry trials should be entered on the Spirometry Data Form. The largest FVC and FEV_1 (all measurements in BTPS corrected) should be selected and reported, even if they come from different trials. Other variables should come from the acceptable trials with the largest sum of FVC and FEV_1.

The Spirometry data form data will be entered in ADEPT. The forms should also be saved in the subject's file.

Technician comments should be made for each trial on the Spirometry Data Form regarding the subject's effort and any technical aspects of the procedure that would affect the interpretation of the trial.

The best three reproducible spirograms will be selected for filing in the subject file. For record keeping all accepted spirograms should be saved in the computer file.

6. QUALITY CONTROL

See Section 4.2 for calibration procedures. Store calibration reports in a file. See Section 4.7 for acceptability and reproducibility criteria.

7. QUALITY ASSURANCE

HEI or NERI may request copies of the Spirometry Data Form, the printed spirograms, or the saved spirograms.

8. INFECTION CONTROL

8.1 A single use, disposable filter will be used for each subject on each study day. The filter may be reused on the same day the same subject. Clearly write a subject identifier on the filter or have the subject take personal possession of the mouth piece and nose clip.

8.2 Any filter which will be used several times should be disconnected from the equipment to prevent excessive moisture buildup. Noseclips should be disposable or disinfected prior to use.

8. 3. The external surface of the equipment should be wiped weekly (more if needed) with a disinfectant solution.

8.4. Washing is mandatory between subjects, and after handling noseclips, filters, mouthpiece. Use universal precautions with body fluids.

9. REFERENCES

American Thoracic Society Standardization of Spirometry. 1994 Update. 1995. Am J Respir Crit Care Med 152:1107-1136.

AARC Clinical Practice Guidelines Spirometry, 1996 Update.1996.Respir Care 41(7): 629-636.

Keystone PFT System Operator's Manual, September 2001.

Miller MR, Hankinson J, Brussasco V, et al. Standardisation of spirometry. 2005. Eur Respir J 26:319-338.

MOSES Standard Operating Procedure (SOP)

SOP # 7 – SPUTUM

TITLE: Sputum induction, sputum processing and sample shipping and analysis

Version: 1.0 **Directory/file[a]:**

Author: Neil Alexis **Signature:** **Date: June 15, 2012**

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[c]:** **Date:**
Revisions/additions:

Signature:

ANNUAL REVIEW[d]

Signature	**Date**

SOP UPDATED TO VERSION [e]

Explanations: [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.
[b] If available.
[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. <u>If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!</u>
[c] Annual review does not change the version of the SOP.
[d] Change the version # as necessary.

SPUTUM INDUCTION, SPUTUM PROCESSING AND SAMPLE SHIPPING AND ANALYSIS

Content:

A: Sputum Induction, page 1
B: Sputum Processing, page 7
C: Shipping supernatant and slides to the UNC core laboratory, page 9
D: Analyses of Supernatant and Slides, page 10
E: QC and QA, page 11

A: SPUTUM INDUCTION

1. SCOPE OF APPLICATION

To safely obtain airway sputum samples with minimum contamination of oral squamous epithelial cells, and to accurately collect and store the sample prior to delivery to laboratory personnel for processing.

2. PREREQUISITES

2.1 Training

1. The individual performing the sputum induction should be familiar with the equipment used and all required steps in the induction procedure.
2. The individual should also be knowledgeable about sample requirements and preparation.
3. Persons performing sputum induction should be proficient in pulmonary function testing, and able to administer fast acting bronchodilators (albuterol) if/when necessary.

2.2 Equipment and supplies

- Devilbiss Ultra-Neb 99 Ultrasonic Nebulizer or a suitable brand/model equivalent (NouVag Nebulizer NOU-3301), disposable nebulizer cups and lids, disposable plastic aerosol tubing, mouthpiece, tissues, container with ice for sample storage, 2 sterile specimen cups with lids, drinking cup, sterile syringes, disposable 15ml ampules of saline (0.9%, 3%, and 10%), mixed up 4% and 5% saline.
- AeroStar BioSafety System/Chamber, BioSafety Systems., Inc (800/421-6556)
- NIOSH-approved N95 Respirator
- Spirometer
- Microscope (Fisher Scientific: Various models available)
- Cytocentrifuge (Shandon Corp or Fisher Scientific)
- Centrifuge (Fisher Scientific: Various models available)
- Sputolysin (Fisher Scientific: 50-230-7313)
- Lab Quake – Tumbler (Fisher Scientific: 13-687-14Q)
- Vortex (Fisher Scientific: NC9612654)
- Hemacytometer (Fisher Scientific: S17040)
- 50 Micron Square, 40 thread diameter nylon mesh (Amazon.com)

- Tradewinds Direct Weighing Funnels (Fisher Scientific: 17-988-492)
- Cytofunnel (Fisher Scientific: 10-355)
- Cytoseal xyl, 4 oz can (Fisher Scientific: 22-050-262)
- 50 mL Tubes (Fisher Scientific: 05526B)
- Cryo-baby (Fisher Scientific: LCR)
- Cyrotags (Fisher Scientific: NC9422709)
- Cryotubes (Fisher Scientific: 89092-262)
- GraLab Electronic Timer (Fisher Scientific: 06-656-1B)
- The testing must be performed in an area with a sink/portable basin the subject can expectorate in to.
- Albuterol must be available.

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS:

3.1 Always use universal precautions when handling body fluids (wear gloves and NIOSH-approved N95 Respirator (optional) (annual test fit required).

2.2 Subjects should never be left unattended once the procedure has started.

2.3 Oxygen and albuterol will be available.

2.4 Sputum induction cannot be performed if the subject is unable to perform acceptable/valid spirometry trials.

2.5 Communicate with the covering physician and processors prior to starting.

4. PROCEDURES:

4.1 Nebulizer preparation

1. The technician should wash his/her hands prior to preparing the nebulizer, and all procedures should be performed in a clean (not sterile) fashion.
2. Insert the nebulizer chamber into the unit so that the notch at the bottom of the chamber is aligned with the locating rib at the front of the unit. When positioned properly, the fill label on the chamber will be facing you.
3. Insert the RF reflector ring (if using the Devilbiss) (metal disc with a hole in the middle and a notch on one side) into the nebulizer chamber. Fill the nebulizer chamber with tap water up to the fill line on the side of the chamber. The unit will not function if the water level is too low. Place the disposable nebulizer cup and lid into the chamber.
4. A length of disposable aerosol tubing, 2 sections or approximately 12” should be attached to the elbow for the air supply, and to the lid on the disposable cup. A second length of tubing, 4 sections, or approximately 24” should be attached to the other port on the lid of the nebulizer cup.
5. The mouthpiece should be inserted on the other end of the aerosol tubing. Place 15ml (a total of 30 ml is made when saline is mixed for the 4% level, use entire volume) of saline at each level into the disposable cup.

6. Turn the unit on. Adjust the output to the highest level the subject can tolerate, ideally no less than halfway.

4.2 AeroStar BioSafety System/Chamber (UCSF only)

1. Open upper, right and left sided panels, and raise table panel.
2. Power on the portable chamber and verify adequate flows and current certification.
3. Place a non-rolling chair in front of the portable chamber.

4.3 Saline preparation

1. 3% saline, 0.9% saline, and 10% saline are available in 15ml disposable ampules/vials.
2. To make 4% saline, mix equal parts of 3% saline and 5% saline.
3. To make 5% saline, mix 18ml of 0.9% saline and 15ml of 10% saline.
4. If saline mixing is done on site, the technician should use good hand washing technique, and all solutions should be handled in a sterile manner (single subject use saline, mix in sterile containers, use new syringe for each subject).
5. It is recommended to use single use 15 ml disposable vials of saline instead of IV saline bags. IV bags require tapping with a syringe which have an inherent risk in their use, and generally are discarded before fully used, hence are wasteful.

4.4 Subject preparation

1. The subject/patient should arrive without having eaten for 2hr.
2. Clear liquids are fine provided they are non-caffeinated.
3. The sputum induction will be performed with the subject/patient seated in a non-rolling chair.
4. **Baseline spirometry should be obtained according to ATS criteria, ie. 3 reproducible flow volume curves. Follow the SOP #6 Spirometry specific for your laboratory.** Enter the data into the Spirometry Data Form.
5. Give the subject breathing instructions for the inhalation of the hypertonic saline. Instruct the subject to relax, to inhale the hypertonic saline through his/her mouth and exhale through the nose. The subject should be told to breathe semi-deep tidal breaths, but not to hyperventilate.
6. Instruct the subject to expectorate any saliva-salt water into a separate specimen cup labeled "waste", i.e. not to swallow any build up of this fluid. This prevents any nausea or discomfort from the saline, and helps to preserve a more acceptable specimen.
7. The 3 step cleansing routine performed at the end of each saline inhalation period should be explained to the subject prior to induction. The procedure consists of: 1. rinse/gargle mouth and discard; 2. scrape/clear the back of the throat and discard; 3. blow the nose.
8. The type of cough (i.e. chesty type cough) should be physically demonstrated to the subject prior to induction. The subject should be told to avoid scraping the back of the throat during the official cough attempts throughout the entire procedure.
9. The technician should demonstrate how to passively move the specimen from the back of the throat forward for expectoration without scraping.

10. The technician should emphasize avoid scraping the back of the throat during cough attempts
11. The subject should be told that during spirometry, a sputum sample may be generated from the forced exertion of the maneuver and this is an acceptable sample to obtain.

4.5 Induction procedure

1. Put 15ml of 3% saline into the disposable nebulizer.
2. Wear proper personal protection equipment (gloves and individual fitted NIOSH-approved N95 Respirator (optional).
3. Turn the chamber (UCSF specific) and nebulizer on and start the timer for 7 minutes.
4. Ensure that the subject is comfortable, and has the mouthpiece properly in his/her mouth.
5. Adjust the flow output to the maximum flow the subject can tolerate. If the subject has an urge to cough, he/she may do so, without scraping, and expectorate the sample into the sterile cup labeled with the subject number. If the cough episode is prolonged, turn off the nebulizer, (chamber), and timer until the subject has stopped coughing and is ready to resume the inhalation
6. At the end of the 7 minutes, turn off the nebulizer, (chamber), and instruct the subject to begin the 3 step cleansing procedure.

    1. Rinse their mouth and gargle thoroughly with water and then spit into the sink/portable basin.
    2. Clear/scrape the throat, i.e. scrape the back of the throat and roof of mouth (demonstrate) and expectorate this into the sink/portable basin.
    3. Blow the nose.

7. Have the subject undergo the actual study sample. The cough must be generated from the chest without scraping the throat. The subject must passively move the sample from the back of the throat for expectoration. **DO NOT HOCK OR SCRAPE** when producing the sample
8. Keep the sample container on ice during the induction procedure
9. After the cough attempt is complete, ie. the subject has no more secretions to produce or the cough sounds "dry", repeat the spirometry – if one acceptable flow volume curve is produced that is enough for the purposes of acquiring the FEV1.
10. If the FEV_1 has not fallen 10% or more from baseline, dispose the remaining saline, and refill the nebulizer with the next concentration of saline, ie. 3→4% saline into the cup.
11. If the FEV1 fall is between 10 to 19% from baseline, perform another (second) spirometry. If the FEV1 is still between 10 to 19% perform another (third) spirometry. If any safety FEV_1 has not fallen 10% or more from baseline, dispose the remaining saline, refill the nebulizer with the next concentration of saline, and continue with sputum induction. Otherwise continue the sputum induction with the same saline concentration, i.e. 3→3% saline.

12. If any safety FEV_1 drops by 20% or more, or if the subject becomes distressed, discontinue the procedure immediately and administer albuterol if necessary. If the procedure is terminated due to FEV_1 criteria, it cannot be re-started that day.
13. After the second inhalation period continue with the 3 step cleansing routine, followed by the cough attempt and spirometry check. If the safety FEV_1 fall is less than 10% continue with the final 7 min. inhalation period using 5% saline. If the FEV_1 fall is between 10-19%, continue with the final 7 min. inhalation period but maintain the saline concentration at 4%. After the inhalation period, continue with the 3 step cleansing procedure, cough attempt and spirometry check.
14. The subject cannot be discharged until the FEV_1 is within 10% of baseline. Assess the subject's lung sounds, and check vital signs and call the physician for evaluation or discharge of the subject.
15. The sample should be delivered to the laboratory for processing immediately upon completion of the induction. If this cannot be done, the sample can be kept at 4 °C for up to 2hr but this should not be the rule, rather the exception.

4.6 Recordkeeping for Spirometry

The results of the baseline spirometry conducted prior to sputum induction (see section 4.4.4) should be recorded on the Spirometry Data Form and reported to NERI through ADEPT. A copy should be stored in the subject's file. The spirometry steps should be recorded on the site-specific sputum induction worksheet. The worksheet should be stored in the subject's file

4.7 Cleaning and infection control

1. Use a new disposable nebulizer cup for each subject each day.
2. Wipe the exterior of the Devilbiss Ultra-Neb 99 Ultrasonic Nebulizer, the Nouvaug or the AeroStar BioSafety System/Chamber (UCSF) with alcohol or another surface disinfecting solution.
3. Change the disposable aerosol tubing between subjects.
4. The nebulizer chamber should be removed from the unit and drained each day.

5. REFERENCES

Pin, I. 1992. Use of Induced Sputum cell counts to Investigate Airway Inflammation in Asthma. Thorax 47 : 25-29

De la Fuentes et al. 1998. Safety of Sputum Induction in Severe Asthma. Am. J. Respir Crit Care Med. 157(4): 1127

DeVilbiss ULTRA-NEB®99 Ultrasonic Nebulizer Instruction Guide

6. APPENDIX

Site-specific sputum induction worksheet

B: SPUTUM PROCESSING

1. WEIGHING AND SELECTION OF SPUTUM SAMPLE

1.1 Weigh an empty 50mL tube with lid and record weight on it.

1.2 Place another 50mL tube upside down on scale and zero the weight.

1.3 Pour sample into the tube and measure the total weight in grams.

1.4 Pour entire sample into Petri dish against a dark background and record color (clear, yellow, green) and characteristics (purulent, visible plugs, aggregates, foamy, viscous).

1.5 Note any evidence of blood. If there is blood, attempt to excise this portion from the sample

1.6 Select out all "plugs" (all opaque/dense material versus the surrounding clear fluid) with pointed forceps and place in pre-weighed 50mL tube – note all plugs are visible or colored. It may useful to use an inverted microscope to identify plug material. It is also useful to raise the Petri dish above eye level and examine the sample through the bottom of the Petri dish. Plugs hidden by foam and bubbles from a top-down view, can be identified with this method.

1.7 Determine selected sample weight (total weight-empty tube weight) and record in grams.

1.8 If no plugs are present, continue to process the entire sample but note this fact on the work sheet as "no plugs selected – processed whole sample"

2. PROCESSING RAW SAMPLE AND SAVING SUPERNATANTS

2.1 Add 0.1% Sputolysin (DTT) in mLs equal to 4X selected sputum sample weight in grams. (For example, 2g of sample would need 8mL of 0.1% Sputolysin added) (NOTE: DTT comes as a 1% solution in 10mL vials. Make the 0.1% solution by diluting 1:10 with PBS (1ml DTT + 9ml PBS). Record date on opened vial and store opened 1% DTT for up to two weeks at 4° C).

2.2 Crystals may form at the bottom of the Sputolysin vial after having been at 4 °C. Be sure to allow the DTT to acclimate to room temperature and if crystal appear at the bottom of the vial, gently attempt to break them up prior to use.

2.3 Vortex sample 15 seconds at low to medium speed and pipet aspirate the sample up and down several times with P1000 pipettor to break up any clumps.

2.4 Place sample on a rotating tumbler for 15 minutes (NOTE: Timing is important).

2.5 After tumbling step, dilute sample with PBS. Use the same volume that was added in step 1. (For example, if 8mL of Sputolysin was added, you would add 8mL of PBS).

2.6 Place sample back on tumbler for 5 minutes

2.7 Filter sample into a new 50mL tube through a 48-53µm nylon mesh filter (from Small Parts, Inc.) lining a funnel (pre-wet the filter with buffer for better adhesion to the funnel and ensure the surface or floor of the filter is smooth, ie. without creases).

2.8 If the sample is high volume, one can perform parallel filtrations with additional filters and tubes. The filtrates can be pooled at the end of these filtrations. The sample should not be aggressively delivered through the filter but rather consistently dripped.

2.9 Spin down cells at 500Xg for 10 minutes in a 4 °C centrifuge

2.10 Volume of supernatant will vary among subjects.

a. If the volume is ≥6 ml, split it into 3 aliquots with 2 ml each and store the rest.
b. It the volume is <6 ml and >2, split into 3 aliquots with similar volume.
c. If the volume is < 2 ml, split it into 2 aliquots with similar volume.
d. All aliquots (i.e. tubes) should be sent to the core laboratory for analyses (at time of shipping).
e. Label the tubes with labels provided by NERI.
f. Freeze aliquots and discard the rest of the supernatant (or store depending on need).

2.11 Add between 0.5ml to 1ml or 2mL of 1X PBS to resuspend cells depending on how viscous/thick the pellet looks. A more viscous pellet will require 1ml, a very viscous pellet may require 1-2ml. A thin or less viscous looking pellet will require 0.5ml of resuspension. The desired concentration for the cell suspension is 1x 10^6 cells/ml for cytospin preparation.

2.12 Record volume of PBS used for resuspension.

3. CELL COUNTS AND VIABILITY TESTING

3.1 Combine 10µL of sample with 10µL of trypan blue stain.

3.2 Mix this suspension well with pipette aspirations (10-15x)

3.3 Be sure the Trypan Blue stain is clean and free of bacteria

3.4 Counting on the hemacytometer should begin immediately –do not delay

3.5 Place 10µL of mixture into the hemacytometer chamber and be careful not to over flow the chamber – 10 ul should fill up the chamber perfectly.

3.6 Count both live (clear) cells and dead (blue) cells in each of the 4 corner grids/quadrants. (NOTE: Exclude, ie. do not count RBC's and squamous epithelial cells (SEC) but do count bronchial epithelial cells). <u>If the cell viability is below 50%, the sample is disqualified and not processed any further.</u>

3.7 Observe or note the number of squamous epithelial cells while performing the cell count – use this as an impression as to whether the sample has a high amount of SECs – if the sample has a high amount of SECs, this will indicate that another filtration step is necessary before continuing any further - if so, refilter the sample as in #7 above and continue.

3.8 If there too many cells to make an accurate cell count, the sample is too concentrated and needs to be diluted. In this case, add a volume of PBS to the sample up to 0.5ml and remix with Trypan Blue and repeat hemacytometry step.

3.9 If there are no cells present or very few, then the sample is too dilute. In this case, the sample needs to be re-centrifuged, and a volume of supernatant removed (up to 0.5ml). Repeat the Trypan Blue mixing and hemacytometry steps again.

3.10 The number of cells appearing on the hemacytometry quadrants should not make it difficult to count, ie. the spacing should be adequate, not too crowded but not too sparse.

3.11 The hemacytometry cell count (cells/ml) formula is: sum of 4 grids/4 X 2 X10^4

3.12 The Total Cell Count (TCC) in the sample = [{sum of 4 grids/4}X2X10^4] X Volume of Sample (ml).

3.13 Once the TCC is calculated determine the sample concentration, ie. if the TCC is 2 x 10^6 cells, and the volume of the sample is 1ml, then the concentration of the sample is 2 x 10^6 cells/ml.

3.14 Determine number of cells/mg (=TCC/weight of selected sample)

3.15 Viability = (live cells/total cells) X 100%

4. CYTOSPINS

4.1 For cytospin preparation the sample needs to be adjusted to 1 x 10^6 cells/ml

4.2 If the current sample concentration is higher than this, addition of buffer is needed –if the sample concentration if less than this, centrifugation and removal of supernatant is required for the adjustment

4.3 Adjust the sample to a final concentration of 1X10^6 cells/mL; mix to resuspend.

4.4 Make 4 slides using 60µL volume of cell suspension (single cytofunnel recommended).

4.5 One can also prepare a range of volumes, ie. a slide at 60ul, one at 65ul, one at 70ul and one at 75 ul.

4.6 Spin for 6 minutes at 450rpm.

4.7 Remove the slides and allow to air dry – do not let the slides sit exposed to the air for longer than necessary –as soon as they are dry continue to the fixation/staining step

4.8 Fix the slides with (95% methanol), and stain 2 slides with Diff Quik /Hema 3 stain set (ten dips in each) and rinse in distilled water.

4.9 Allow to air dry and then mount with Cytoseal and coverslip.

4.10 Add label provided by NERI to each of the four slides.

4.11 Store slides in a hard cover slide box at room temperature prior to shipping.

5. PREPARING CELLS FOR EXTRACTION OF TOTAL RNA

5.1 Spin down cells in a 1.5mL microcentrifuge tube at 500Xg for 5 minutes and discard 1X PBS. In a fume hood, add 1mL of Trizol reagent to cell pellet. Gently pipet up and down to break up the cells. Label and freeze at -80°C for later extraction of total RNA.

6. RECORDKEEPING FOR SPUTUM SUPERNATANTS AND SLIDES

A Sputum Supernatant and Slide Tracking Form should be filled out for every sputum sample processed and stored in the subject folder. The form also needs to be entered into ADEPT, so the samples collected can be tracked by the system. At time of shipping ADEPT will generate a shipping form specific for the sputum samples that are being shipped.

Results of the analyses from the analytic laboratory will be provided to NERI and entered in the ADEPT database.

C: STORING AND SHIPPING SUPERNATANT AND SLIDES TO THE UNC CORE LABORATORY

1. SUPERNATANT

1.1 Samples should be stored in cryovials (2 ml) at -70 °C (see 2.2.11).

1.2 Tubes should be shipped on dry ice in a hard box to avoid breakage. Include the appropriate shipping form, which will be generated by ADEPT.

1.3 Shipping of samples should be done in batches according to all appropriate DOT rules for shipping human biological samples. The central lab at UNC requests **that each site notify the lab via email** (heather_wells@med.unc.edu) that a shipment has been initiated.

1.4 Ship to:
Dr. Neil Alexis/Ms. Heather Wells,
US EPA Building, UNC Chapel Hill,
104 Mason Farm Rd.,
Chapel Hill, NC, 27599
Tel: 919-966-9915

2. CYTOSPIN SLIDES

2.1 Slides should be stored at room temperature (see 4.4.11)

2.2 Four cytospin slides from each induced sputum sample will be generated: two are fixed and stained and two are fixed, but not stained.

2.3 **Ship all four slides from a sputum sample.** If the staining is of poor quality the core laboratory staff will fix the unstained slides.

2.4 Slides should be shipped in hard case slide boxes to avoid breakage.

2.5 Slides should be shipped in batch every 2 weeks or at a TBD frequency. Slides should be labeled with computer generated labels and not hand written. Include the appropriate shipping form, which will be generated by ADEPT.

2.6 The central lab at UNC requests that **each site notify the lab via email** (heather_wells@med.unc.edu) that a shipment has been initiated.

2.7 Shipping of slides should be done according to all appropriate DOT rules for shipping human biological samples. Include the appropriate shipping form, which will be generated by ADPET

2.8 Ship to:

Dr. Neil Alexis/Ms. Heather Wells,
US EPA Building, NC Chapel Hill,
104 Mason Farm Rd,
Chapel Hill, NC, 27599
Tel: 919-966-9915

D: ANALYSES OF SUPERNATANT AND SLIDES

1. SUPERNATANT

Samples will be analyzed in duplicate for the selected soluble markers (indicated in the MOSES protocol). These are: total protein (analyzed by Comassie Plus Protein Assay); IL-6, IL-8, TNF-α, and CD40L (by immunoassay). The results of the analysis will be submitted to NERI.

2. CYTOSPIN SLIDES

2.1 The central lab will select one stained slide as the primary slide for differential cell count reading and the other slide will serve as a back up in case of damage to the primary slide or if the primary slide is considered of poor quality.

2.2 The central reading lab will use a 3 reader system. Two readers (Reader 1 and Reader 2) will be assigned to the study and each will perform a count on every primary slide. A third reader (Reader 3) will be used if there is a >10% discrepancy in cell types between Reader 1 and 2. Reader 3's counts will be averaged with the reader who was within 10% of his counts.

2.3 A maximum of 600 cells will be counted in order to count a minimum of 400 non-squamous cells, differentiating between eosinophils, neutrophils, macrophages, epithelial cells, lymphocytes and metachromatic cells. If 400 non-squamous cells are not counted then the cytospin slide will be classified as a poor quality slide and noted as such on the counting worksheet. In this instance the second or back up stained slide will be used for counting. If the counts from this slide also result in less than 400 non-squamous cells being counted a similar entry will be made on the counting worksheet.

2.4 All absolute cell count data will be recorded on hard copy worksheets then transposed to an electronic spreadsheet. The electronic spreadsheet will automatically compare Reader 1 and 2's counts and determine those that fall outside of the 10% discrepancy criteria. It is to be determined whether the central lab or a data management team will calculate the differential cell percentages based on the absolute cell counts. All data will be submitted to NERI.

E: QUALITY CONTROL AND QAULITY ASSURANCE

1. QUALITY CONTROL

Slide Reading QC: The core laboratory electronic data capture spreadsheet will automatically flag all reader 1 versus reader 2 differences in the major cell types (Mac/PMN/Eos) that exceed 10%. All discrepancies are resolved by reader 3 and entered into the spreadsheet. Reader 3 must be within 10% of reader 1 or 2 in order for the data to be averaged and reported. Captured data are transmitted to the sponsor using an agreed upon transmission system or portal system. Prior to data transmission, the data capture spreadsheet is QCed by our lab technician for data entry errors, obvious out of range values and typos. The spreadsheet is electronically transmitted to the sponsor at agreed upon intervals throughout the duration of the study or whenever the sponsor requests an up to date data transfer. The final master spreadsheet will be sent to the sponsor at study completion.

2. QUALITY ASSURANCE

HEI may audit the results of the sputum analyses conducted at the core laboratory and request copies of the analysis records. HEI also may audit the procedures followed by the individual research centers.

MOSES Standard Operating Procedure (SOP)

SOP # 11 – PHLEB SCREEN

TITLE: Phlebotomy for Screening Visit

Version: 1.0 **Directory/file[a]:** //

Author: LabCorp **Signature:** **Date: June 5, 2012**

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

ANNUAL REVIEW[C]	
Signature	**Date**

SOP UPDATED TO VERSION [d]	

Explanations: [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. <u>If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!</u>

[c] Annual review does not change the version of the SOP.

[d] Change the version # as necessary.

PHLEBOTOMY FOR SCREENING VISITS

1. SCOPE AND APPLICATION

The purpose of this SOP is to describe the procedure of blood drawing and blood processing, shipping, and storage during the screening visit. This information is provided in the attached MOSES Laboratory Manual prepared by LabCorp.

Blood collected at screening will be analyzed for hematology, chemistry panel (serum), and nicotine and cotinine. In addition whole blood will be stored for genotyping.

Below is a description of what LabCorp will provide to each center and overview of the Laboratory Manual's content.

2. DESCRIPTION OF THE KIT AND LABELS PROVIDED BY LABCORP

LabCorp provides one kit for each subject that undergoes screening. The kits are shipped to each center in bulk (about 8-10 per center at the beginning). Additional kits can be reordered directly from LabCorp using the Supply Reorder Form (attached to the Manual).

Each kit contains all the supplies needed for one phlebotomy procedure (with the exclusion of the syringe):

- two 21 gauge eclipse needles, 2 adult vacutainer holders, 2 band aids, 2 alcohol pads
- one 3.5 mL SST yellow top tube (prelabeled)
- one 5mL white cap plastic transfer vial (prelabeled)
- two 3 mL lavender top EDTA tubes (prelabeled)
- one non-sterile pipette
- one 6 place tube holder absorbent material
- one biohazard specimen bag
- one FedEx Diagnostic pack shipper
- <u>one Laboratory Test Request Form</u> (with barcode matching the barcode on the tubes' labels) and one Unscheduled Visit Laboratory Test Request Form
- two extra tube labels

2.1 The kit does not contain

- **one 3 mL lavender top EDTA tube needed for collecting blood to archive on site for genotyping.** Also needed are two 2 mL PP microtubes (Sarstedt #72/694.007 or similar.) These should be provided by each center.

3. OVERVIEW OF MOSES INVESTIGATOR MANUAL

The Investigator Manual is preceded by a Welcome page with Lab Corp personnel contact information. The Manual starts with an Action Plan which includes a General Introduction with courier pickup and delivery information and a description of the Laboratory Test Request Form (page 9), which needs to be included in the package to be shipped to LabCorp). The Study Specific Instructions follow and are organized in 6 chapters.

Chapter 1 provides courier information. Chapter 2 contains the list of scheduled tests. Chapter 3 provides examples of Test Requisition Forms. <u>Chapter 4 describes the phlebotomy</u>

procedure (i.e., Specimen Collection and Preparation.) Chapter 5 summarizes the main points for packaging and shipping the blood samples. Chapter 6 provides the turnaround time for reporting the results. (Please note that the turnaround time for nicotine/cotinine represents the worst-case scenario.) Details about labels, shipping, and reporting of results are provided below.

3.1 Labels and Laboratory Requisition Forms (see page 9 of the Manual)

The tube labels and the Laboratory Test Request Form must have the following information. Both need to be filled out by hand.

- Subject ID
- Subject's date of birth (day, month –first 3 letters, year- four digits) and gender
- Collection date (day, month –first 3 letters, year- four digits)
- Collection time

The Unscheduled (or retest) Visit Laboratory Test Request Form is the same as the standard form, but offers the possibility of selecting the test(s) that needs to be repeated. This should be used if a subject needs to be re-screened.

3.2 Shipping Instructions (see pages 27-28 of the Manual)

LabCorp provides Fed Ex shipping labels (including some for Saturday Delivery) for shipping the tubes (see example on Page 16). Use these labels for shipping the package with the tubes to LabCorp. Make a copy of the Laboratory Test Requisition form included in the kit before sealing the package. Affix subject ID label on the copy and store it in the subject file. Immediately after you have initiated a shipment fax the Shipment Alert Form (Appendix 5) to LabCorp (the fax number is provided on the form).

3.3 Reporting of Results by LabCorp (see page 29 of the Manual)

Results of the analyses are provided by fax to each center as soon as they are available. Each center's fax number is in the LabCorp database. If it changes, the new number needs to be communicated to LabCorp. NERI will access the data electronically and import them directly into ADPET.

4. RECORDKEEPING

The following documents should be filed in each subject's file:
Copy of the Test Request Form
LabCorp results reports

5. ATTACHMENTS

- Laboratory Manual: HEI MOSES Common Protocol 10-01-04 dated 07MAY2012
- Appendices
  1. Holidays Observed by LabCorp Clinical Trials
  2. Reference Ranges
  3. Laboratory Accreditation
  4. CV of Medical Director
  5. Shipment Alert Form
- Supply Reorder Form for ordering additional kits

WELCOME FROM YOUR PROJECT TEAM!

LabCorp Clinical Trials has been selected by Health Effects Institute to perform the laboratory testing for MOSES Common Protocol 10-01-4.

As such, we have the pleasure of providing you and your team with all the necessary information and instructions in order to make our collaboration as successful as possible.

The LabCorp team members designated for this study are identified below. We encourage you to contact us as frequently as necessary to answer any questions and address any concerns. We are here to provide all needed study assistance and support.

Your Project Monitor will be your first avenue of communication on most study related items.

LabCorp Clinical Trials operates Monday through Friday between 8:00 AM and 6:00 PM Eastern Standard Time (EST). All calls received after business hours, on holidays, or on weekends will be forwarded to the Clinical Trials voice mailbox. If a message is left during this time, your call will be returned promptly on the next business day. There is also an on-call phone 919-602-2139 that is carried by our Monitoring Team for EMERGENCIES ONLY. Emergency on-call hours extend from 6:00 PM to 10:00 PM EST, Monday through Friday, and 9:00 AM to 5:00 PM (EST) on Saturdays and Sundays.

All individuals associated with the clinical portion of this study should carefully review all applicable instructions and procedure descriptions prior to submitting or collecting samples. If there are any questions or concerns, please contact your Project Monitor, listed below.

We wish you great success with your study and look forward to the receipt of your samples.

Sincerely,
Abigail Berens
Project Manager

Project Monitor	Tamika Hawkins	TEL: FAX: E-MAIL:	919-474-4368 919-474-4385 Hawkit2@ labcorp.com
Project Manager	Abigail Berens	TEL: E-MAIL:	952-563-4072 Berensa@labcorp.com
Assistant Project Manager	Alicia J Maynard	TEL: FAX: E-MAIL:	919-474-4365 919-474-4385 Johnsa1@labcorp.com

LabCorp Clinical Trials – Cranford, New Jersey:	1-877-788-8861

LABORATORY MANUAL FOR SCREENING PHLEBOTOMY

Health Effects Institute
MOSES Common Protocol 10-01-4

Sponsor: Health Effects Institute
101 Federal Street, Suite 500
Boston, MA 02110

Central Laboratory Facility: LabCorp Clinical Trials
750 Walnut Ave.
Cranford, NJ 07016

LabCorp CLINICAL TRIALS

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SUMMATION OF CHANGES

Description of Change	Section	Date Added	Version
Initial Release	N/A	07May2012	1

ACTION PLAN

Courier Information

Select the Correct Sampling Kit

Complete the Laboratory Requisition Form

Collect and Prepare the Specimens

Package for Transport

Result Report

For the transportation of samples from your site to the lab, one or more couriers may have been selected. If you receive additional or supplemental information with courier instructions, please review the material and then file in Chapter 1.

Organize pickup with the selected courier company

- Latest Booking Time: This time is provided by the courier company and is the latest possible time to arrange a pickup of samples for that day. To ensure the timely pickup and delivery of your samples to the central laboratory, it is imperative to alert the courier company before this "Latest Booking Time". However, we advise all sites to pre-alert the courier company the day before sample collection.
- Booking Number: Once you have scheduled your pickup, you should be given a booking or pickup number. Please document this number as confirmation of your request.
- Latest Pickup Time: This is the latest possible time during a day for the courier to collect the samples from your site and meet the specified delivery time to the central laboratory. This time is based on the routine service provided.
- Problems with Pickup: If you experience any problem with the pickup of your samples (e.g. courier is not showing up), please contact LabCorp immediately. Be prepared to provide the booking number and the airway bill number so we can work with the courier company to resolve the problem. Do not wait until the next day to inform LabCorp of the issue, as this can delay the processing of your samples and possibly compromise the integrity of the specimens.

Saturday Delivery

- If samples are to be received at LabCorp Clinical Trials on Saturday, special packaging instructions apply. Please refer to Chapter 1.

Holidays

- Please check your local holidays before shipping samples, as customs and courier services may not be operational prior to, during or following an observed holiday in your country.
- Before scheduling a shipment, also check the public holidays of the country to which the samples are being shipped.
- The LabCorp facilities are closed on Sundays, and on certain holidays (See Appendix 1).
- It is advisable to avoid shipments immediately prior to any of the dates listed in Appendix 1. To ensure a timely delivery, please schedule your shipment no later than 3 days before any holiday.

FOR SPECIFIC COURIER INFORMATION REGARDING YOUR STUDY, PLEASE REFER TO CHAPTER 1.

- You were provided with sampling kits and some bulk supplies.
- Each kit contains the required materials to collect, prepare, and return the samples to LabCorp Clinical Trials.
- The requisition form and barcode labeled items, included inside each kit box, are preprinted and contain the same barcode number. Do not transfer the contents between kits.
- All label information must be completed prior to shipping the samples.

PLEASE MAKE SURE YOU HAVE SELECTED THE CORRECT SAMPLING KIT:

- Check PROTOCOL
- Check KIT TYPE
- Check EXPIRATION DATE

If the incorrect sampling kit is selected for the visit, samples may be missing or drawn unnecessarily. Also, the results will be recorded and reported under the wrong visit. Verification of those samples in question may delay the reporting of results.

In case your kits are about to expire

Order new kits using the reorder form provided in Appendix 5.
Expired kits can be destroyed as per local regulations.

In case you have an unsuccessful collection

Should an item be broken or wasted, replace it with an item from the bulk supplies.
There are additional bar-coded labels in each collection kit. Affix one of the extra bar-coded labels in a way that can be easily scanned (e.g. lengthwise for a tube). Make sure the “Lot Expiration” barcode is not covered. Complete the information required on the label, including the name of the testing required.

Unscheduled Visits

Most protocols allow for an Unscheduled or Retest Visit. This kit contains a requisition form and all the components required to collect all testing for the specific protocol. Collect only those samples needed for testing at an Unscheduled/Retest Visit. On the requisition form, mark the required test(s) and complete all patient information. Process the samples as defined in Chapter 4 of this manual.

Reordering supplies

To reorder supplies, make a copy of the study-specific Reorder Form provided in Appendix 5.
Complete the Supply Reorder Form and fax or e-mail to the fax number / e-mail address listed on top of the form.

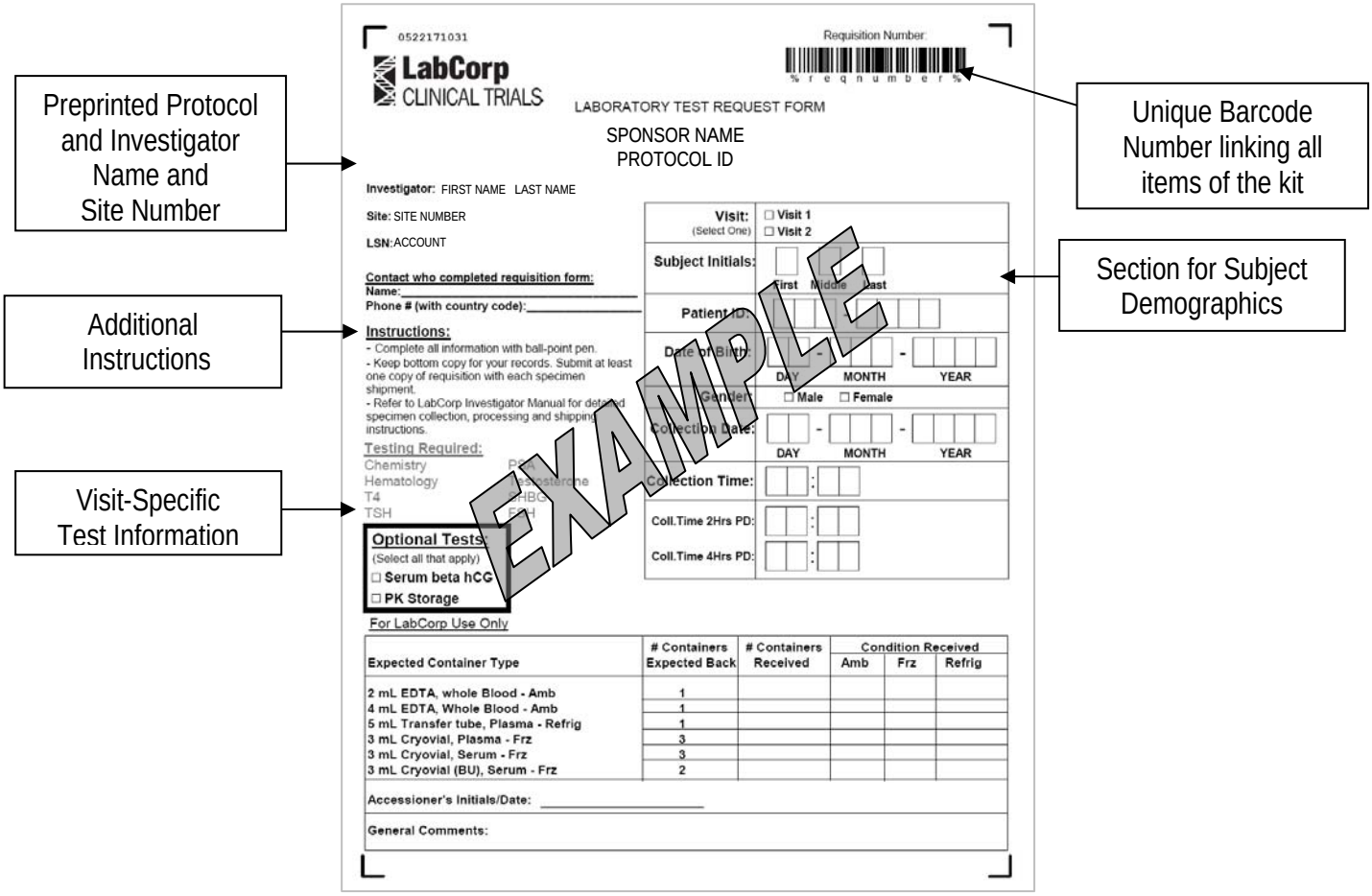
When reordering supplies, please allow a minimum of 5 to 7 working days
for assembly and delivery of your supplies.

FOR EXAMPLES OF STUDY-SPECIFIC REQUISITION FORMS, PLEASE REFER TO CHAPTER 3.

The Laboratory Requisition Form is included in the sampling kit

- Specimens submitted for testing **must** have an accompanying requisition form.
- The barcode number on the requisition form **must match** the barcode number on the preprinted label.
- The patient and collection information on the requisition form **must match** the patient information on the label.
- The patient information **must** be written legibly and completely.
- All requested patient and collection information **must** be entered for each visit to ensure proper sample processing within the laboratory.

NOTE: If there are any discrepancies as outlined above and/or any discrepancies involving patient demographics, verification from your facility will be required. This could delay result reports.



INCLUDE ONE COPY OF REQUISITION IN EACH SHIPMENT

RETAIN ONE COPY OF REQUISITION FOR YOUR RECORDS

COMPLETE ALL DEMOGRAPHIC INFORMATION AS REQUESTED ON THE REQUISITION

1. Use the International Date Format: (ex. 15-OCT-2011)

   For month format (MMM), please use following abbreviations:

JAN (January)	JUL (July)
FEB (February)	AUG (August)
MAR (March)	SEP (September)
APR (April)	OCT (October)
MAY (May)	NOV (November)
JUN (June)	DEC (December)

2. Use the 24 Hour Time Format: (ex. 14:30)

3. IF APPLICABLE:

   - Be sure to mark the correct Visit.
   - Be sure to mark any Optional Testing

4. Please do not insert any information into the "For LabCorp Use Only" section.

NOTE: Please do not order any testing other than what is indicated on the requisition. LabCorp Clinical Trials cannot perform non-protocol testing without the written authorization from the sponsor.

- All samples must arrive with a matching requisition enclosed in the specimen bag.
- All samples must be identified with the corresponding Barcode/Subject Label.
- Specimen Requirements have been carefully determined. It is critically important that the instructions in this manual and on the test requisition form are followed precisely. Examples of Specimen Requirements may include:
    - Sample Type (e.g. serum, plasma, whole blood, urine, tissue)
    - Tube/Container Type (e.g. Red Top Tube [no preservative], Sodium Heparin, Formalin)
    - Temperature (e.g. Ambient, Refrigerated, Frozen)
    - Amount of sample (e.g. minimum required or full draw - no short samples)

NOTE: Shipments are subject to local, national, and international laws and regulations. Individuals preparing shipments are responsible for ensuring all applicable requirements, laws and certifications are met. The shipment boxes, provided to you by LabCorp or the courier, are compliant with IATA guidelines.

The technical information presented in this manual does not qualify as regulatory training or certification. Any questions regarding certification in the preparation, handling or shipment of dangerous goods should be directed to the appropriate authorities or regulatory agencies.

For a list of the International Air Transport Association (IATA) approved training schools, please contact your local office (details can be found at www. iata.org/about/offices) or the IATA main facility in Montreal, Canada (+ 1 514 874 0202).

- All study related data will be forwarded as per specifications from the sponsor.
- Any missing demographic information or discrepancies could delay the transfer of data.

FOR STUDY-SPECIFIC RESULT REPORTING,
PLEASE REFER TO CHAPTER 6.

Health Effects Institute

MOSES Common Protocol 10-01-4

Study-specific Instructions

LabCorp CLINICAL TRIALS

COURIER INFORMATION

Courier information will be provided to you separately. Upon receipt and for future reference, please file all courier documentation in this section of your manual.

For Specimen Packaging and Transport instruction,
please refer to Chapter 5

FEDERAL EXPRESS COURIER INFORMATION

Federal Express is selected for the transport of your biological samples. Federal Express airbills, with pre-printed site and LabCorp information, have been provided.

Web-site Address:

You can track your shipment by entering your airbill number on the following web site: www.fedex.com.

For Specimen Packaging and Transport instruction, please refer to Chapter 5

Note:

Federal Express will not accept any shipment without the outer cardboard box.
LabCorp supplies a bulk supply of cardboard/styrofoam shippers at initiation. If you need to replenish your supply of styrofoam shippers, please contact your LabCorp monitor or complete and fax the Supply Reorder Form located in Appendix 5.

Avoid Weekend Sample Transport:

To avoid transporting samples over the weekend, we advise scheduling patient visits with laboratory assessments Monday through Thursday.

In the case of a pickup on Friday, it is essential to mark "SATURDAY DELIVERY" on the FedEx airbill *and* to attach several Saturday Delivery Labels to each box/courier bag.

To assure your samples will be delivered on Saturday, please adhere to the following procedure:

1. When scheduling your pickup, advise the operator that shipment is for Saturday Delivery.
2. Place a Saturday Delivery Label on each side of the shipping box/courier bag.
3. Do not put labels over any shipment markings which are already on the box.
4. Make certain to mark the 'SATURDAY Delivery' box on the FedEx airbill.
5. Inform the driver performing the pickup that the shipment is for Saturday Delivery.

FEDERAL EXPRESS COURIER INFORMATION

AIRBILLS:

Airbill and labels for Ambient shipments within the United States

Reminder: If specimens are shipped on Friday, the site must check the "SATURDAY Delivery" box on the airbill and attach several Saturday Delivery Labels to each package. Specimens may not arrive at the laboratory until Monday if Saturday delivery is not requested and/or Saturday Delivery Labels are missing.

Federal Express observes certain major holidays. Please review the holiday list in Appendix 1 to avoid scheduling subjects when FedEx service is unavailable.

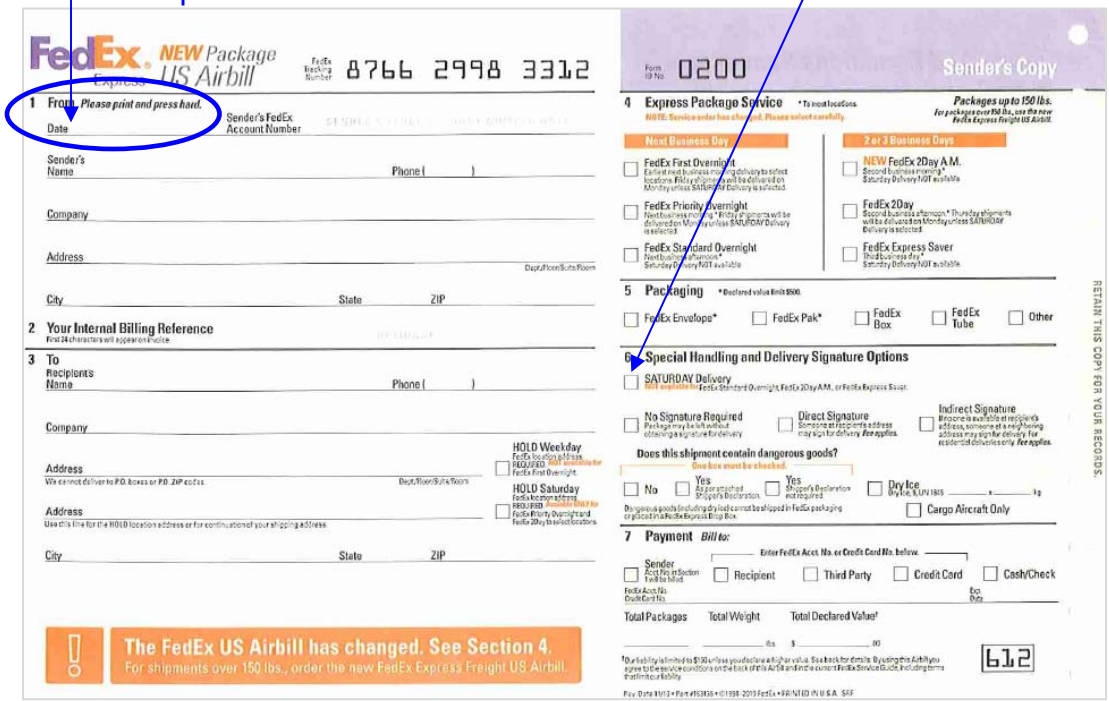
The Federal Express pickup time should be determined the morning of each subject visit. Contact Federal Express at 1-800-GO FEDEX (1-800-463-3339) for schedules and available pickup times specific for your area. Notify the Federal Express dispatcher that you require a pickup and provide the following information:

- ☎Your name
- ☎Your address
- ☎Exact location of the container

Section #6
For Friday Shipments: mark "SATURDAY Delivery"

Airbill for shipments within the United States

"Date" of shipment must be indicated



← Example - Saturday Delivery Label

SCHEDULE OF ASSESSMENTS

Visit Name/Visit Number				Screen	Unscheduled
Visit Interval				N/A	N/A
Visit Type				S	U
Test Panel/Name	**Specimen Type**	**Sample Tube/ Cryovial (Coming back)**	**Samples permitted to be batched**		
Chemistry Panel	Serum	5 ml Transfer Vial	No	A	A, O
Hematology Panel	Whole Blood	3 ml EDTA Tube	No	A	A, O
Quantitative Blood Nicotine/Cotinine	Whole Blood	3 ml EDTA Tube	No	A	A, O

Notes:

S: Scheduled / T: Termination / U: Unscheduled / R: Retest / O: Optional / A: Ambient / F: Frozen / C: Cooled

SCREEN TEST REQUISITION EXAMPLE

0203021042

Requisition Number:

%r e q n u m b e r %

LabCorp CLINICAL TRIALS

LABORATORY TEST REQUEST FORM

EXAMPLE

Investigator:

Site:

LSN:

Contact who completed requisition form:
Name: _____
Phone # (with country code): _____

Instructions:
- Complete all information with ball-point pen.
- Keep bottom copy for your records. Submit at least one copy of requisition with each specimen shipment.
- Refer to LabCorp Investigator Manual for detailed specimen collection, processing and shipping instructions.

Testing Required:
Chemistry
Hematology
Nicotine/Cotinine

Visit:	**Screen Visit**
Patient ID:	☐☐☐☐☐
Date of Birth:	☐☐ - ☐☐☐ - ☐☐☐☐ (DAY MONTH YEAR)
Gender:	☐ Male ☐ Female
Collection Date:	☐☐ - ☐☐☐ - ☐☐☐☐ (DAY MONTH YEAR)
Collection Time:	☐☐ : ☐☐

For LabCorp Use Only

Expected Container Type	# Containers Expected Back	# Containers Received	Condition Received		
			Amb	Frz	Refrig
5 mL Transfer Tube, Serum - Amb	1				
3 mL EDTA, Whole Blood - Amb	2				

Accessioner's Initials/Date: _____

General Comments:

UNSCHEDULED TEST REQUISITION EXAMPLE

0884334707

Requisition Number:

%reqnumber%

LabCorp CLINICAL TRIALS

LABORATORY TEST REQUEST FORM

EXAMPLE

Investigator:

Site:

LSN:

Contact who completed requisition form:
Name:_____
Phone # (with country code):_____

Instructions:
- Complete all information with ball-point pen.
- Keep bottom copy for your records. Submit at least one copy of requisition with each specimen shipment.
- Refer to LabCorp Investigator Manual for detailed specimen collection, processing and shipping instructions.

Optional Tests:

☐ **Chemistry**
☐ **Hematology**
☐ **Nicotine/Cotinine**

(Select all that apply)

Visit:	**Unscheduled Visit**
Patient ID:	☐☐☐☐☐
Date of Birth:	☐☐ - ☐☐☐ - ☐☐☐☐ (DAY MONTH YEAR)
Gender:	☐ **Male** ☐ **Female**
Collection Date:	☐☐ - ☐☐☐ - ☐☐☐☐ (DAY MONTH YEAR)
Collection Time:	☐☐ : ☐☐

For LabCorp Use Only

Expected Container Type	# Containers Expected Back	# Containers Received	Condition Received		
			Amb	Frz	Refrig
5 mL Transfer Tube, Serum - Amb	1				
3 mL EDTA, Whole Blood - Amb	2				

Accessioner's Initials/Date: _____

General Comments:

SPECIMEN COLLECTION AND PREPARATION

General Reminders

- Throughout specimen collection and handling, proper universal precautions should be practiced. Precautions such as collection gloves, protective eye wear, and lab coats should be worn throughout the collection and specimen handling procedure. Particular caution should be observed during venipuncture.

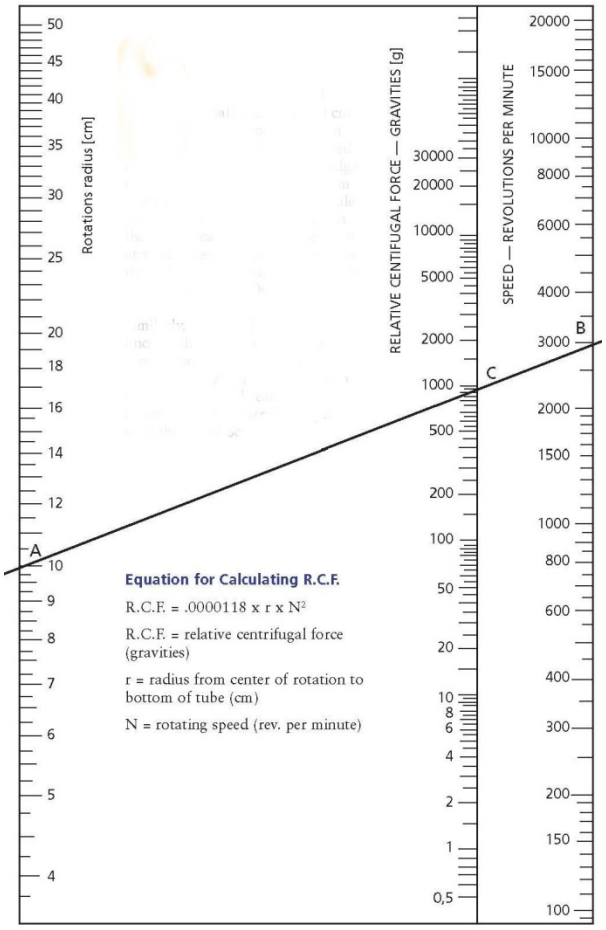
- In order to minimize the potential for cross-contamination between different additives in the tubes, it is suggested that tubes without additives be collected before tubes containing an anticoagulant substance.

- Do not submit hemolyzed samples. (not applicable for tubes with anti-coagulant substances).

SPECIMEN COLLECTION AND PREPARATION

CENTRIFUGATION

Guidelines for the Conversion of Relative Centrifugal Force (g) to RPM (Rotations per Minute)



Radius (cm)	G-force	RPM
10	1200	3280
12	1200	2990
14	1200	2770
16	1200	2590
18	1200	2440
20	1200	2310
22	1200	2200
24	1200	2110
26	1200	2030
28	1200	1950
30	1200	1890

Radius (cm)	G-force	RPM
10	1500	3660
12	1500	3340
14	1500	3090
16	1500	2890
18	1500	2730
20	1500	2590
22	1500	2470
24	1500	2360
26	1500	2270
28	1500	2190
30	1500	2110

Note: If you need further assistance, please call your LabCorp Project Monitor.

SPECIMEN COLLECTION AND PREPARATION

VISITS	TESTS	COLLECTION TUBES	INSTRUCTIONS / STABILITY	TUBES TO BE SENT BACK	SHIPPING TEMP.	SHIPPING Frequency / Courier / Location
Screen Unscheduled	**Chemistry Panel**	 3.5 mL SST Tube (Yellow Top)	Immediately after sample is drawn, gently invert the tube 180° and back, 5-6 times. Allow tube to stand in the upright position for a minimum of 30 minutes but no longer than 2 hours. Centrifuge the tube for 10 minutes at 1300 – 2000 *x g* (RCF). Using a disposable pipette, transfer serum into a 5 mL Transfer Tube. Refrigerate Transfer Tube if shipment is delayed for more than 8 hours.	 5 mL Transfer Tube	AMBIENT	On Day of Collection Via FedEx To LabCorp Clinical Trials 750 Walnut Ave Cranford, NJ 07016

Screen Unscheduled	Hematology Panel	 3 mL EDTA Tube (Lavender Top) (Whole Blood)	Immediately after sample is drawn, gently invert the tube 180° and back, 8-10 times. Use a pencil to identify the slides with the Patient ID.	 3 mL EDTA Tube	AMBIENT	On Day of Collection via FedEx to 750 Walnut Ave. Cranford, NJ 07019

Screen Unscheduled	Nicotine & Cotinine	 3 mL EDTA Tube (Lavender Top) (Whole Blood)	Immediately after sample is drawn, gently invert the tube 180° and back, 8-10 times.	 3 mL EDTA Tube (Whole Blood)	AMBIENT	On Day of Collection via FedEx to 750 Walnut Ave. Cranford, NJ 07016
Screen Unscheduled **Genotyping samples are not sent back to LabCorp for these visits**	Genotyping **Genotyping testing is not performed at LabCorp**	 3 mL EDTA Tube (Lavender Top) (Whole Blood)	Immediately after sample is drawn, gently invert the tube 180° and back, 8-10 times. Divide 2 aliquots of about 1.5 mL each into 2 mL PP microtubes (Sarstedt #72/694.007 or similar) **LabCorp is not providing kits or supplies for Genotyping and samples are not sent to LabCorp for testing. Please refer to your Sponsor contact for questions**	 1.5 mL of Whole Blood per tube	FROZEN	Store on site at -80°C for future DNA Extraction **DO NOT send Genotyping samples to LabCorp**

SPECIMEN PACKAGING FOR TRANSPORT

Study-specific ATTENTION POINTS:

- All samples have to be identified with the corresponding Barcode/Subject Label.
- All tubes and containers must be tightly closed to avoid leakage during transport.
- Samples of multiple subjects may be shipped in one container and should remain in their individual specimen bags, along with corresponding requisition forms.
- Check local and national holidays (See Appendix 1).
- In the event samples cannot be shipped on the Day of Collection (or outside cut-off times), store on site and forward the next day according to specified shipping requirements.

  - Keep serum chemistries *refrigerated*
  - Keep whole blood for hematology at *ambient* temperature

- You must fill out all transport documents completely
  - Transport document(s) are provided and are pre-printed with site and LabCorp information. (See Chapter 1 - Courier Services)
  - Sign and Date where indicated.
  - Retain a copy for your records.

- Forward Shipment Alert Form
  - On the day that you ship your specimens, make a copy of the Shipment Alert Form located in Appendix 5.
  - Complete and fax the Shipment Alert Form to the attention of your monitor.

SPECIMEN PACKAGING FOR TRANSPORT

Non-Infectious AMBIENT Shipment

SHIPPING BOX

Ambient Samples:

1. Insert samples into absorbent wrap.
2. Place wrapped samples inside the largest compartment of specimen bag and seal.
3. Insert a copy of the completed requisition form in the outer pocket of the specimen bag. (Retain one copy for your records.)
4. Place the specimen bag into the LabCorp ambient kit box and close.
5. Place the kit box into the courier bag and seal.
6. Complete the transport documents. (Retain one copy for your records.)
7. Insert transport documents into airbill pouch, affix pouch to the outside of the courier bag and submit to driver.
8. Complete and fax Shipment Alert Form to LabCorp.

NOTE: Samples of multiple subjects may be shipped in one container and should remain in their individual specimen bags, along with corresponding requisition forms.

REPORTING AND TURNAROUND TIME

Reporting

As per the request of the sponsor, study result reports will be faxed to you on a daily basis.

The complete results of one patient's visit will be divided into 2 different reports, which will be faxed separately. (i.e. 1 for Chemistry and Hematology results and 1 for Nicotine/Cotinine results.)

Missing demographic information or discrepancies could delay result reports.

Upon receipt of the report, carefully check the subject demographics against the CRF. If any discrepancy is noticed, contact the LabCorp monitor(s) immediately.

Turnaround Time (TAT)

Report	Test Name	TAT from receipt (to include shipment between LabCorp locations), counted in working days (TAT may be extended if repeat testing is required.)
Safety	Chemistry	within 2-3 working days after receipt of samples at LabCorp
	Hematology	within 2-3 working days after receipt of samples at LabCorp
Nicotine/ Cotinine	Nicotine Cotinine	Within 7-10 working days after receipt of samples at LabCorp (Note: Official TAT is listed as 7-10 business days. The lab will normally report within 4-5 business days, unless there is a delay due to volume and demand for the test)

Legend:

L: Low, below normal reference range
H: High, above normal reference range
LA: Low Alert
HA: High Alert
LP: Low Panic
HP: High Panic

Ex: Exclusion Value
M: Protocol Specific Markedly Abnormal Value

The investigator should act upon the flagged results as required by the protocol.

For more information about the results provided, please contact your LabCorp monitor.

When contacting LabCorp, ALWAYS have the Requisition Number available, since this is the LabCorp primary identifier.

US Holidays Observed by LabCorp Clinical Trials

- LabCorp is closed on the listed Holidays, as well as Sunday.
- Please keep these dates in mind as you prepare to schedule subjects for blood draws.
- Remember to contact courier(s) for their list of holidays and reduced services.

HOLIDAY	2012
New Year's Day	Sunday, January 1 (observed Monday, Jan. 2)
Memorial Day	Monday, May 28
Independence Day	Wednesday, July 4
Labor Day	Monday, September 3
Thanksgiving Day	Thursday, November 22
Christmas Day	Tuesday, December 25

HOLIDAY	2013	2014
New Year's Day	Tuesday , January 1	Wednesday, January 1
Memorial Day	Monday, May 27	Monday, May 26
Independence Day	Thursday, July 4	Friday, July 4
Labor Day	Monday, September 2	Monday, September 1
Thanksgiving Day	Thursday, November 28	Thursday, November 27
Christmas Day	Wednesday, December 25	Thursday, December 25

REFERENCE RANGES

Esoterix Clinical Trial Services, A division of LabCorp
www.esoterix.com

Protocol Reference Range Definitions

ESN: 101904 Health Effects Institute, MOSES Common Protocol 10-01-4
Unit Type: Conventional, Sex: Both, Age: >= 18 years, < 150 years

Parent Test Name	Test Name	Test Code	Result Type	Sex	Age	Panic Low	Alert Low	Normal Range	Alert High	Panic High	Unit
Auto Diff	Basophils, Absolute	BASOAA	String	Both	17y - 150y			0 - .2			10^3/uL
	Basophils, Percent	BASOPA	Numeric	Both	17y - 150y			0 - 3			%
	Eosinophils, Absolute	EOSNAA	String	Both	17y - 150y			0 - .4			10^3/uL
	Eosinophils, Percent	EOSNPA	Numeric	Both	17y - 150y			0 - 7			%
	Lymphocytes, Absolute	LYMPAA	String	Both	17y - 150y			.7 - 4.5			10^3/uL
	Lymphocytes, Percent	LYMPPA	Numeric	Both	17y - 150y			14 - 46			%
	Monocytes, Absolute	MONOAA	String	Both	17y - 150y			.1 - 1			10^3/uL
	Monocytes, Percent	MONOPA	Numeric	Both	12y - 150y			4 - 13			%
	Neutrophils, Absolute	NEUTAA	String	Both	17y - 150y	0.5	1	1.8 - 7.8	14		10^3/uL
	Neutrophils, Percent	NEUTPA	Numeric	Both	17y - 150y			40 - 74			%
	Nucleated RBC, Absolute	NRBCAA	String	Both	All			- 0			10^3/uL
	Nucleated RBC, Percent	NRBCPA	Numeric	Both	All			- 0			%
Chemistry Panel	AG Ratio	TAGR	Numeric	Both	All			.7 - 2			Ratio
	ALT (SGPT)	TALT	Numeric	Female	All			0 - 40	500		IU/L
				Male	All			0 - 55	500		
	AST (SGOT)	TAST	Numeric	Both	All			0 - 40	500		IU/L
	Albumin	TALB	Numeric	Both	2y - 59y		2	3.5 - 5.5	6		g/dL
					59y - 69y		2	3.6 - 4.8	6		
					69y - 79y		2	3.5 - 4.8	6		
					79y - 89y		2	3.5 - 4.7	6		
					89y - 150y		2	3.2 - 4.6	6		
	Alkaline Phosphatase	TALP	Numeric	Female	14y - 20y			45 - 300	1,000		IU/L
					20y - 60y			25 - 150	1,000		

Note: All age ranges are inclusive on the low end and exclusive on the high (i.e. an age range of 18y - 50y includes patients from their 18th birthday up to their 50th birthday). All tests in the protocol are displayed, even if no reference range is defined.

Protocol Reference Range Definitions

ESN: 101904 Health Effects Institute, MOSES Common Protocol 10-01-4
Unit Type: Conventional, Sex: Both, Age: >= 18 years, < 150 years

Parent Test Name	Test Name	Test Code	Result Type	Sex	Age	Panic Low	Alert Low	Normal Range	Alert High	Panic High	Unit
Chemistry Panel	Alkaline Phosphatase	TALP	Numeric	Female	60y - 150y			25 - 165	1,000		IU/L
				Male	14y - 20y			60 - 400	1,000		
					20y - 60y			25 - 150	1,000		
					60y - 150y			25 - 160	1,000		
	Amylase	TAMY	Numeric	Both	All		5	28 - 100	400		U/L
	Bicarbonate	TCO2	Numeric	Both	All		10	20 - 32	40		mEq/L
	Blood Urea Nitrogen	TBUN	Numeric	Both	18y - 40y		2	6 - 20	75		mg/dL
					40y - 60y		2	6 - 24	75		
					60y - 90y		2	8 - 27	75		
					90y - 150y		2	10 - 36	75		
	Calcium	TCAL	Numeric	Both	All	7		8.5 - 10.6		13	mg/dL
	Chloride	TCL	Numeric	Both	All		75	96 - 109	115		mEq/L
	Creatine Kinase	TCK	Numeric	Female	All			24 - 173			IU/L
				Male	All			24 - 204			
	Creatinine	TCRE	Numeric	Female	15y - 150y			.57 - 1	7.5		mg/dL
				Male	15y - 150y			.76 - 1.27	7.5		
	Direct Bili	TDBL	Numeric	Both	All			0 - .4			mg/dL
	Gamma Glutamyl Transferase	TGGT	Numeric	Female	All			0 - 60	650		IU/L
				Male	All			0 - 65	650		
	Globulin	TGLB	Numeric	Both	All			2 - 4.5			g/dL
	Glucose, Serum	TGLS	Numeric	Both	All	40		65 - 99		500	mg/dL
	Iron	TFE	Numeric	Female	9y - 150y		10	35 - 155	260		ug/dL
				Male	9y - 150y		10	40 - 155	260		
	LDH	TLDH	Numeric	Both	All			100 - 250			IU/L
	Lipase	TLIP	Numeric	Both	All			0 - 59			U/L
	Magnesium	TMAG	Numeric	Both	All		1	1.6 - 2.6	3.5		mg/dL

Note: All age ranges are inclusive on the low end and exclusive on the high (i.e. an age range of 18y - 50y includes patients from their 18th birthday up to their 50th birthday). All tests in the protocol are displayed, even if no reference range is defined.

Protocol Reference Range Definitions

ESN: 101904 Health Effects Institute, MOSES Common Protocol 10-01-4
Unit Type: Conventional, Sex: Both, Age: >= 18 years, < 150 years

Parent Test Name	Test Name	Test Code	Result Type	Sex	Age	Panic Low	Alert Low	Normal Range	Alert High	Panic High	Unit
Chemistry Panel	Phosphorous	TPHS	Numeric	Female	9y - 19y			2.5 - 5.3			mg/dL
					19y - 150y			2.5 - 4.5			
				Male	9y - 19y			2.5 - 5.6			
					19y - 150y			2.5 - 4.5			
	Potassium	TK	Numeric	Both	All	2.5		3.5 - 5.5		6.5	mEq/L
	Sodium	TNA	Numeric	Both	All	120		134 - 144		160	mEq/L
	Total Bili	TTBL	Numeric	Both	All			.1 - 1.2	12	17	mg/dL
	Total Protein	TTP	Numeric	Both	All			6 - 8.5			g/dL
	Uric Acid	TUA	Numeric	Female	18y - 150y			2.5 - 7.1	15		mg/dL
				Male	18y - 150y			3.7 - 8.6	15		
Estimated Glomerular Filtratio	eGFR	TGFRW	Numeric	Both	All			>/= 60			mL/min/1.73 m^2
	eGFR - If African American	TGFRB	Numeric	Both	All			>/= 60			mL/min/1.73 m^2
Hematology Panel	Hematocrit	HCT	String	Female	17y - 150y	18.1		34 - 44		64.4	%
				Male	17y - 150y	18.1		36 - 50		64.4	
	Hemoglobin	HGB	String	Female	17y - 150y	6.1	7.1	11.5 - 15	19.9	21.4	g/dL
				Male	17y - 150y	6.1	7.1	12.5 - 17	19.9	21.4	
	Mean Cell Volume	MCV	String	Both	11y - 150y			80 - 98			fL
	Mean Corpuscular Hemoglobin	MCH	String	Both	17y - 150y			27 - 34			pg
	Mean Corpuscular Hgb Conc	MCHC	String	Both	17y - 150y			32 - 36			g/dL
	Platelet Count	PLT	String	Both	17y - 150y	31	50	140 - 415	799	999	10^3/uL
	Red Blood Cells	RBC	String	Female	17y - 150y		2.76	3.8 - 5.1	6.99		10^6/uL
				Male	17y - 150y		2.76	4.1 - 5.6	6.99		
	White Blood	WBC	String	Both	17y - 150y	1.1	2	4 - 10.5	19.9	49.9	10^3/uL

Note: All age ranges are inclusive on the low end and exclusive on the high (i.e. an age range of 18y - 50y includes patients from their 18th birthday up to their 50th birthday). All tests in the protocol are displayed, even if no reference range is defined.

Protocol Reference Range Definitions

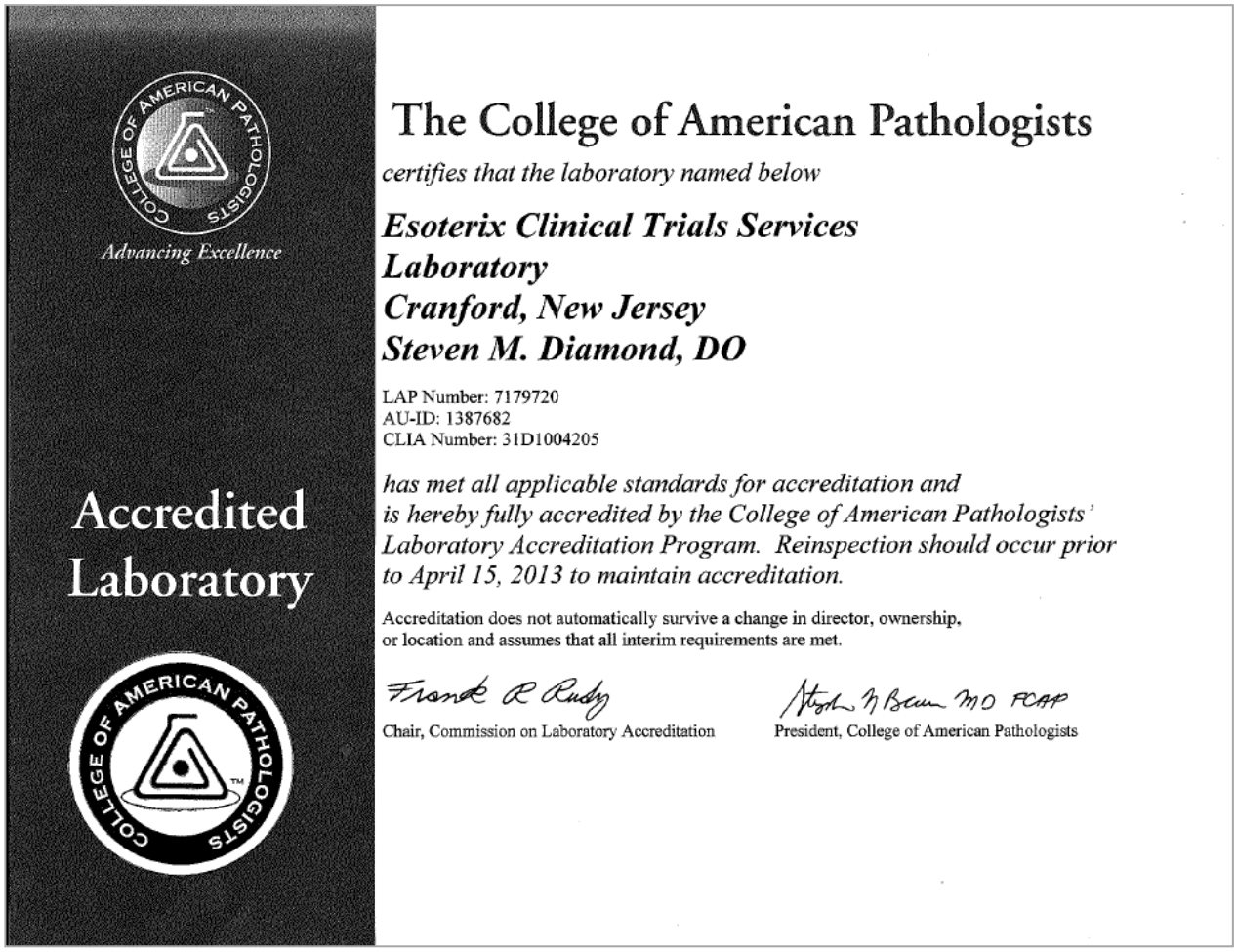
ESN: 101904 Health Effects Institute, MOSES Common Protocol 10-01-4
Unit Type: Conventional, Sex: Both, Age: >= 18 years, < 150 years

Parent Test Name	Test Name	Test Code	Result Type	Sex	Age	Panic Low	Alert Low	Normal Range	Alert High	Panic High	Unit
	Cells										
Lipid	Cholesterol	TCHL	Numeric	Both	0y - 19y			100 - 169			mg/dL
					19y - 150y			100 - 199			
	HDL	THDL	Numeric	Both	All			40 -			mg/dL
	LDL Calc	TLDLC	Numeric	Both	All			0 - 99			mg/dL
	Triglycerides	TTRG	Numeric	Both	All			0 - 149			mg/dL
Nicotine and Cotinine	Cotinine	COTINS	Numeric								ng/mL
	Nicotine	NICOTS	Numeric								ng/mL
	CBC Comments	CBCCOM	String								

Note: All age ranges are inclusive on the low end and exclusive on the high (i.e. an age range of 18y - 50y includes patients from their 18th birthday up to their 50th birthday). All tests in the protocol are displayed, even if no reference range is defined.

LABORATORY ACCREDITATION

CAP-Cranford, NJ



Advancing Excellence

Accredited Laboratory

The College of American Pathologists

certifies that the laboratory named below

Esoterix Clinical Trials Services Laboratory
Cranford, New Jersey
Steven M. Diamond, DO

LAP Number: 7179720
AU-ID: 1387682
CLIA Number: 31D1004205

has met all applicable standards for accreditation and is hereby fully accredited by the College of American Pathologists' Laboratory Accreditation Program. Reinspection should occur prior to April 15, 2013 to maintain accreditation.

Accreditation does not automatically survive a change in director, ownership, or location and assumes that all interim requirements are met.

Chair, Commission on Laboratory Accreditation

President, College of American Pathologists

LABORATORY ACCREDITATION

CLIA-Cranford, NJ

CENTERS FOR MEDICARE & MEDICAID SERVICES
CLINICAL LABORATORY IMPROVEMENT AMENDMENTS

CERTIFICATE OF ACCREDITATION

LABORATORY NAME AND ADDRESS	CLIA ID NUMBER
ESOTERIX CLINICAL TRIALS SERVICES 750 WALNUT AVENUE CRANFORD, NJ 07016	31D1004205
	EFFECTIVE DATE 07/01/2011
LABORATORY DIRECTOR	EXPIRATION DATE
STEVEN M DIAMOND DO	06/30/2013

Pursuant to Section 353 of the Public Health Services Act (42 U.S.C. 263a) as revised by the Clinical Laboratory Improvement Amendments (CLIA), the above named laboratory located at the address shown hereon (and other approved locations) may accept human specimens for the purposes of performing laboratory examinations or procedures.
This certificate shall be valid until the expiration date above, but is subject to revocation, suspension, limitation, or other sanctions for violation of the Act or the regulations promulgated thereunder.

CMS
CENTERS for MEDICARE & MEDICAID SERVICES

Judith A. Yost, Director
Division of Laboratory Services
Survey and Certification Group
Center for Medicaid and State Operations

27 certs2_060411

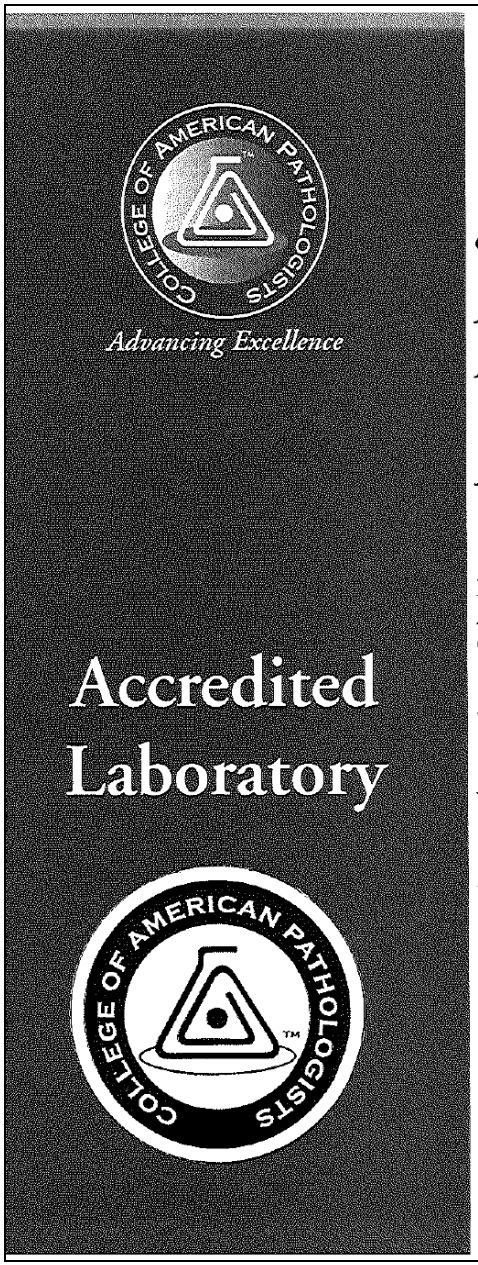
If you currently hold a Certificate of Compliance or Certificate of Accreditation, below is a list of the laboratory specialties/subspecialties you are certified to perform and their effective date:

LAB CERTIFICATION (CODE)	EFFECTIVE DATE	LAB CERTIFICATION (CODE)	EFFECTIVE DATE
VIROLOGY (140)	04/03/2007		
GENERAL IMMUNOLOGY (220)	07/01/2003		
ROUTINE CHEMISTRY (310)	07/01/2003		
URINALYSIS (320)	07/01/2003		
ENDOCRINOLOGY (330)	07/01/2003		
TOXICOLOGY (340)	08/23/2004		

FOR MORE INFORMATION ABOUT CLIA, VISIT OUR WEBSITE AT WWW.CMS.HHS.GOV/CLIA
OR CONTACT YOUR LOCAL STATE AGENCY. PLEASE SEE THE REVERSE FOR
YOUR STATE AGENCY'S ADDRESS AND PHONE NUMBER.
PLEASE CONTACT YOUR STATE AGENCY FOR ANY CHANGES TO YOUR CURRENT CERTIFICATE.

LABORATORY ACCREDITATION

CAP-Burlington, NC



Rec'd 5-3-11

The College of American Pathologists

certifies that the laboratory named below

Laboratory Corporation of America
Regional Laboratory & Center for Esoteric Testing
Burlington, North Carolina
William F. Hancock, MD

LAP Number: 1396901
AU-ID: 1179680
CLIA Number: 34D0655059

has met all applicable standards for accreditation and is hereby fully accredited by the College of American Pathologists' Laboratory Accreditation Program. Reinspection should occur prior to February 14, 2013 to maintain accreditation.

Accreditation does not automatically survive a change in director, ownership, or location and assumes that all interim requirements are met.

Frank R Rudy
Chair, Commission on Laboratory Accreditation

Stephen M Beck MD FCAP
President, College of American Pathologists

LABORATORY ACCREDITATION

CLIA-Burlington, NC

CENTERS FOR MEDICARE & MEDICAID SERVICES
CLINICAL LABORATORY IMPROVEMENT AMENDMENTS

Rec'd 10-27-10

CERTIFICATE OF ACCREDITATION

LABORATORY NAME AND ADDRESS

LABORATORY CORPORATION OF AMERICA
1447 YORK COURT
BURLINGTON, NC 27215

LABORATORY DIRECTOR

WILLIAM F HANCOCK JR MD

CLIA ID NUMBER

34D0655059

EFFECTIVE DATE

10/20/2010

EXPIRATION DATE

10/19/2012

Pursuant to Section 353 of the Public Health Services Act (42 U.S.C. 263a) as revised by the Clinical Laboratory Improvement Amendments (CLIA), the above named laboratory located at the address shown hereon (and other approved locations) may accept human specimens for the purposes of performing laboratory examinations or procedures.
This certificate shall be valid until the expiration date above, but is subject to revocation, suspension, limitation, or other sanctions for violation of the Act or the regulations promulgated thereunder.

CMS
CENTERS for MEDICARE & MEDICAID SERVICES

Judith A. Yost, Director
Division of Laboratory Services
Survey and Certification Group
Center for Medicaid and State Operations

f02 certs2_092510

If you currently hold a Certificate of Compliance or Certificate of Accreditation, below is a list of the laboratory specialties/subspecialties you are certified to perform and their effective date:

LAB CERTIFICATION (CODE)	EFFECTIVE DATE
BACTERIOLOGY (110)	07/27/1995
MYCOBACTERIOLOGY (115)	07/27/1995
MYCOLOGY (120)	07/27/1995
PARASITOLOGY (130)	07/27/1995
VIROLOGY (140)	07/27/1995
SYPHILIS SEROLOGY (210)	07/27/1995
GENERAL IMMUNOLOGY (220)	07/27/1995
ROUTINE CHEMISTRY (310)	07/27/1995
URINALYSIS (320)	07/27/1995
ENDOCRINOLOGY (330)	07/27/1995
TOXICOLOGY (340)	02/04/2003
HEMATOLOGY (400)	07/27/1995
ABO & RH GROUP (510)	07/27/1995

LAB CERTIFICATION (CODE)	EFFECTIVE DATE
ANTIBODY NON-TRANSFUSION (530)	07/27/1995
ANTIBODY IDENTIFICATION (540)	07/27/1995
HISTOPATHOLOGY (610)	07/27/1995
ORAL PATHOLOGY (620)	07/27/1995
CYTOLOGY (630)	10/26/2006
RADIOBIOASSAY (800)	10/26/2006

FOR MORE INFORMATION ABOUT CLIA, VISIT OUR WEBSITE AT WWW.CMS.HHS.GOV/CLIA
OR CONTACT YOUR LOCAL STATE AGENCY. PLEASE SEE THE REVERSE FOR
YOUR STATE AGENCY'S ADDRESS AND PHONE NUMBER.
PLEASE CONTACT YOUR STATE AGENCY FOR ANY CHANGES TO YOUR CURRENT CERTIFICATE.

CV MEDICAL DIRECTOR-Cranford, NJ

CURRICULUM VITAE
Steven Diamond, DO, MBA, FCAP

3/17/11

Board Certification:	American Board of Pathology Anatomic and Clinical Pathology (Recertified) Hematology	 11/75 1/09 5/77
Board Registry:	America Board of Medicolegal Death Investigators	 5/20/02
Education:	Chicago College of Osteopathic Medicine Midwestern University Chicago, IL	DO 6/70
	University of Wisconsin Madison, WI	Path Resident 1970 – 1971
	United States Navy Philadelphia, PA	Pathologist 1971 – 1973
	Thomas Jefferson University Philadelphia, PA	Path Resident 1973 – 1975
	Wharton School of Business University of Pennsylvania Philadelphia, PA	MBA 1992 – 1994
State Licenses:	New Jersey Pennsylvania Missouri New York	
Medical Societies:	College of American Pathologists Pennsylvania Association of Pathologists National Association of Medical Examiners American Board of Medicolegal Death Investigators	
Professional Services:	Director of Laboratories Hunterdon Medical Center 2100 Wescott Dr. Flemington, NJ 08822	1/1999-present
	Medical Director Esoterix Inc., Clinical Trials Laboratory 750 Walnut Avenue Cranford, New Jersey 07016	7/2004-present
	Laboratory Director Accupath Diagnostic Laboratories, Inc Trenton, New Jersey 08691	8/2007-present
	Associate Director Miller-Keystone Memorial Blood Bank Bethlehem, PA 18017	1/1994-present
	Medical Examiner of Hunterdon County 2100 Wescott Drive Flemington, New Jersey 08822	1/2003-present
	Medical Director, Tandem Laboratories 115 Silva Street Ewing, New Jersey 08628	6/2008-present

CURRICULUM VITAE
Steven Diamond, DO, MBA, FCAP

Publications:

1. Allen HB, Johnson BL, Diamond SM: Smoker's Wrinkles?: JAMA 225:1067
2. Diamond SM: Primary Adenocarcinoma of Renal Pelvis: Mil Med 139:959
3. Takeda M, Diamond SM: Cytological Diagnosis of Malignant Melanoma Metastatic to Endometrium: Acta Cytol 22:503

Employment History:

Warren Hospital 185 Roseberry Street Phillipsburg, NJ 08865	7/1975-12/1998
Hunterdon Medical Center 2100 Wescott Drive Flemington, NJ 08822	1/1999-present

CV MEDICAL DIRECTOR-Burlington, NC

CURRICULUM VITAE

Name: William Franklin (Frank) Hancock (Jr)
1303 West Davis Street
Burlington, NC 27215

Education:

College Name of College or University	Major	Attended From	 To	Degree
Campbell College, Buies Creek, NC	Liberal Arts	1961	1962	
University of Tennessee, Knoxville, TN	Liberal Arts	1962	1963	
University of North Carolina, Chapel Hill, NC	Chemistry	1963	1964	BA
University of North Carolina, Chapel Hill, NC	Medicine	1964	1968	MD
Medical College of Virginia, Richmond, VA	Pathology	1964	1968	Internship
Medical College of Virginia, Richmond, VA	Pathology	1969, 1971	1973	Residency (interrupted by Viet Nam conflict)

Job Experience:

Institution	Title	Job Duties	From	To
LabCorp, Burlington, NC	Medical Director, Staff Pathologist	Hematology, Urinalysis, Coagulation, Immunohematology, Anatomical Pathology, Toxicology, General Lab consultation, QA	1990	Present
LabCorp, Center for Molecular Biology and Pathology, Research Triangle Park, NC	Temporary Medical Director for the state of Florida	Hematology, Urinalysis, Coagulation, Anatomical Pathology, General Lab consultation, QA	9/07	2/08
LabCorp/Triad Clinical Lab, Winston Salem NC	Medical Director	General Lab, QA	2003	Present
LabCorp/Greensboro Stat Laboratory, Greensboro NC	Medical Director	Hematology, Chemistry, Coagulation, QA	2003	Present
Norfolk Diagnostics Laboratory, Norfolk, VA	Medical Consultant (Limited)	General Lab	1985	Present
Chatham Hospital, Siler City, NC	Medical Director	General Lab/Pathology	1974	Present
Halifax Memorial Hospital, Roanoke Rapids, NC	Pathologist/Lab Director	Clinical Lab/Pathology	1979	1982

Updated: Nov 2011

Signature: William F Hancock MD Date: 11-01-2011

Wilson Carolina Medical Center Laboratory, Wilson, NC	Medical Director	General Lab	1985	1991
Halifax Community College, Roanoke Rapids, NC	Medical Technology Program	Director/Consultant	1980	1983
Lee County Hospital, Sanford, NC	Associate Pathologist	Pathology	1973	1974

Awards, Licenses, Board Certifications:

Award, Certification	Date	Certification Number	Specialization
National Board of Medical Examiners	1972	99574	Medicine
American Board of Pathology	1973	Lifetime Membership	Anatomical & Clinical Pathology
North Carolina Medical Board	1973	15932	
New York State Department of Health	1990	HANCW1	Director
Virginia State Medical Board	1972	22572	MD
Tennessee State Medical Board	11/27/07	42842	MD
Georgia State Medical Board	5/09/08	61085	MD
South Carolina Medical Board	8/07/08	TL31240	MD
Maryland State Medical Board	1987	192930	MD
District of Columbia	2009	MD038373	MD

Updated: Nov 2011

Signature: William Hamrick Jr MD Date: 11-01-2011

North Carolina Medical Board

Physician License Certificate

Next Renewal Date **11/14/2012**

Certificate # **4055**

The physician named below is currently licensed with the NC Medical Board and has paid the fees as required by the general statutes of North Carolina, section 90-13.1 and rules promulgated pursuant thereto.

License No: 15932

William Franklin Hancock Jr. MD
1303 West Davis St
Burlington, NC 27215-2149

R. David Henderson

Executive Director

Below is your renewal certificate for your wallet. Please detach and discard this portion.

North Carolina Medical Board

Next Renewal Date

11/14/2012

Certificate # **4055**

This is to certify that the physician named below is currently licensed with the NC Medical Board and has paid the fees as required by section 90-13.1 and rules promulgated pursuant thereto.

William Franklin Hancock Jr. MD

License No: 15932

R. David Henderson

Executive Director

William F. Hancock Jr MD 11-01-2011

FORMS

Supply Reorder Form

To reorder supplies, use the LabCorp pre-printed, study-specific reorder forms.

Complete the Supply Reorder Form and fax to the fax number listed on top of the form.

Please allow a minimum of 5 to 7 working days for assembly and delivery of reordered supplies. For regions outside the United States, allow 7 to 10 working days.

LabCorp CLINICAL TRIALS

SHIPMENT ALERT FORM

On the Day of Shipment, please complete this form and fax to:

LabCorp Clinical Trials
FAX: 919-474-4385

ATTENTION: Tamika Hawkins
(Phone: 919-474-4368)

Sponsor / Protocol: Health Effects Institute/
MOSES Common Protocol 10-01-4

Investigator Last Name: _____

SiteNumber: _____

Contact Person at Site: _____

Phone Number: _____

Date of Shipment: _____/_____/_____
DD / MMM / YYYY

Number of Boxes Shipped
to Cranford, NJ: _____

FedEx Airbill Number(s): _____

_____

LSN: 101904

LabCorp Monitor: Tamika Hawkins, Phone # 919-474-4368
LabCorp Project Manager: Abigail Berens, Phone # 952-563-4072

LabCorp CLINICAL TRIALS

SUPPLY REORDER FORM

Health Effects Institute – MOSES Common Protocol 10-01-4 **Site Number:** **Investigator:**	**By Fax: 919-474-4385** **Attn: Tamika Hawkins** **By Email: Hawkit2@labcorp.com** **LSN: 101904**

1. Kits

QUANTITY	KIT TYPE
	Screen Kit

QUANTITY	KIT TYPE
	Unscheduled Kit

2. Documents

QUANTITY	DESCRIPTION	CTX MNEMONIC
	Investigator Instructions Manual	INMAN

3. Transport Material

QUANTITY	DESCRIPTION	CTX MNEMONIC
	LabCorp Kit Box – Small	KBXS
	FedEx Diagnostic Shipping Pak	FEDEX
	FedEx Saturday Delivery Labels	SADEL
	FedEx Preprinted Ambient Airbill to Cranford, NJ	FATCJ

4. Additional Material

QUANTITY	DESCRIPTION	CTX MNEMONIC
	3.5 mL SST Tube	GL3.5
	5 mL White Cap Transfer Vial	TVW5
	3 mL EDTA Lavender Tube	LAV3
	Non Sterile Pipette	PIPST
	6 Place Tube Holder Absorbent Material	AQPK
	Biohazard Specimen Bag	SPBAG
	21 Gauge Eclipse Needle	21G1N
	Adult Vacutainer Holder	AVAC
	Band Aid Strip	BANDA
	Alcohol Pad	ALCOH

SECTION TO BE COMPLETED BY THE SITE	
Order placed by:	
Order date:	
Phone number:	
Date supplies required*:	

FOR LABCORP USE ONLY	
Date received:	
Order handled by:	
Date input CTPM/CTX:	
SWO number (CTX only):	
Expected delivery date:	

ALLOW AT LEAST 5 TO 7 WORKING DAYS FOR DELIVERY OF SUPPLIES

MOSES Standard Operating Procedure (SOP)

SOP # 12 – PHLEB EXP

TITLE: Phlebotomy for Exposure Visits

Version: 1.0 **Directory/file[a]:** //

Author: Mark Frampton **Signature:** **Date: April 30, 2012**

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

ANNUAL REVIEW[C]

Signature	**Date**

SOP UPDATED TO VERSION [d]

Explanations: [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. <u>If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!</u>

[c] Annual review does not change the version of the SOP.

[d] Change the version # as necessary.

PHLEBOTOMY FOR EXPOSURE VISITS

1. SCOPE AND APPLICATION

The purpose of this SOP is to describe the procedure of blood drawing and blood processing, distribution, and storage during the pre-exposure, exposure, and post-exposure visits.

2. PREREQUISITES

2.1 Equipment and Supplies

- A winged 21 gauge blood collection set is used along with a multiple-tube collection adapter.
- 1 Sodium Citrate tubes 4.5 ml BLUE TOP for discard
- 3 Sodium Citrate tubes 4.5 ml, BLUE TOP (FLOW CYTOMETRY, MP-TISSUE FACTOR, vWF MULTIMERS, SOLUBLE MARKERS, AND ARCHIVING)
- 1 EDTA tube 4 ml, LAVENDER TOP (Clinical lab: CBC, diff, platelets)
- 2 ml PP microtubes (Cryotubes, Fisher Scientific, 21-403-201)
- Additional supplies:
    - Gloves
    - Alcohol prep
    - Gauze pad
    - Tourniquet
    - Bandaid
    - Sharps container
    - Ice bucket

2.2 Training Requirements

Training in phlebotomy is required.

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATION

3.1 It is preferred that blood draws are performed on the left arm. Allowing for FMD BAU to be performed on the right arm.
3.2 Draw in order: discard tube, 3 Blue top tubes and a Lavender top tube.
3.3 Minimize the amount of time the vein is occluded.
3.4 Clean stick and flow is critical avoid activating the platelets.
3.5 Do not make a fist or repeatedly open and squeeze the hand. This will elevate potassium and ionized calcium and activate platelets.
3.6 Do not do more than three (3) sticks.
3.7 Discard the first 1 mL before drawing the citrate (blue top) tubes.
3.8 Fill all addictive containing tubes to full.
3.9 Invert tubes to ensure proper mixing. DO NOT SHAKE TUBES.
3.10 Process bloods as soon as possible

4. PROCEDURES

4.1 Blood Drawing

1. Choose the arm not used for blood pressure or fore-arm flow measurements; preferably use the left arm for blood drawing.
2. Locate the vein in the ante-cubital area. If a tourniquet is used, release it and re-tie it when ready to draw. The tourniquet should not be in place for longer than a minute before blood flows.
3. A clean stick is critical to keep the platelets from activating. It is acceptable to pull back, advance the needle or make slight lateral movements to locate the vein. Deep probing or "digging" is not.
4. If the subject has difficult veins, lowering the arm or applying a warm pack, is acceptable. Pumping the hand or making a fist are not desirable. They elevate potassium and ionized calcium and will activate platelets.
5. If first stick is not successful repeat the stick on the same (left) arm. If that is not successful try the other arm. Do not do more than three trials. If all unsuccessful consult the study physician.
6. Citrate (blue top) tubes must be filled to the full extent the vacuum allows in order to maintain the correct ratio of anticoagulant to blood. Discard the sample if there is a venous collapse or stoppage of blood flow while filling the citrate (blue top) tubes. Try a new tube and if not successful, a second stick will be required.
7. In the event that a second stick is required, remove the tourniquet and wait at least 3 minutes before reapplying it. If citrate is the first to be drawn, a discard tube must be used.

4.2 Order of Drawing

- 1 discard tube containing at least 1 ml of blood. Can be BLUE or RED TOP but not purple top (to avoid contamination with EDTA).
- 3 BLUE TOP, must be filled completely, invert 3-4x (do not shake)
- 1 LAVENDER TOP, invert 8-10x

4.3 Tube Handling and Storage (see Blood Flow Diagram in Table 1)

FIRST BLUE TOP (Tube #1)

- Flow cytometry: Within 5 minutes of draw, withdraw 200 ul of citrated whole blood and add to 1800 ul HEPES buffer (previously prepared by flow lab), mix with a gentle tap (DO NOT VORTEX OR INVERT). This diluted sample is kept at room temperature and sent to the flow lab. It should be processed within 30 minutes of the dilution (see SOP for Flow Cytometry). The time interval between blood draw and start of staining should not be more than 1 hour, and should be as consistent as possible during the study.
- Plasma for MP-Tissue Factor and vWF multimers: Spin the remaining whole blood at 1500 x g for 15 minutes at room temperature. Aliquot into 200 ul aliquots in 2 ml PP microtubes (Sarstedt #72.694.007 or similar) (should be approximately 10 tubes).
    - 5 tubes will be used for MP-TF and

- 5 tubes will be used for VWF multimers.
- Any excess plasma can be aliquoted in a similar fashion and sent with above samples.

Store all samples at -80ºC. Batch ship MP-Tissue Factor to Nigel Mackmann (see page 5).

SECOND BLUE TOP (Tube #2)

- Soluble markers, AssayGate: Put on ice, and then spin at 1000 x g for 10 minutes at 4°C within 30 minutes of blood collection. Aliquot 1.5 ml of the plasma and store at -80°C. The sample is stable for up to a year. Batch ship (minimum number of samples is 36) to AssayGate, Inc (see page 6). Save the remainder in 1.5 ml aliquots for archiving.

THIRD BLUE TOP (Tube #3)

- Archiving: Spin at 1000 x g for 10 minutes at 4°C within 30 minutes of blood collection. Aliquot (1.0 ml per tube) plasma and store at -80°C.

LAVENDER TOP (Tube #4)

- Send immediately to the clinical lab for CBC/differential/platelet count.

4.4 Recordkeeping

The PRE-EXP-POST Blood Tracking Form should be filled out for every blood drawing and stored in the subject folder. Use one form for each blood draw. The form also needs to be entered into ADEPT, so the samples collected can be tracked by the system. At the time of shipping ADEPT will generate a shipping form specific for the blood samples that are being shipped.

Results of the analyses from the analytic laboratories will be provided to NERI and entered in the ADEPT database.

4.5 Shipping

Samples should be shipped in batches. All samples from one subject should be shipped at the same time. Each shipment should include the appropriate Shipping Form.

5. QUALITY CONTROL

6. QUALITY ASSURANCE

HEI or NERI may request copies of the blood tracking form.

7. REFERENCES

None

8. APPENDICES

PRE-EXP-POST Visit Blood Tracking form

Table 1. Blood Flow Diagram for Phlebotomy SOP - April 30, 2012

TESTS	COLLECTION TUBES	INSTRUCTIONS / STABILITY	TUBES TO BE SHIPPED	SHIPPING TEMP.	SHIPPING Frequency / Courier / Location
Discard	4.5 mL (Blue) Sodium citrate tube	To ensure proper filling of the collection tube when using a winged blood collection set for venipuncture, and a coagulation tube is the first tube to be collected, a discard tube should be drawn first Discard tube. This discard tube does not need to be completely filled.		N/A	
Platelet activation MP-Tissue Factor and vWF multimers	4.5 mL (Blue) Sodium citrate tube (#1)	Keep at ROOM TEMPERATURE Within 5 minutes of draw, withdraw 200 ul of citrated whole blood and add to 1800 ul HEPES buffer (previously prepared by flow lab) Mix with a gentle tap. (DO NOT VORTEX OR INVERT). Send to the flow lab for processing. It should be processed within 30 minutes of the dilution (see SOP for Flow Cytometry). The time interval between blood draw and start of staining should not be more than 1 hour, and should be as consistent as possible during the study. Spin the remaining whole blood at 1500 x g for 15 minutes at room temperature. Using a disposable pipette, remove plasma (from the top) without disturbing the cells. Transfer plasma as follows (should be 10 tubes): 5 tubes will be used for MP-TF 5 tubes will be used for VWF multimers. Any excess plasma can be aliquoted in a similar fashion and sent with above samples. Store all samples at -80ºC in upright position, until frozen shipment. DO NOT SHIP THE TUBES FOR VWF MULTIMERS (store on site)	To Flow Cytometry Lab (0.2 ml blood +1.8 ml HEPES) 200 uL X 5 For MP-TF (total 1 ml blood) 200 uL X 5 For vWF multimer (total 1 ml blood)	FROZEN	N/A SHIP MP-TF TUBES IN BATCHES No minimum number of samples required. All samples from one subject should be shipped together (FedEX) Nigel Mackman, Ph.D. Department of Medicine 335A Mary Ellen Jones Building University of North Carolina at Chapel Hill 98 Manning Drive CB#7035 Chapel Hill NC 27599 Tel :919 843 3961

TESTS	**COLLECTION TUBES**	INSTRUCTIONS / STABILITY	TUBES TO BE SHIPPED	SHIPPING TEMP.	SHIPPING Frequency / Courier / Location
Soluble markers	 4.5 mL (Blue) Sodium citrate tube (#2)	Keep in ICE Spin at 1000 x g for 10 minutes at 4 °C within 30 minutes of blood collection. Using a disposable pipette, remove plasma (from the top) without disturbing the cells. Aliquot plasma as follows: 1.5 ml into a 2- ml PP microtube (Sarstedt #72.694.007 or similar). Store at -80 °C. The sample is stable for >1 month. Batch ship to AssayGate, Inc.	 1.5 mL For Soluble Markers	FROZEN	SHIP TUBES IN BATCHES Minimum number: 36 tubes Send as soon as 4 subjects have completed al the exposures (FedEX) To Assay Gate 9607Dr. Perry Road Ijamsville, MD 21754 Phone:301-874-0417 Fax: 301-560-8288 E-mail: orders@assaygate.com EID# 20-319-1767
Archive	 4.5 mL (Blue) Sodium citrate tube (#3)	Keep in ICE. Spin at 1000 x g for 10 minutes at 4 °C within 30 minutes of blood collection. Using a disposable pipette, remove plasma (from the top) without disturbing the cells. Aliquot plasma as follows: Aliquot 1.0 ml plasma per 2- ml PP microtube (Sarstedt #72.694.007 or similar). Will likely have enough for 2-3 tubes. Store on site at -80 °C.	 1.0 ml per tube For archiving	N/A	
CBC/ differential/ platelet	 4 mL(Lavender) EDTA-tube (#4)	Keep at ROOM TEMPERATURE. Send to the clinical lab for CBC/differential/platelet count.	 To Clinical Lab (Rm Temp)	N/A	

MOSES Study
Pre- Exp- Post- Blood Tracking Form

A1. Subject ID Label:

A2. Visit: ☐PRE1 ☐EXP1 ☐POS1
☐PRE2 ☐EXP2 ☐POS2
☐PRE3 ☐EXP3 ☐POS3

A3. Collection & Processing Date: __ __ - __ __ __ - __ __ __ __

B1. Details:

Collection					Processing
Tube	**Yes**	**No**	**Reason (if N) / Comments**	**Time**	
Discard					
Platelet Activation/MP-Tissue Factor and vWF multimers (Blue, #1)	☐	☐			
Soluble markers (Blue, #2)	☐	☐			
Archive (Blue, #3)	☐	☐			
CBC/differential/platelet (Lavender, #4)	☐	☐			

Tube #1	**Yes**	**No**	**Reason (if N) / Comments**
To Flow Cytometry Lab	☐	☐	
MP-TF tube #1	☐	☐	
MP-TF tube #2	☐	☐	
MP-TF tube #3	☐	☐	
MP-TF tube #4	☐	☐	
MP-TF tube #5	☐	☐	
vWFM tube #1	☐	☐	
vWFM tube #2	☐	☐	
vWFM tube #3	☐	☐	
vWFM tube #4	☐	☐	
vWFM tube #5	☐	☐	

Tube #2	**Yes**	**No**	**Reason (if N) / Comments**
For Soluble Markers	☐	☐	

Tube #3	**Yes**	**No**	**Reason (if N) / Comments**
Plasma microtube #1	☐	☐	
Plasma microtube #2	☐	☐	
Plasma microtube #3	☐	☐	

Tube #4	**Yes**	**No**	**Reason (if N) / Comments**
To Clinical Lab	☐	☐	

This form will be entered into the database

MOSES Standard Operating Procedure (SOP)

SOP # 13 – FLOW

TITLE: Immunostaining and Flow Cytometry of Platelets, Microparticles and Conjugates

Version: 1.0 **Directory/file[a]:** //

Author: Mark Frampton **Signature:** **Date: June 5, 2012**

Revised by: **New version[b]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New version[b]:** **Date:**
Revisions/additions:

Signature:

Revised by: **New versionb:** **Date:**
Revisions/additions:

Signature:

ANNUAL REVIEW[c]

Signature	**Date**

SOP UPDATED TO VERSION [d]

Explanations: [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. <u>If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!</u>

[c] Annual review does not change the version of the SOP.

[d] Change the version # as necessary.

IMMUNOSTAINING AND FLOW CYTOMETRY PLATELETS, MICROPARTICLES AND CONJUGATES

1. SCOPE and APPLICATION

The scope of this document is to provide a description of the procedure for processing and analyzing the blood for flow cytometry. Specifically

Platelets will be assayed and counted both in their resting and activated states. In addition, to counting, fluorescence intensities will also be measured. Microparticles of various types (platelet and endothelial) will also be counted. Microparticles generally increase with activation of the cell of origin. Increases in platelet-leukocyte conjugates are generally viewed as an indication of increased thrombotic risk and will also be measured.

2. PREREQUISITES

The SOP assumes familiarity with the operation of flow cytometer. The following subsections provide a list of equipment and supplies needed as well as required training needed to operate the instrument.

2.1 Equipment

1. BD LSRII Flow Cytometer using Blue 488 laser 515/20 nm band pass, Red 633 laser 620/20 nm band pass, and Green 532 laser 575/25 nm band pass options.

2.2 Material and supplies

1. **Becton Dickinson Biosciences**
   2350 Qume Drive, San Jose, CA 95131, (877-232-8995)
   - CD42b FITC (# 555472) lot 88692
   - CD14 PE (#345497) lot 19020
   - CD31 PE (# 340297) lot 12937
   - CD62E PE (#551145) lot 70065
   - CD62P PE (#348107) lot 17481
   - CD142 PE (#550312) lot 09356
   - CD154 PE (#340477) lot 85313
   - CD45 APC (#340943) lot 22350
   - CD42b APC (#551061) lot 12588
   - SORP-NIST size beads (special order)

2. **Spherotech Inc.**
   27845 Irmalee Circle**,** Unit 101**,** Lake Forest, IL 60045**, (**800-368-0822)
   - AccuCount Beads (Spherotech # ACBP-20-10 special order) Notify the Rochester site of the precise bead count for your lot (see the product sheet).
   - Rainbow Beads (Spherotech #RCP-60-5) Notify the Rochester site of your lot number.

3. **General suppliers (Fisher Scientific or other)**
   - TRAP -6 (Bachem H-8365)
   - HEPES: 1M (Gibco/Invitrogen #15630-106)
   - Miscellaneous analytical grade chemicals
   - 0.1 µm 150 ml filter units (Nalgene #565-100) or 0.1 MillexVV (PVDF) syringe filters (both are sterile packaged and do not require pre-wetting).
   - 4 ml amber glass sample vials (Wheaton #224982 or similar)
   - Sterile, capped polystyrene flow tubes (Falcon # 352058) or similar

- Adjustable pipettors and sterile tips

4. **Stock Solutions:**
   a) **Dulbecco's Phosphate Buffered Saline with azide (1 liter)**
   (This buffer is used only for making antibody stocks. It can also be purchased as a concentrate.)
   0.2 g KCl
   0.2 g KH_2PO_4
   8.0 g NaCl
   2.16 g Na_2HPO_4
   10 ml 10% NaN_3 (made in PBS, keeps in refrigerator for 1 year)
   Bring to 1 liter with distilled water and adjust to pH 7.4.

   b) **HEPES Buffered Saline** (1 liter)
   0.15 M NaCl (8.766 g)
   0.01 M KCl (0.373 g)
   0.001 M $MgSO_4$ (0.120 g)
   0.01M HEPES (10 mL of manuf.stock (1.0 M))
   Bring to 1 liter with distilled water
   Adjust to pH 7.4 (usually a few drops of 5 N NaOH)
   Filter through 0.1□filter into sterile bottle and refrigerate. Good for 2-3 weeks.
   Note: Sufficient quantities for the day's use must be re-filtered through a 0.1µm **filter to insure clean microparticle data.**

   c) **TRAP-6 Stock (0.015M)**
   Add 445 µl HEPES buffer to the bottle containing 5 mg of TRAP-6. Vortex until dissolved. Dispense in 10 ul aliquots and store in -20°C freezer.

   d) **TRAP-6 Working Solution**
   Add 790 µl of HEPES buffer to the 10 ul aliquot, vortex and keep on ice for the day's use.

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATION

Standard BSL-2 procedures for handling of human blood samples should be followed.

4. PROCEDURES

4.1 Preparation of Antibody Cocktails

Antibody cocktails should be prepared for individual tubes as indicated in the Table below. Antibody titers will be initially determined by the MOSES Flow Core at the University of Rochester for the lot numbers listed above. Other sites may adjust titers as needed due to instrument variations. As lot numbers expire, each site will be responsible for cross-over and adjustment of titers.

Once titered, these amounts are combined in the correct proportions so that 30 ul of the combination is used for each stain tube (60ul in tube #4 and 5, see table). The remainder of volume is made up with PBS/azide.

In tubes #7 and 13, TRAP is not included in the antibody cocktail and is added separately at the time the assay tubes are set up. It is **<u>not necessary</u>** to adjust the total volume to 30 µl in these tubes due to the addition of TRAP.

Note: Since the antibody combinations are made up in bulk, it would be unnecessarily bothersome (and prone to error) to make up the Ab combinations with a different volume to take into account the TRAP additions. Since we are not adjusting for each subject's platelet count anyway, the titer should be more than sufficient to accommodate this. The concentration of CD42b drops 0.1%, CD62P drops 0.2% when the total volume shifts from 80 to 81 ul. In the tube used for compensation, it is irrelevant, since we're only looking to get a truly positive population to gate on.

Sufficient amounts of each combination can be made up in bulk and stored refrigerated (do not freeze), in sterile brown vials to last no longer than 6 months. This enables ease of daily setup and better consistency than trying to aliquot out microliter quantities to each tube. Multiply the individual titers listed below by a convenient amount for your exposure schedule.

Current titers at the MOSES Flow Core at the University of Rochester yield these combinations for each tube:

Tube #	Diluted Blood	Antibody Cocktail	TRAP	Antibody Cocktail Combinations				Purpose
				FITC	APC	PE	PBS/azide	
1	*	*	*	3 ml filtered HEPES buffer				Contamination check
2	*	*	*	1 drop NIST in 0.5 ml filtered HEPES buffer				MP gating
3	50 ul	*	*	No Antibodies, unstained cells			30 ul	Comp neg & FMO for 6,7,8
4	100 ul	60 ul	*	*	15 ul CD45	6 ul CD14	39 ul	FMO for #2
5 (count) [a]	100 ul	60 ul	*	15 ul CD42b	15 ul CD45	6 ul CD14	24 ul	Conj
6 (count) [a]	50 ul	30 ul	*	7.5 ul CD42b	*	15 ul CD62P	7.5 ul	Plat, MP
7 (count) [a]	50 ul	30 ul	1 ul	7.5 ul CD42b	*	15 ul $CD62P^{trap}$	7.5 ul	Stim plat, MP
8 (count) [a]	50 ul	30 ul	*	7.5 ul CD42b	*	7 ul CD31	15.5 ul	Plat, MP
9 (count) [a]	50 ul	30 ul	*	*	*	5 ul CD62E	25 ul	Plat, MP
10 (count) [a]	50 ul	30 ul	*	*	*	20 ul CD142	10 ul	Plat, MP
11 (count) [a]	50 ul	30 ul	*	*	*	20 ul CD154	10 ul	Plat, MP
12 [b]	50 ul	30 ul	*	7.5 ul CD42b	*	*	22.5 ul	Comp & FMO for 3,4,5
13 [b]	50 ul	30 ul	10 ul	*	*	15 ul $CD62P^{trap}$	15 ul	Comp & FMO for 3,4,5
14 [b]	50 ul	30 ul	*	*	7.5 ul CD42b		22.5 ul	Comp
15	NA	*	*	1 drop Rainbow in 0.5 ml HEPES buffer				Calibration

[a] Add 50 ul Accu-count beads to Tubes 5 thru 11 **AFTER** staining and prior to acquisition on the cytometer.
[b] Tubes 12 thru 14 need to run only at the baseline exposure days for compensation or FMO purposes.

It is critical that all buffers and sample tubes be at room temperature before adding blood as platelets will release microparticles if cooled.

4.2 Handling of Blood Samples at the Collection Site

Prior to collection of blood: Fresh filter HEPES working buffer using a 0.1 um syringe filter and allow to come to room temperature. Add 1800 µl of the filtered HEPES to a sterile tube and bring to the collection site. If not already done, prepare the working TRAP solution.

At the collection site: With 5 minutes of the blood draw, Add 200 µl citrated blood to the 1800 µl HEPES buffer, while submersing the pipette tip under the buffer. Do not layer the blood at the bottom of the buffer as it will not mix. Mix only with a gentle tap. Keep at room temperature. **Note:** It is very important that all blood samples be **handled gently** and processed **as soon as possible**. The percent platelet activation increases slightly with time. Be consistent in the time lag before processing.

4.3 Setting up the Assay Panel for a Run

4.3.1 Panel Set Up

1. Fresh filter approximately 50 ml of HEPES buffer.
2. **Prior to collection of blood** set up panel tubes for Flow cytometry assay and add appropriate volumes of reagents and antibody cocktails to sample tubes as described here and in the table above. If panel tubes are set up in advance and have been refrigerated, allow them to warm to room temperature before using.
3. Place 3 ml of filtered HEPES buffer in tube #1. It is essential to use 3ml since you will use this tube to rinse between samples, which are filled to 3 ml.
4. Place 0.5 ml of filtered HEPES buffer in #2 for later addition of NIST beads
5. Add 0.5 ml of HEPES buffer in #15 for later addition of Rainbow beads.
6. Prepare panel **tubes 4 and 5 with 60 ul**, **tubes 3 and 6 through 14 with 30 ul** of appropriate antibody cocktail (see table).

   (Be sure to place the microtip at the bottom of the staining tube before releasing. Neither the antibodies nor the blood will flow completely down the side of the tube.)
7. Add 1 µl TRAP to tube #7 and 10 µl to #13.

Note: At this point, wrap the tube rack in foil. Refrigerate if not used within 15 minutes. Make sure to warm to room temperature before proceeding to the next step (staining).

4.3.2 Staining

1. Add 100 µl of the diluted blood into tubes #4 and 5. The force of this entry is all the mixing it gets, further agitation causes microparticle release.

2 Add 50 µl diluted blood into tubes #3 and 6-14.

3 Cover with foil and let incubate for 20 minutes at room temperature.

4 After incubation, add 3 ml of filtered HEPES buffer to tubes #3-14 (the sample tubes containing blood).

5 **<u>Vigorously</u> vortex the AccuCount bead bottle.** Add 50 µl of AccuCount beads to tubes #5 thru 11.

6 Add 1 drop of NIST beads to tube #2 and 1 drop of Rainbow beads to tube #15. Cap all tubes. (If caps are not tight, you may use parafilm. The tubes will later be mixed by inversion.)

7 Acquire the samples on the Flow cytometer as soon as possible. These tubes may be held at room temperature in the dark for up to 1 hour before reading with negligible change. Do not refrigerate (platelets will release microparticles).

4.4 Initial Setup of Cytometer (LSR) Template and Labeling Conventions

1. The flow cytometer should be optimized according to site protocols.

2. Use filtered sheath fluid if possible. If your instrument has the FSC PMT option as opposed to the standard diode, use it.

3. The experiment name will use the **study/date**, e.g. MOSES053012. This name will lay hidden when you export the data. The name is not critical but will allow you to file your own data in folders.

4. Rename 'Specimen 1' using the site/subject 5 digit code/time code, e.g. 13218V3. Here 1 is the site code for Rochester, 3218 is the unique subject code. V3 is a time code used only by the flow lab (V3 = pre-exposure sample, 1st visit, V4 = exposure sample 1st visit, V5 = post-exposure sample, V6, V7, V8 are used for the 2nd visit and V9, V10, V11 for the 3rd visit.) These codes will be correlated with the official study codes on the analysis spreadsheets before submission to NERI.

5. Add 15 sample tubes, and enter the lasers and voltages you've previously determined.

6. Open the "Experiment Layout" and fill in the template as follows:

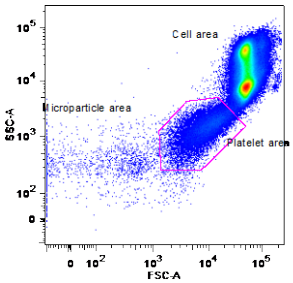
	Label (FITC)	Label (APC)	Label (PE)
Specimen_001	Blue B 515/20 A	Red C 620/20-A	Green E 575/25-A
01	HEPES buffer	HEPES buffer	HEPES buffer
02	NIST	NIST	NIST
03	Unstained	Unstained	Unstained
04		CD45	CD14
05	CD42b	CD45	CD14
06	CD42b		CD62P
07	CD42b		CD62Ptrap
08	CD42b		CD31
09			CD62E
10			CD142
11			CD154
12	CD42b		
13			CD62Ptra
14		CD42b	
15	Rainbow	Rainbow	Rainbow

Note: The labels (Blue B, etc) are preset on our instrument, they may be different on yours which is why it is essential that your template lists the antibodies.

7. Compensation settings should be zero (0%) for all three fluorescent parameters. Compensation will be done with the FLOJO software offline using the platelet area.

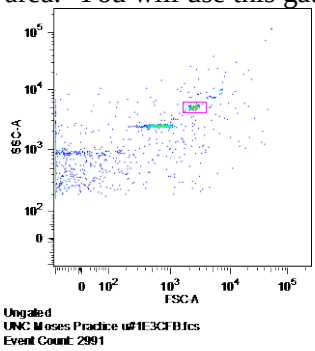
8. Data is collected on FSC (log), SSC (log) settings with the threshold set at 10^2 for both FSC and SSC. All parameters will use **area** rather than height. Height should be collected but will not be used for analysis at this time.

9. Using tube #3, adjust the scatter axes such that all areas of interest are in the collection area. Be sure the entire cell area is enclosed. Set a rough gate on the platelet area.

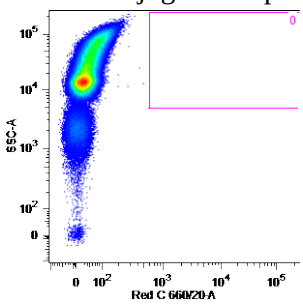


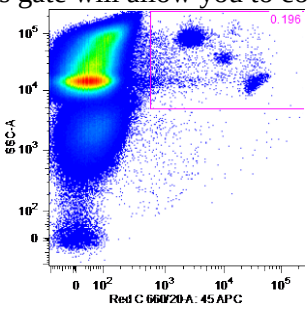
Using a mid-range LOW flow rate, acquire 20,000 platelet events. Note the time elapsed. This should take between 4 and 6 minutes. If longer, slightly raise the flow rate to achieve this window.

10. Using tube #2 (NIST beads) gate the 1u bead area. You will use this gate as a guide to keep settings uniform. (See below for exceptions.)



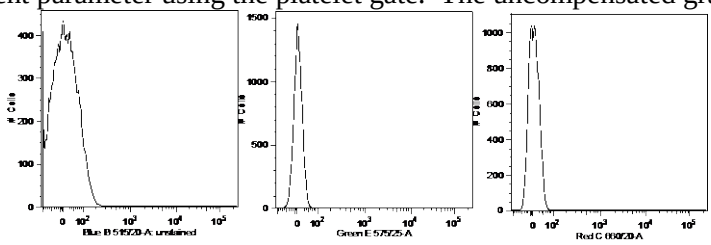
11. Make a plot of SSC-A vs. 45 APC. Gate the CD45+ cell events. This gate will be empty for now. When you have conjugate samples this gate will allow you to count sufficient events in this area.

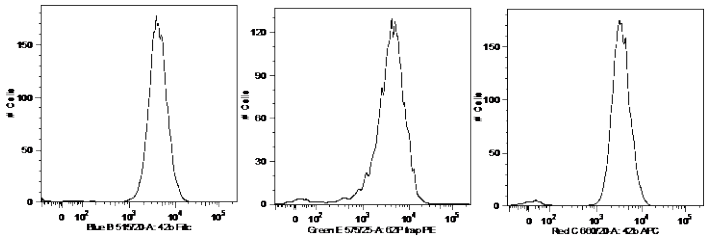




12. While compensations are set at 0%, you need to make sure that the voltages have been set appropriately. Using tube #3 (unstained cells) and 12, 13, and 14 (single stains) check each

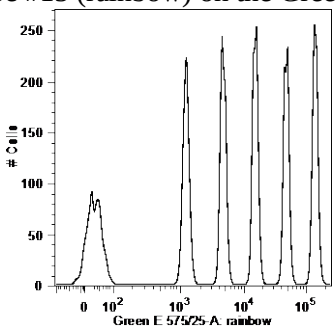
fluorescent parameter using the platelet gate. The uncompensated graphs should look similar





to these.

13. Now check tube #15 (rainbow) on the Green(PE) channel and you should have 6 (six) clearly defined peaks.



If necessary go back and adjust the voltage for this channel, cross-checking with tube #13 (the 62Ptrap stained cells).

14. **Save the template.**

4.5 Regular Cytometer Acquisition for a Sample run

1. Open the acquisition template and check tube #1 (HEPES buffer) to assure that system has been appropriately cleaned. Do not proceed until satisfied. If cleaning is necessary, prepare another tube 1 for use in the panel.
2. Check tubes #2 and 3 to assure that the scatter settings are reasonable. Occasionally a subject will have a greater cell pattern spread and despite the correct NIST bead gate, the scatter will need to be adjusted. It is critical that the entire cell population is on screen.
3. Begin acquiring samples starting with #1 (HEPES buffer). Stop acquisition when pattern at 60 seconds. Counts here will be used to track noise levels.
4. Move to tube #2 (NIST) acquiring 5,000 or more **'total'** events. Rinse with the filtered HEPES buffer tube (#1).

   Note: It is important that remixing of the sample tubes (#3-14) be done only by gently inverting the tube. Do not vortex or shake the tube as this may cause additional microparticles to be released.
5. Remix tube #3 (unstained sample) by inversion and acquire 100,000 '**total**' events while running at **LOW** flow rate (sufficient to get about 10,000 platelet events). Rinse with the filtered HEPES buffer tube (#1).

6. Change the acquisition to collect 5,000 '**45+ events**' and adjust the flow rate to **HIGH**.
7. Acquire tubes 4 and 5, remixing before reading. It will take several minutes to read each tube. Tube 4 is only an FMO, you may stop collection at 2,000 events if you desire. Rinse with the filtered HEPES buffer tube (#1) between each sample.
8. Before acquiring tube #3, change the acquisition to 20,000 'platelet' events. Coincident events in the MP region can be a problem, so run on **LOW** flow rate. It will take several minutes to read each tube. Do not exceed 6 minutes. If the subject has a low platelet count, it is safer to stop reading at 6 minutes and analyze fewer events than to read with a HI flow rate and compromise the singularity of each event.
9. Continue reading through #14. (FMO tubes (12,13,14 may be stopped at 5000 events, if desired).
10. Tube 15 has no cells so stop acquisition at 10,000 '**total'** events.
11. Export files ad FCS 3 files, NOT as an "Experiment" file. (this is important since tube-specific labeling info will not transfer to FloJo if exported as a FACSDiva Experiment file.)

4.6 Recordkeeping

Upload files in FCS 3 format for analysis to the secure dropbox folder "Ozone Study/Flow Cytometry Data/individual site" using your site-specific code. Maintain a backup copy for your lab. The core laboratory will download the files and conduct the analyses as described in their SOP. The results will be transmitted to NERI and entered in the ADEPT system.

5. QUALITY CONTROL

Follow calibration and maintenance procedures for flow cytometer as recommended by the manufacturer.

6. QUALITY ASSURANCE

HEI may request lab records and conduct audits of the individual laboratories and core laboratory.

7. REFERENCES

Li,N. Goodall, AH and P. Hjemdahl " A sensitive flow cytometric assay for circulating platelet-leukocyte aggregates" Brit J Haemat (1997) 99: 808-816

Janes, SL, Wilson DJ. Chronos, N and AH Goodall "Evaluation of Whole Blood Flow Cytometric Detection of Platelet bound Fibrinogen on Normal Subjects and Patients with Activated Platelets" Throm & Haem (1993) 70(4): 659-666.

Ault, KA "The Clinical Utility of Flow Cytometry in the Study of Platelets" Dartmouth Cytometry Course, July 2000.

Goodall, AH and J Appleby "Flow-cytometric analysis of platelet-membrane clycoprotein expression and platelet activation" in Platelets and Megakaryocytes, Vol.1. Gibbins, JM and Mahaut-smith MP (eds) Humana Press 2004

Jimenez JJ, Jy W, Mauro LM, Horstman LL, Bidot CJ and YS Ahn "Endothelial Microparticles (EMP) as Vascular Disease Markers" Adv.Clin.Chem. 39, 131-157 (2005).

MOSES Standard Operating Procedure (SOP)
SOP # 21 ECG - Screening

TITLE: 12-Lead Electrocardiogram Recording during Screening Visit

Version: 1.0 **Directory/file[a]:** //

Author: MOSES Investigators **Signature:** **Date: March 23, 2012**

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

ANNUAL REVIEW[c]

Signature	**Date**

SOP UPDATED TO VERSION [d]

Explanations: [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!

[c] Annual review does not change the version of the SOP.

[d] Change the version # as necessary.

12-LEAD ELECTROCARDIOGRAM RECORDING DURING SCREENING VISIT

1. SCOPE AND APPLICATION

The purpose of this SOP is to provide guidelines to record a standard 12-Lead Electrocardiogram (ECG) during the screening visit. It is important to obtain an acceptable recording in order to properly assess the health of the subject's heart. The purpose of the procedure is to determine whether the subject has a normal ECG and should be included in the study.

This document should be reviewed annually. Any revisions should be properly documented in the cover form of this SOP.

2. PREREQUISITES

This SOP provides background information of the electrocardiogram machine and instructions to record a standard 12-Lead ECG. The following subsections provide a list of equipment and supplies needed to take the measurement, as well as required training needed to operate the instruments.

2.1 Equipment and supplies

UNC

Marquette MAC PC (Marquette Electronics Inc., 8200 West Tower Avenue, Milwaukee, Wisconsin 53223, 414/355-3790)

UCSF

Marquette Mac 15 with 12SL Analysis Program Statements (Marquette Electronic Inc., 8200 West Tower Avenue, Milwaukee, Wisconsin 53223. 414/355-3790)

UR

Philips PageWriter Trim III (Philips Medical Systems, 3000 Minuteman Rd Andover, MA 01810

Common

1. ECG Cables
2. ECG Pads
3. Alcohol Pre Pads
4. Razor Blade
5. Adult Gown (optional)

2.2. Training

The operator conducting the test should be trained in operating the instrument and performing the test in a standardized manner. The test should be performed in a quiet environment.

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

1. Expose the subject's skin briefly to prepare and place electrode pads and electrodes.

2. For female subjects, place leads V3-V6 under the breast rather than on the breast.
3. Have the subject lie quietly and comfortably during the test.
4. Do not immerse any part of the instrument in water.

4. PROCEDURES

4.1 Settings:

UNC

1. Power on
2. When the system boots up, it requires the operator to press any key to continue.
3. System self- test: done automatically
4. Follow displayed instructions to enter subject data

UCSF

Main Power switch: "O" is off. "I" is on.

1. Power on
2. System self- test:
    1. Displays software program using "Pgm 008A Self Test 12"
    2. Then, displays "A1 FD M Ram = 512K"
        a) 512K is the memory amount available.
    3. Then, displays "Task V1+II+V5… " or "Most recent ECG not saved on diskette…"
    4. Follow displayed instructions

UR

1. Power on.
2. System self-test completes automatically.
3. Push Patient ID button to enter subject data.

4.2 Subject Preparation

1. Describe ECG procedure to subject.
    a) Inform subject of the nature of electrode placement and receive permission to proceed and touch subject.
    b) Have subjects remove jewelry and clothing from the waist up and change into a gown, cover the chest with blanket or towel, particularly with female subjects.
2. Expose the skin only as necessary to prepare and affix the electrodes.
3. Have subject lie on his/her back.
4. Prepare skin and place electrodes as described below in the "Chest and Limb Electrode Placements Sites."
5. Instruct the subject to lie still, breathe regularly, and not to cross his/her arms and legs, and not to speak during the exam.

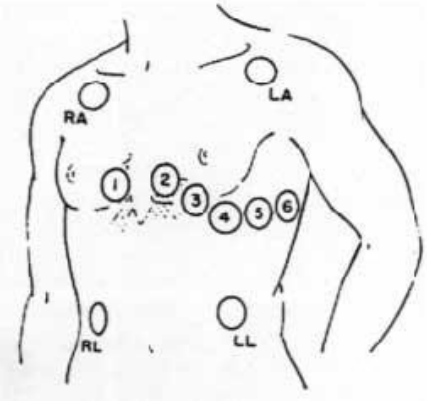
4.3 Skin Preparation

1. Clean the skin where the electrode pads will be placed with an alcohol pad to

remove any excess oil from the skin.

2. Rub the skin well to remove the epidermal skin layer.
3. Shave the hair from each electrode site as needed to improve conductivity, adherence to skin and to minimize discomfort when removing the electrode.
4. Place an electrode on the prepared site.

4.4 Chest and Limb Electrode Placement Sites



Leadwire	Position
V1	Fourth intercostal space at the right border of the sternum.
V2	Fourth intercostal space at the left border of the sternum.
V3	Midway between locations V2 and V4.
V4	At the mid-clavicular line in the fifth intercostal space.
V5	At the anterior axillary line on the same horizontal level as V4.
V6	At the mid-axillary line on the same horizontal level as V4 and V5.
RA and LA	Right and left arm just below the clavicle, or midway between the elbow and the shoulder of both limbs.
RL and LL	Right and left lower abdominal quadrant, or above ankles or upper leg as close to the torso as possible.

1. To locate the V1 position (fourth intercostal space):
2. Place your finger on top of the jugular notch.
3. Move your finger slowly downward about 1.5 inches (3.8 centimeters) until you feel a slight horizontal ridge or elevation. This is the "Angle of Louis" where the manubrium joins the body of the sternum.
4. Locate the second intercostal space on the right side, lateral to and just below the Angle of Louis.
5. Move your finger down two more intercostal spaces to the fourth intercostal space which is the V1 position.

4.5 Input subject information

UNC

1. The first field is for last name, enter "Moses"Study ID number, age, gender, and race (from Home and Health questionnaire), height and weight (from Vital Signs and Blood Pressure Screening Data Form)
2. The second field is for first name, enter "screen"
3. The third field is for ID, enter the subject number.
4. Toggle through the remaining fields, entering height and age.

UCSF

1. Study ID number, age, gender, and race (from Home and Health questionnaire), height and weight (from Vital Signs and Blood Pressure Screening Data Form)

UR

1. Push Patient ID button.
2. Enter MOSES Study ID in ID box.
3. Turn trim knob to move cursor to next box.
4. Leave Last Name box and First Name box blank
5. Push trim knob to check male or female in Gender box.
6. Enter age into Age box.
7. Press ALT O
8. Enter physician name.
9. Enter operator initials in Operator ID box.
10. Push Enter key.

4.6 Record ECG

4.6.1 Obtain a resting, supine ECG (baseline)

UNC

1. Press the "12-lead" key and display
   a) "Acquiring data" message is displayed when ECG recording. The software will prompt the user if there are any bad leads or other artifacts to allow for resolution prior to analyzing ECG.
   b) "Analyzing ECG"
   c) "Printing Reports"
2. Storing ECG
   This is automatically done. When the disk is full, the software prompts you for permission to delete. This must be done when the disk is full.

UCSF

Confirm a diskette is in the drive

Press the "RECORD ECG" key

1. Automatically done
   a) "ECG Acquisition Complete" message is displayed when ECG recording completed.

b) "Analyzing ECG"
c) "Printing Reports"

2. Storing ECG is automatically done

a) "Processing ECG For Storage"
b) "Write to Diskette"
c) "Storage to Diskette Complete. Type any key to Continue"

UR

1. Preview live ECG on screen for good electrode quality (green color).
2. If quality okay, press AUTO button.
3. 12 lead ECG is acquired and displayed on screen.
4. Press Enter key to record, save, and print ECG for physician's review.
5. Push Copy button to print second copy for CRC.

4.7 After the procedure

1. Remove the electrodes and any remaining gel.
2. Subjects can carry on as usual. There are no restrictions.
3. Show the ECG printout to the physician on call.
4. **Physician should evaluate the ECG:**
   a) If normal the physician should sign it.
   b) If any abnormalities, such as T-segment depression, arrhythmias, or others abnormalities are detected in the ECG printout, the ECG should be reviewed and signed by a cardiologist.

4.8 Corrective action

In consultation with the cardiologists, the study physician will make the decision whether the subject should be included in the study.

4.9 Recordkeeping

File the signed ECG printout in the subject file.

5. QUALITY CONTROL

Perform routine maintenance and safety checks as recommended by the manufacturer of the device

UNC. The device receives an annual safety check.

6. QUALITY ASSURANCE

As part of the QA, HEI or NERI may request a copy of the ECG printout and the maintenance/safety checks records

7. INFECTION CONTROL

1. Dispose of single use hypo-allergenic electrodes.
2. Wipe the subject's skin and reusable electrodes with a tissue or damp cloth to clear them of electrode paste/gel.
3. Clean the instrument exterior surfaces with a clean, soft cloth and a mild dishwashing detergent diluted in water.
4. Dry the instrument exterior surfaces with a clean cloth or paper towel.
5. Hand washing between subjects.
6. Practice universal precautions.

8. REFERENCES

1. Marquette MAC PC Analysis System Operator's Manual. Marquette Medical System.

MOSES Standard Operating Procedure (SOP)

SOP # 22- HOLTER

TITLE: High Resolution Holter Recording

Version: 1.0 **Directory/file[a]:** //

Author: W. Zareba **Signature:** **Date: June 8, 2012**

Revised by: M Costantini **New version[b]: 2** **Date: October 5, 2012**

Revisions/additions: Changed the process for informing the Core Laboratory that a Holter card shipment is initiated (Section 4.5).

Signature:

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

ANNUAL REVIEW[C]

Signature	**Date**

SOP UPDATED TO VERSION [d]

Explanations:

[a] Incorporate the version of SOP into the file name, e.g., Holter V1 sop.

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. <u>If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!</u>

[c] Annual review does not change the version of the SOP.

[d] Change the version # as necessary.

HIGH RESOLUTION HOLTER RECORDING

1. SCOPE and APPLICATION

The purpose of this SOP is to describe the procedure for Holter ECG recording continuously over a prolonged 24-30 hour period for the purpose of quantifying cardiac arrhythmias, heart rate variability, ST segment and repolarization changes.

2. PREREQUISITE

a. Equipment and Supplies
   - Compact Flash card
   - H12+ 24-hour Digital Recorder
   - AA Alkaline or AA Lithium battery
   - Monitoring electrodes
   - LeadForm Patient Cable available in Standard and Large sizes
   - Carrying Case
   - Skin prep supplies (not shown)

b. Training Requirements
   Enrolling sites will be trained to prepare and apply the Holter recorder and to transfer data to the ECG Core Lab.

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATION

Skin preparation is the most important action before applying the Holter – see section 4.1 – Hook up information

4. PROCEDURES

The Holter recorder will be programmed to operate continuously in a high-resolution mode for up to 30 hours. During this long-term recording, there will be repeated 10-minute periods recorded in resting supine position: at baseline (pre-exposure), immediately after exposure, 3 hours after exposure, during night hours, and next morning. The 10-minute recordings will be started at the time indicated per study protocol. The purpose of the resting supine recordings is to obtain clean uninterrupted by noise ECG to be used to quantify several parameters of interest.

All testing will be done using the Mortara H12+ recorder. Instructions on how to use the recorder and electrode hook-up are described below.

4.1 Hook-up Instructions

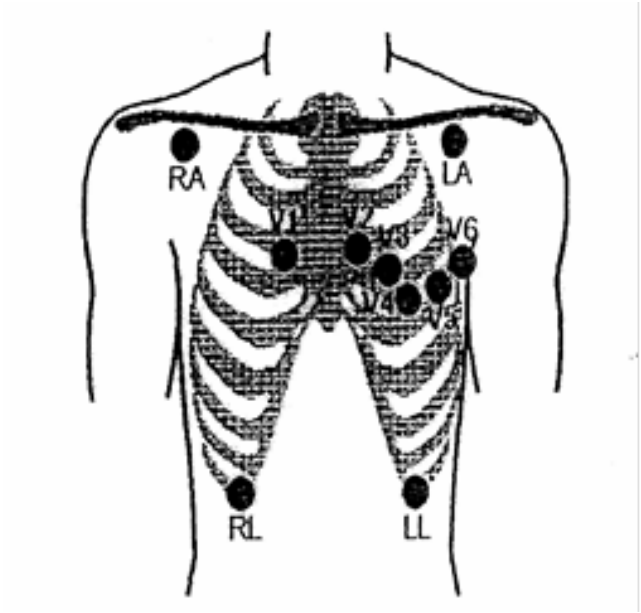
1. Attach electrodes to the 12 lead wires for the recorder.

2. Perform appropriate skin preparation as follows:
   a. Shave hair from the skin at electrode site
   b. Wipe skin with alcohol pads

c. Abrade the electrode site with the abrasive pad (found inside hook-up kits)

3. Apply electrode wires to the skin as follows:

- RA or R - Four inches away from the right side of the sternum, below the clavicle
- LA or L – Four inches away from the left side of the sternum, below the clavicle
- V1 or C1 - 4th intercostal space, right of sternum
- V2 or C2 - 4th intercostal space, left of sternum
- V3 or C3 - between V2 and V4
- V4 or C4 - 5th intercostal space, left midclavicular line
- V5 or C5 - 5th intercostal space, anterior axillary line
- V6 or C6 - 5th intercostal space, left midaxillary line
- RL or N - 8th intercostal space, right anterior axillary line
- LL or F - 8th intercostal space, left anterior axillary line



4.2 Attaching Subject Cable

Insert the connector block into the side of the H12+ monitor. To do this, you must place the cable connector in a completely straight position, parallel to the monitor. It is very easy to bend those gold pins located in the inside of the monitor so caution is need when connecting the cable. Do not force the cable head into the monitor.

4.3 To Insert Battery and Flash Card

Open the top cover of the H12+. Insert the compact flash card and a new AA battery. A battery will be included in the electrode kit that has been purchased. To open the battery door, hold the latch down that is located on the side of the monitor, then, slide the battery door until it stops. The battery goes in the circular opening with the positive end up. At this time you may also insert the flash card. There is a slot for a 512mb card to be placed. Push the card down until it locks in place. The card will only go in one way. To remove this card, simply press the small black button that is located directly next to the flash card slot. This will then eject your flash card. To close the cover, place the battery door on the H12+ and slide the door in the opposite direction until the door snaps in place.

4.4 Using the Keypad

Three keys are available on the right for navigating through the LCD screens and for entering the subject ID and event markers during the recording.
These include the Up/Right, Down, and Enter keys.
During Subject hook-up, the Down and Up keys are used to scroll through the Main menu options, To enter the subject ID and to set the time/date and language.
The Enter key is used to select a Main menu option and sub-menus displayed on the LCD screen.

4.4.1 Main Menu options

The main menu includes the following options.

1. Lead Check
2. Display ECG
3. Enter ID
4. Record
5. Configure

1. **LEAD CHECK (checking impedance)** is the first option displayed on the LCD screen after patient hook- up.

2. From the Main Menu, scroll to **LEAD CHECK** using the **Down** or **Up** key. Press the Enter key to select this option. A graph will appear showing all the leads and their impedances. A full bar means optimal lead electrode to skin contact quality. The bar should be at or above the center line. If not, re-prep and replace the electrode. Press any key to return to Main Menu.

3. **DISPLAY ECG** is used to visually inspect the leads before starting a recording. Press the enter key to select this option. After verification of all leads, press the **Enter** key to return to the Main menu.

4. **ENTER ID** is used to enter the subject ID in the subject record before starting a recording. From the Main menu, scroll to ENTER ID using the Down or Up key. Press the Enter key to select this option.

5. To enter the subject ID, the cursor is moved to the desired letter or digit, and then selected by pressing the Enter key. The cursor is located initially in the upper-left corner of the screen over the number 0.

6. To move the cursor one letter or digit to the right on a line, press the Up key. To move the cursor down one line, press the Down key.

7. If the cursor is on the bottom line, press the Down key moves the cursor to the top line. When entering the subject ID, the Up key is used to move the cursor to the right. The cursor cannot be moved in the left or up direction.

8. To enter a space, move the cursor to the last line and position the cursor over the blank space following the last letter. Press the Enter key to input a space in the subject ID.

9. To delete a letter or digit in the subject ID, position the cursor over Del on the bottom line. Press the Enter key delete the last letter or digit entered in the subject ID.

10. To end subject ID entry, position the cursor over End on the bottom line. Press the Enter key to store the subject ID.

4.4.2 How to Set Date, Time, and Date Format

1. Configure (see diagram below) is used to set the current date and time, the date format and language (set to English). These settings typically are set before the initial subject recording on the H12+ and do not need to be set on a per subject basis. Time zone changes or a year change requires date and/or time to be re-configured.

2. Press the Down (arrow) key to scroll to the **CONFIGURE** option. Select the **CONFIGURE** by pressing the **Enter** key. Select **DATE/TIME** and if necessary, set the minutes, hours, day, month, year, date format, and done (you need to press the enter key at DONE to proceed to the next step)

3. Use the **Down** or **Up** key to scroll to the desired option and press the **Enter** key. When setting the date or time, increase the value by pressing the **Up** key.
   To decrease the value, press the **Down** key. When the correct value is displayed on the screen, press the **Enter** key. A **SET SECONDS** option does not exist. If you want to reset the seconds, set the minutes first.

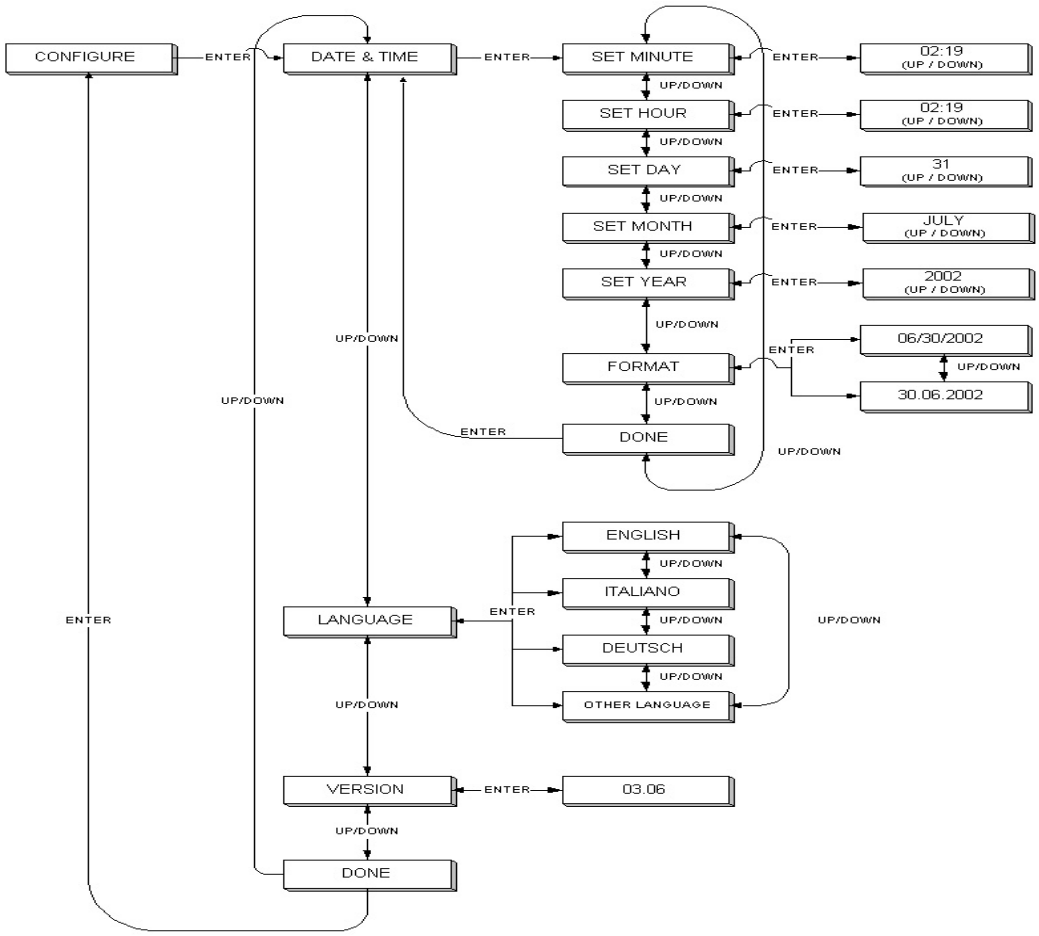
   Press the **Enter** key at the instant you want the seconds to be reset.

4. **FORMAT** provides two options for the date format: month/day/year or day/month/year. From the configuration menu, scroll to **FORMAT** using the **Down** or **Up** key.

   Scroll from one option to the other by using the **Down** or **Up** key. Press the **Enter** key to select the desired format and return to the **Configure** menu. To return to the **Configure** menu, scroll to **DONE** using the **Down** or **Up** key and press the **Enter** key.

5. Check that the date and time displayed are correct.

Diagram of Configuration Settings



4.4.3 How to Start a Recording

Make sure that the time on the Holter Clock matches the time on the lab clock. If it does not, make the proper adjustments so they read the exact same

time. In any event, the time recorded on the checklist must be the actual time that appears on the Holter

Use the **Up** or **Down** keys to scroll through the **Main Menu** until **RECORD** is displayed. Press the **Enter** key to select **RECORD.**

During normal operation, the current time is displayed in the middle of the screen for the entire 30-hour recording. If at any time, the subject notices that the time is not advancing, do not remove the card and continue the entire 30-hour recording.

If during the recording the battery door is opened or RECORD is selected in error prior to the desired start of recording, a new formatted card will need to be installed.

4.4.4 Supine Recordings

The subjects should be lying down in a quiet room for 10 minutes as pre-specified by the protocol:

- 10 minutes before the start of the exposure
- 5 minutes after the end of the exposure
- 3 hours after at the end of the exposure
- 30 minutes after the subjects arrives to the clinic on the post-exposure day.

4.4.5 Ending a Recording Session

At the end of the recording session, remove the battery. After doing this you may remove the electrodes and lead wires. **Do Not** remove the lead wires prior to removing the battery. This will cause artifact in the recording that may affect any analysis of the recording.

4.4.6 Troubleshooting

The following table lists messages and solutions that may occur during patient hook-up and recording:

Message	Solution
'LOW BATTERY'	Replace existing battery with a fully charged battery.
'CARD ERROR'	Flash card error. Contact per study instructions.
'NO CARD'	Flash card not detected in the card slot. Install a formatted card.

'CARD NOT FORMATTED'	No H12+ volume label found on card. Contact per study instructions.
'CARD NOT ERASED'	Data detected on flash card. Reformat the card using H-Scribe software. Contact per study instructions.
'Reformat card with FAT16 / FAT not FAT32'	Compact Flash card has been formatted with FAT32 (which is not supported). Reformat the CF card with FAT16 or FAT. Using H-Scribe software to reformat the CF card will not correct the error. Contact per study instructions.
Any single lead (RA or LA or V1 …)	Single lead fail. Check if the lead wire is off or the electrode needs to be replaced.
Any lead combination (RA, V1, V3…)	More than one or all leads in fail. Check the lead wires and electrodes.
Recording ends early	May be due to battery quality or a large amount of artifact during recording which draws more power. Check the data recorded by acquisition at H-Scribe.
Recorder inoperable	Contact per study instructions.

4.5 Record keeping and shipping

Once a subject is enrolled, the core laboratory technician (Att Patricia Negri) should be informed by e-mail of the projected plan for collecting the Holter data.

Once a recording session ends, the Holter data card should be shipped to the University of Rochester core lab for analyses within 24 business hours.

With each Holter data card, the center must send

- the appropriate shipping form and
- daily checklists (that is the Exposure Day and the Post-exposure Day checklists) indicating the times of the procedures.

If the shipping form and the checklists are not sent along with the card, the center will be contacted, and the card will be analyzed only after the shipping and daily log forms are received. The ECG core lab keeps a log of the Holter cards received by the centers.

Remove the flash card immediately and mail it by FedEx to:

Heart Research Follow Up Program,
University of Rochester Medical Center
Attention: Kelley Cenamme
265 Crittenden Blvd., CU 420653,
Rochester, NY 14620

Phone: 585-273-3342
Fax: 585-273-5283
E-mail: Kelley.Cenname@heart.rochester.edu

At the time of shipping, the ADEPT Data Manager system will notify by e-mail the following core laboratory staff that a shipment has been initiated:

Kelly Cenamme: Kelley.Cenname@heart.rochester.edu
Betty Mykins: Betty.Mykins@heart.rochester.edu
Michal Weiss: Michal.Weiss@heart.rochester.edu

The results of the analysis of each Holter recording will be completed within 2 weeks from receipt of the card and enrolling centers will be provided with a copy of Holter report sent as PDF by e-mail. The enrolling centers will be informed immediately about any life-threatening cardiac arrhythmias detected on the Holter.

Research output from Holter analyses will be provided in batches to the MOSES Data Management Center (at NERI) according to agreed-upon procedures.

5. QUALITY CONTROL

Recommended Patient Cable Leads Cleaning:

- Do use clean, lint-free cloth with alcohol-free germicidal disinfectant.
- Don't use alcohol or solvents
- Don't use abrasive cleaners or materials

Patient Cable Maintenance:

- Check patient cables for cracks or breakage prior to use.
- Don't use tape on the patient cable
  Tape residue will cause hardening and can introduce cracks
- Patient cables should be stored by looping them loosely
  Don't pull or stretch the cables
  Don't wrap cables tightly

Exterior Visual Inspection

- Check connectors for loose, bent or corroded contact Pins outside the holter
- Inspect covers for warping, surface damage or missing hardware
- Check for any other form of damage

Battery compartment

- Check battery connection terminals for debris

6. QUALITY ASSURANCE

HEI or NERI may request copies of daily log (or checklists) and of the analyses of the Holter recording. HEI may conduct a QA audit of the core laboratory responsible for the analyses of the Hoter recording.

MOSES Standard Operating Procedure (SOP)

SOP # 31 – BAU

TITLE: Brachial Artery Ultrasound to Evaluate Vascular Function (Brachial artery reactivity and Doppler)
Version: 1.0 **Directory/file[a]:** //

Author: Peter Ganz **Signature:** **Date: May 24, 2012**

Revised by: Peter Ganz **New version[b]: 2** **Date: December 23, 2012**

Revisions/additions: Removed nitroglycerin from supply list (2.1.2) Clarified that the "Sweep speed should be set at 25 mm/sec" (rather than 50 mm/sec) (Section 4.2.) Changed text to reflect 25 mm/sec (rather than 50) (Section 4.5.4.) Removed Section 4.6 regarding nitroglycerin and adjusted Record Keeping to become Section 4.6. Removed instructions on nitroglycerin use (Sections 4.3.10 and 4.7.2.) Changed RL to LL for suggested lead placement (4.1.4.) Specified that the core laboratory analyzes only the Doppler flow images for the first 3 full cardiac cycles following cuff release. (Sections 4.5.4 and 4.7.2.) Added comment about blood pressure taken at beginning of procedure (Section 4.4.5) Added comment that extra baseline images can be taken to ensure quality and instructed how to label and save them (Section 4.4.6) Added comment on how to record a repeated procedure (Section 4.7.3) Changed the process for informing the Core Laboratory that a card shipment is initiated: ADEPT sends an e-mail (Section 4.7.5.)

Signature:

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

ANNUAL REVIEW[c]	
Signature	**Date**

SOP UPDATED TO VERSION [d]	

Explanations: [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. If the revision is

major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!

[c] Annual review does not change the version of the SOP.

[d] Change the version # as necessary.

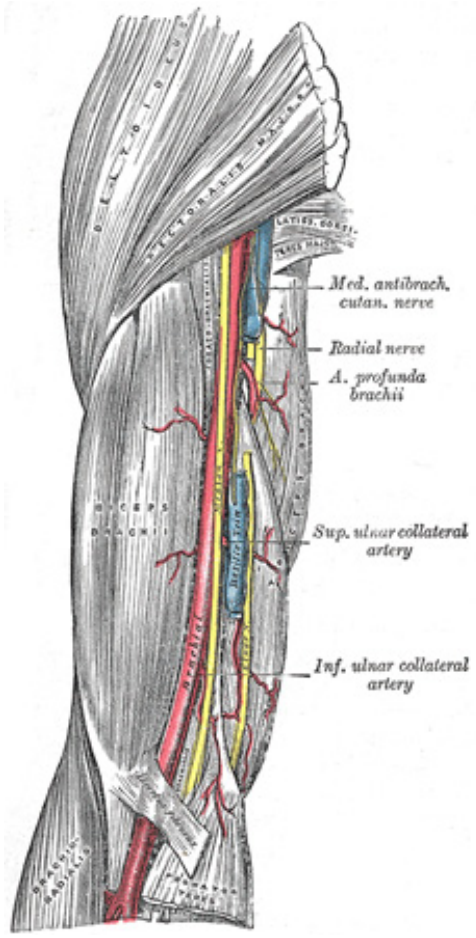
BRACHIAL ARTERY ULTRASOUND TO EVALUATE VASCULAR FUNCTION

1. SCOPE and APPLICATION

This is a test which uses B-mode ultrasound to evaluate endothelium-dependent (flow-mediated) and endothelium-independent (nitroglycerin-mediated) dilation of the brachial artery. Flow-mediated, endothelium-dependent vasodilation is used as a measure of the bioavailability of endothelium-derived nitric oxide. Nitroglycerin-induced, endothelium-independent vasodilation assesses vascular smooth muscle responsiveness to an exogenous nitric oxide donor. Interpreted together, these measurements enable investigators to determine whether an intervention affects endothelial function and/or vascular smooth muscle function.

To assess flow-mediated, endothelium-dependent vasodilation, measurements of the diameter of the brachial artery are taken under basal conditions and during reactive hyperemia following 5 minutes of forearm occlusion. Reactive hyperemia is the transient increase in blood flow that occurs following a period of ischemia. Increased flow is a physiologic stimulus that releases nitric oxide from the endothelium. Ischemia is induced by inflating a blood pressure cuff on the forearm to suprasystolic pressures for 5 minutes. The ischemia causes vasodilation of the downstream resistance vessels. Deflation of the cuff results in increased blood flow through the brachial artery to accommodate the dilated resistance vessels. Flow-induced release of nitric oxide from the endothelium causes the brachial artery to dilate.

Endothelium-independent vasodilation is assessed by measuring the brachial artery diameter under basal conditions and 3 minutes following the administration of sublingual nitroglycerin (0.4mg). This exogenous nitric oxide donor causes the brachial artery to dilate, even in the absence of functional endothelium. Nitroglycerin is not administered to those individuals with significant bradycardia (HR< 60), hypotension (systolic blood pressure <100mm Hg) or to subjects who recently used a PDE-5 inhibitor.



Brachial Artery Anatomy

The brachial artery is the major blood vessel in the upper arm. It commences at the lower margin of the tendon of the teres major, and, passing down the arm, ends about 1 cm. below the bend of the elbow, where it divides into the radial and ulnar arteries. At first the brachial artery lies medial to the humerus; but as it runs down the arm it gradually gets in front of the bone, and at the bend of the elbow it lies midway between its two epicondyles. [Henry Gray (1821–1865). Anatomy of the Human Body. 1918]

In up to 20% of individuals, the brachial artery divides into the

radial and ulnar arteries above the antecubital fossa. In this case it is particularly important to image above the bifurcation.

Artery Wall Anatomy

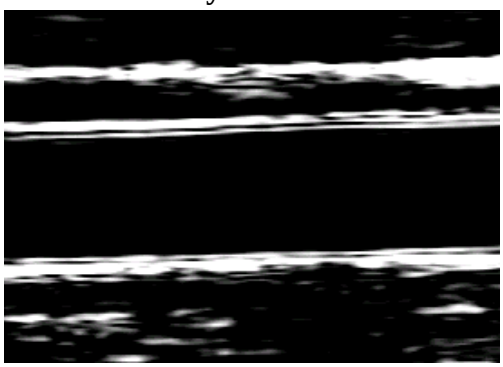
The arterial wall is comprised of three distinct layers – intima, media, and adventitia.

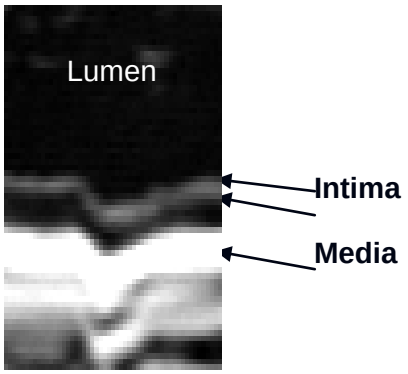
- The inner layer, tunica intima, is composed of a single layer of endothelial cells with a basement membrane.
- The middle layer, tunica media, is a thick layer of smooth muscle cells and collagenous fibers usually arranged in a circular fashion.
- The outermost layer, tunica adventitia, is composed of connective tissue.

Vascular Ultrasound

Duplex vascular ultrasonography comprises gray scale (B mode) imaging and Doppler velocity analysis. Ultrasound images are generated using a pulse echo system. The depth of the tissue interface is determined by the time between pulse generation and returning echo. Each returning echo is displayed as a gray dot on the video screen using a brightness mode (B mode). A two dimensional image is created by combining the reflected echoes into a single display.

Brachial Artery





Doppler Shift

Doppler ultrasound is a technique for making non-invasive measurements of blood flow velocity. To measure the velocity of blood, ultrasound is transmitted into a vessel and the sound that is reflected from the blood is detected. Since the blood is moving, the sound undergoes a frequency (Doppler) shift. This is described by the Doppler equation.

Doppler Shift $F = (2Vf \cos\theta)/c$

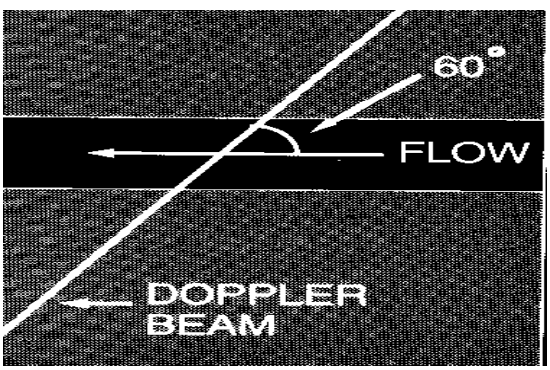
Blood Velocity $V = (cF)/(2f\cos\theta)$

(F = Doppler Shift; V = Blood velocity; f = transmitted frequency; θ = Doppler angle; c = sound velocity)

The Doppler frequency shift is proportional to the cosine of the Doppler angle. The maximum possible Doppler shift will occur when the ultrasound beam is directed straight down the barrel of the vessel. Therefore, a Doppler angle of 0˚ produces the maximum possible Doppler shift because cosine 0˚ = 1. Since cosine 90˚ = 0, a Doppler shift is not produced when the Doppler angle is 90˚. Since cosine 60˚ = 0.5, the most accurate blood velocities are seen when the Doppler angle is 60˚or less.

Acute Doppler angles (<60˚) must be obtained in order to obtain accurate velocity measurements. This is because the cosine values change slowly at acute angles (below 60˚) and rapidly at larger angles (above 60˚). Erroneous estimates of the Doppler angle cause errors in blood flow measurements. Consequently, in order for accurate blood velocity calculations, it is imperative that the Doppler angle is 60˚ or below (optimally 60˚ in every case).

Doppler Angle (θ)	Cosine Value
0	1
10	0.98
20	0.93
30	0.87
40	0.77
50	0.64
60	**0.50**
70	0.34
80	0.17
90	0



The ultrasound curser must be parallel to the artery and the Doppler angle must be less than 60˚ (as seen in image).

2. PREREQUISITES

2.1 Equipment and Supplies

2.1.1 Equipment

1) Duplex ultrasound for simultaneous acquisition of B-mode diameter and pulsed-wave Doppler velocity signals.
2) ECG gating capability
3) Vascular probe with high frequencies (9-15 MHz)
4) Probe holder is optional

2.1.2 Supplies

1. BP gauge (e.g CE0050, Hokanson/Tycos)
2. BP cuff (e.g. SC5, Hokanson)
3. Ultrasound gel (e.g. 7025901, Aquasonic)
4. Electrodes (e.g. 7024706, BioTac)

2.2 Training Requirements

1. At least 50 FMD/Doppler studies performed by each vascular ultrasound technician.
2. At least 10 FMD/Doppler studies approved by the FMD Core Lab at SFGH.

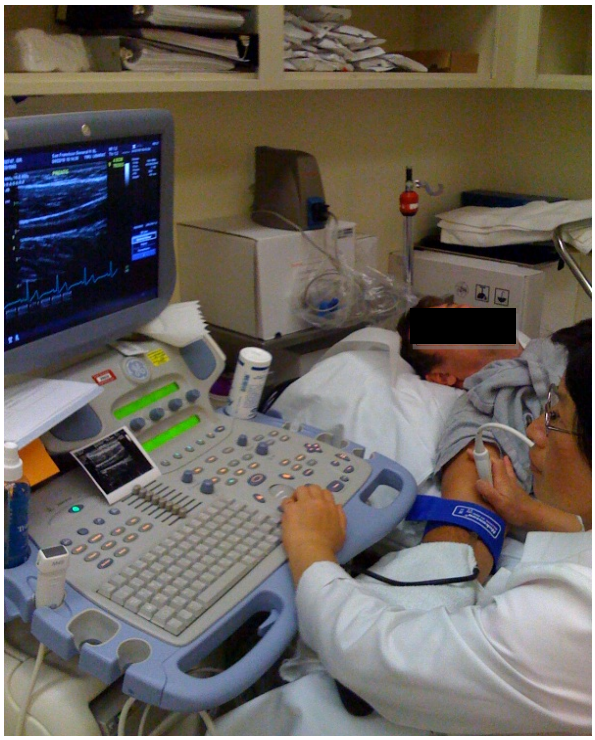
3 CAUTIONARY NOTES OR SPECIAL CONSIDERATION

Nitroglycerin experiments are NOT to be performed if the patient's BP is < 100/60 or if the patient has taken sildenafil (Viagra), vardenafil (Cialis) or tadalafil (Levitra) within 48 hours of the ultrasound study.

4 PROCEDURES

4.1 Study Preparation

1. Patients are asked to refrain from cigarette smoking, caffeine intake, illicit drug use, and exercise for 12 hours before the study. They are asked to have a low fat (25-30%) lunch on the day of the procedure.
2. Patient should rest for 10 minutes prior to study, laying supine on a table in a quiet, temperature controlled room (70 to76 ˚F).
3. The right arm is extended from the torso at an angle of 80-90 degrees and the wrist is placed in a 'thumbs-up' position.
4. A 3-lead ECG is placed on the patient (typically RA, LA & LL leads)
5. A 2.5 inch pneumatic cuff is placed on the on the widest part of the forearm (typically about 1 - 2 cm distal to the antecubital fossa).
6. An appropriate sized sphygmomanometric (blood pressure) cuff is placed on the left arm above the antecubital fossa in order to acquire blood pressure measurements throughout the study



4.2 Equipment Set-Up

1. Turn on all equipment power: ultrasound console, blood pressure monitor, automated blood pressure cuff for rapid inflation and deflation (if available) and thermal printer.
2. Enter patient information (Study ID #, Visit #l, Study date and time) on main ultrasound screen.
3. Ultrasound requirements:
    - Fast frame > 25 Hz
    - High frequency transducer
4. Use the ultrasound control panel to set depth (typically 4cm) and gain settings to appropriate levels.
    - Brachial artery should be in the center of the screen.
    - Lumen should be dark.
    - Intima and media should be clear on both the anterior and posterior walls.
    - Compression (Dynamic Range), Focal Zone and Time Gain Compression should be held constant during the study and setting replicated during any subsequent studies.
    - Pulse Wave Doppler is used for capture of hyperemic velocity. Sweep speed should be set at **25** mm/sec. The Cursor should be placed in the middle of the brachial artery and cursor size should be standardized (1/3 the diameter of the brachial artery).

4.3 Initial Image Acquisition

1. **Scan location**: The brachial artery can be best imaged with high-resolution ultrasound at the elbow (antecubital fossa) since it lies quite superficial. However, it can be inadvertently compressed when the ultrasound probe is held over this location. Thus ideally, the artery should be imaged 5 to 9 cm above the antecubital fossa (3 to 7 cm from the leading edge of a 4 cm line at array transducer) where it forms a straight segment, free of major branches. The artery lies deep in the biceps and is less likely to be compressed at this level. In addition, anatomic landmarks such a small arterial branches, tendons, or fascial planes, are readily visualized and can help the sonographer maintain the same position over the selected artery segment.
2. **Ultrasound gel** is placed in the appropriate location.
3. **Image orientation and display**: The right brachial artery is located and imaged above the antecubital fossa in the longitudinal plane. Confirm arterial location with Doppler signal. All images should be oriented so that on the monitor, the hand is on the left side of the monitor and the shoulder is on the right side of the monitor. Note the approach.
4. **Focal Zone**: Set the focal point at far wall of vessel. Vessel depth should be deeper than 1 cm. One should see a good double-line sign (see 5. Below). One needs to have the vessel as horizontal as possible with an available 10 mm long, straight segment. Curvy or slanted segments have much greater variability of measurements.

5. **Ideal Image**: A segment with clear anterior and posterior intima-media interfaces between the lumen and vessel wall is selected for continuous imaging. **Double line sign** represents optimal beam alignment for seeing wall interfaces; blood-intima boundary and media-adventitia boundary; produce an ultrasound image that will allow the reader to measure the brachial artery diameter from "trailing edge" of adventitia-media interface of near wall to the "leading edge" of the media-adventitia interface of the far wall; **double-line sign is needed for at least 1 cm**; view the image as if you were the reader.

6. **Optimal Probe Angle:** To optimize measurement of both the B-mode and Doppler signals, the probe should be fixed at an angle of approximately 60 degrees.
7. Ideally, a device (probe holder – see picture to right) is used to hold the transducer probe in the same position throughout the study. A probe holder is recommended, since it is difficult particularly for inexperienced operators to keep the probe in the same position throughout the study (probe holders may need to be custom-built). Some experienced operators may prefer to image without the probe holder.
8. During the image acquisition, anatomic landmarks such as veins and fascial planes are noted to assist in maintaining the same image of the artery throughout the study. The landmarks, the angle of the probe, and the distance from the antecubital fossa are used in obtaining identical images on subsequent studies. Identify skin landmark with "x" to reproduce location.
9. Print a hard copy of the baseline B-mode image and save for use during subsequent studies. Note anatomic landmarks used which can be referenced for positioning during subsequent studies.
10. Timer should be used to identify the time points of cuff inflation, cuff deflation and time point of image acquisition. For example, using the timer, we would find Cuff Inflation at 0:00, Cuff Deflation at 5:00, FMD at 30 seconds at 5:30, FMD at 45 seconds at 5:45, FMD at 60 seconds at 6:00 etc. In addition to displaying the timer, please annotate images on the mid right edge of the monitor screen using the following abbreviations:
    - **BSL**: Baseline B-mode image
    - **BSL Dopp**: Baseline Doppler Image
    - **Re BSL**: Repeat Baseline B-mode image
    - **Re BSL Dopp**: Repeat Baseline Doppler Image
    - **RH Dopp**: Doppler during initial reactive hyperemia
    - **RH 30 sec** (B-mode image 30 seconds post cuff deflation)
    - **RH 45 sec** (B-mode image 45 seconds post cuff deflation)
    - **RH 60 sec** (B-mode image 60 seconds post cuff deflation

- **RH 75 sec** (B-mode image 75 seconds post cuff deflation)
- **RH 90 sec** (B-mode image 90 seconds post cuff deflation)
- **RH 105 sec** (B-mode image 105 seconds post cuff deflation)
- **RH 120 sec** (B-mode image 120 seconds post cuff deflation)

4.4 Baseline Imaging

1. Once image is optimized using the techniques described above, print a hard copy of the B-mode image. Make a note of anatomic landmarks for localization on future studies.
2. Confirm appropriate cursor placement and probe position/angle. Confirm image quality on pulse wave Doppler.
3. Note probe position (including distance (cm) from antecubital fossa, medial or lateral approach, and angle of the probe in the sagittal plane) on the patient's Case Report Form (CRF.)
4. Confirm that images will be recorded on MO (magneto-optical) discs. VHS tapes are not adequate.
5. After a 10 minute rest period acquire an initial blood pressure and heart rate on the left arm and then acquire baseline B-mode and Doppler images. The B-mode image should consist of a 5 beat loop recorded to the optical disk (or 5 still frames B-mode captured at the "R" wave). The loop recording should display ECG, as the intent is to analyze 5 end-diastolic (R-wave) images. This should be labeled **BSL.** For Doppler, a minimum of 5 consecutive cardiac cycles should be recorded on baseline imaging. This should be labeled **BSL Dopp**.
6. Repeat baseline B-mode and Doppler images. Additional baseline B-mode images can be taken and submitted to ensure demonstrating the double-line signs for a quality measurement. These images should be labeled RE BSL 2, RE BSL 3.

4.5 Reactive Hyperemia Imaging (RH)

1. Inflate forearm pressure cuff to 200mmHg (and at least 50mmHg above baseline systolic blood pressure). Note time of cuff inflation on patient CRF.
2. After 4 minutes 30 seconds of cuff inflation, begin recording pulse wave Doppler. Note that the scale may need to be adjusted to capture the increased velocities associated with hyperemia. Specifically, the scale may have to be set > 250 cm/s to capture the peak of the first cardiac cycle.
3. At exactly 5 minutes, deflate the cuff. Annotate RH at time of cuff deflation on monitor.
4. In order to obtain 25 mm/sec recordings, images are frozen and acquired at cuff deflation. Such frozen images can only accommodate 3 – 5 cardiac cycles depending on the heart rate (note that we only analyze the first 3 full beats). Then, the 2D ultrasound mode should be switched on to capture the 30 second FMD image.
5. Note the HR and BP at this time on the patient's CRF and store still images to optical disk.
6. Between 30 seconds and 120 seconds, acquire 5 beat loop B-mode images (or still frame B-mode images captured at the "R" wave) every 15 seconds. This should be labeled **RH 30, RH 45, RH 60, RH 75, RH 90, RH 105 and RH 120.** Store to optical disk.

4.6 Repeating the RH procedure if Necessary

1. If the RH Doppler image does not capture at least two full cardiac cycles demonstrating cuff release effects on blood flow velocity, the procedure must be started again from the beginning (section 4.4).
2. Remove the forearm cuff pressure. Have the participant rest lying down for 20 minutes then start by retaking the blood pressure and heart rate and repeat the full study from baseline B-mode and Doppler images through the final RH 120 second image. Label the images the same as you would for a full study.
3. If the procedure is repeated, use a new CRF and make a note in the comment section that the study was restarted due to missing cuff release Doppler image. Submit only the CRF for the completed study. Do not delete the first set of images. All should be saved onto the same CD (see Section 4.7.3.)

4.7 Recordkeeping

After completion of the exam

1. Complete the CRF and make a copy.
2. Save file in DICOM format and copy file to CD.
   Create a separate folder for each of the following:
   - **BSL**: Baseline B-mode image
   - **BSL Dopp**: Baseline Doppler Image
   - **Re BSL**: Repeat Baseline B-mode image
   - **Re BSL Dopp**: Repeat Baseline Doppler Image
   - **RH Dopp**: Doppler during initial reactive hyperemia
   - **RH 30 sec** (B-mode image 30 seconds post cuff deflation)
   - **RH 45 sec** (B-mode image 45 seconds post cuff deflation)
   - **RH 60 sec** (B-mode image 60 seconds post cuff deflation
   - **RH 75 sec** (B-mode image 75 seconds post cuff deflation)
   - **RH 90 sec** (B-mode image 90 seconds post cuff deflation)
   - **RH 105 sec** (B-mode image 105 seconds post cuff deflation)
   - **RH 120 sec** (B-mode image 120 seconds post cuff deflation)

   The folders should use the label names (e.g. a folder should be called BSL, BSL Dopp, Re BSL, Re BSL Dopp, RH Dopp, RH 30 sec, RH 45 sec etc.)

   As noted under section 4.5, for each Doppler recording, send a frozen image that depicts at least 3 cardiac cycles. For the FMD B-mode images, you can send 3 end-diastolic images. The cursor on the ECG has to be positioned on the R-wave of QRS to confirm that these are end-diastolic frames. Alternatively, you can send clips of at least 3 full cardiac cycles at each time point with the ECG displayed and we will select the end-diastolic frame ourselves.
3. Use one CD for each complete BAU procedure (there should be 6 CDs per subject). Use label provided by NERI (which should match the label on the CRF). If the procedure is repeated, it should be recorded with the first attempted procedure on the same CD. If it does not fit due to size of files, another CD can be copied and submitted labeled with the patient ID and the comment "CD #2".

4. Send pre- and post-exposure CDs and copies of the CRFs as soon as the post-exposure visit is completed to:

   Peter Ganz, MD
   Chief, Division of Cardiology
   Room 5G1 Cardiology
   San Francisco General Hospital
   1001 Potrero Avenue
   San Francisco, CA 94110

5. At the time of shipping the ADEPT data management system will notify by e-mail the PI of the core lab via email (GanzP@medsfgh.ucsf.edu) that a shipment has been initiated. The core laboratory may change the frequency of shipping during the course of the study.

5. QUALTIY CONTROL

5.1 Phantom monitoring. Phantom monitoring must be performed every 6 months to ensure quality control. Phantom images to take:

- Gray scale
- Axial resolution
- Lateral resolution
- Depth calibration
- Horizontal calibration
- Vessel simulation
- Fill out appropriate paperwork and then send forms to address above. (NOTE: Phantom monitoring needs to be purchase; forms to record Phantom monitoring needs to be developed)

6.2 Machine/Maintenance/Upgrade log
When the machine used for scanning is serviced in any way, a record of this should be appropriate paperwork and then send forms to address above.

7. QUALTIY ASSAURANCE

HEI may request copies of the CDs and may conduct an independent analysis of the images provided by each of the three research centers. HEI may also audit the data generated at the core laboratory.

8. REFERENCES

None

MOSES Standard Operating Procedure (SOP)

SOP # 32 – BAU Analysis

TITLE: Brachial Artery Ultrasound to Evaluate Vascular Function (Brachial artery reactivity and Doppler)
Version: 1.0 **Directory/file[a]:** //

Author: Peter Ganz **Signature:** **Date: January 27, 2014**
Claire Mills

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

ANNUAL REVIEW[C]

Signature	**Date**

SOP UPDATED TO VERSION [d]

Explanations: [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. <u>If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!</u>

[c] Annual review does not change the version of the SOP.

[d] Change the version # as necessary

BRACHIAL ARTERY ULTRASOUND ANALYSIS

1. TRACKING OF STUDIES AND DATA ENTRY

A spreadsheet with 3 columns has been created to track the BAU studies: 1) the date the sample came into the core lab's possession 2) the date of study analysis and 3) the date the study's analysis results are entered into the NERI database.

The originating site provides the core lab one CD and one BAU Case Report Form (CRF) for each ultrasound. The CRF contains information taken and reused by the scanning sonographer to replicate location of the artery and direction of the scan for reproducibility when scanning the sequential studies. Additionally, information on blood pressures and heart rates before and after the study and heart rates during Doppler image acquisition are included along with timing information. Once all six studies for a participant arrive into the core lab, the goal is to measure all PreExposure BAUs on the same day to ensure consistency in measurements. The Exposure BAUs are also measured on one day.

The core lab analyzes the images provided on the CD along with the CRF information documenting all analysis information onto a BAU Measurement Form. Study data are entered into the online NERI database from the BAU Measurement Forms periodically in batches (see the data from of Brachial Artery Ultrasound Measurement Appendix 1.) If a data point is missing, NERI has asked for -9 to be entered for the missing data. Once the data are entered into the database, the original BAU CRF and Measurement Forms are compared to the data entered into NERI to ensure data entry accuracy. The Core lab does no extra analysis beyond measurement calculations and is therefore blinded to statistical study analysis.

2. METHODOLOGY

Endothelial dysfunction has been well documented to be a primary etiology of atherosclerosis. [1-2] Measurements to assess endothelial function consist of flow mediated dilation and reactive hyperemia. The required measurements consist of baseline diameter before cuff inflation, reactive hyperemia diameter following cuff deflation, and the velocity time interval measurements of the reactive hyperemia from Doppler images. The MOSES core lab will utilize edge detection software produced by Medical Imaging Applications LLC[3] (Coralville, Iowa) which is now considered a standard for flow mediated dilation (FMD) and Doppler analysis.[4-10]

2.1) For the Baseline Diameter utilized in Percent Change Calculations:

Two baseline measurements are taken. Each measurement consists of three cardiac cycles taken at end-diastole, defined by the R wave on ECG. The average of the three cardiac cycles analyzed yields the measurements for Baselines 1 and 2. These two baseline measurements are averaged and are

referred to as the Baseline diameter. An artery diameter is measured by the edge detection software over the span of a minimum of 1 centimeter of vessel.

2.2) For Dilation Diameter Measurements and Calculations:

The reactive hyperemia diameters are analyzed at 30, 45, 60, 75, 90, 105 and 120 second time-points following cuff deflation. As with baseline images, three R waves are analyzed and averaged for the diameter measurements at each time-point. Again, a measurement is measured by the edge detecting software over the span of a minimum of 1 centimeter of vessel. A FMD expressed as percent change in artery diameter is calculated for each of these time-points using the following equation[5]:

$$FMD(\%) = \frac{peak\ diameter - baseline\ diameter}{baseline\ diameter}\ x\ 100$$

2.3) VELOCITY TIME INTEGRAL WITH DOPPLER

Reactive hyperemia, a measure of microvascular function, yields prognostic information beyond FMD.[11]. The studies capture both baseline Doppler and reactive hyperemia Doppler images. The core lab uses the VTI program from Medical Imaging Applications LLC to analyze the wave forms. An average of 3 cardiac cycles measured from R wave to R wave is utilized as the baseline VTI.

The reactive hyperemic VTI is produced the same way with the Doppler image immediately following cuff release. The *first* three full cardiac cycles from R wave to R wave are averaged to produce the RH VTI value.

3. QUALITY CONTROL

3.1) To ensure the accuracy of the data entry, once the data are entered into the database, the original BAU CRF and Measurement Forms are compared to the data entered into NERI. The person who enters the data for the six studies for one participant double checks the original source forms against what has been entered into NERI immediately after the six are completely entered. The Core lab does no extra analysis beyond measurement calculations and is therefore blinded to statistical study analysis.

We plan to discern the reproducibility of our analyses and of the brachial studies in the following ways:

3.2) Variability of our analyses <u>and</u> of the brachial studies: We will define the variability of the 3 pre-exposure studies for each subject. We will specifically focus on the variability in a) the brachial artery diameters before cuff inflation, b) the percent change in FMDs post cuff release and c) the VTIs. The variability in these measurements reflects the sum of any variability in a subject's physiological state, variability in the sonographer's performance and variability in our brachial artery analyses.

3.3) In order to specifically pinpoint the variability in our brachial artery analyses, we will define intra-observer variability for our (i.e. the core lab's) measurements of baseline

diameters, the percent change in FMDs and VTIs as follows: Each 10 patients generate 60 brachial FMD/VTI studies (i.e. each subject has 3 pre-exposure and 3 post-exposure studies). At ≥ 2 weeks after the initial analysis, from each site (UNC, Rochester and UCSF), we will randomly select four out of these 60 studies to re-analyze four items: the Baseline diameters, the RH VTI and two FMD percent change calculations at the 45 and 60 second time-points. Thus, 1 of 15 studies overall will be re-analyzed for intra-observer variability. As the quality of our analyses is linked to the quality of the data we receive from each site, we will balance this analysis by sites. We will work with the NERI statistician to calculate the variability in our measurements.

3.4) We realize that the technical quality of brachial artery studies is variable (in MOSES or in other studies), even at different time-points within a single study. We developed a list of objective metrics to judge the technical quality of each MOSES study, at each individual time-point and will assign a technical quality grade to each MOSES study at each time point, before the data are unblinded. It might potentially prove useful should there be any need to eliminate studies that introduce "noise".

These levels are defined as below:

Level 1 – Excellent

Horizontal positioning of vessel
Artifacts do not interfere with intimal definition
Over 1 centimeter of clearly demonstrable double walls on both near and far walls showing that the vessel has been transected by the ultrasound beam at the widest diameter of the artery
Minimum of 5 QRS complexes recorded (demonstrated by the ECG)

Level 2 – Adequate

Double line sign on one wall and clear intimal layer (even if without a clear definition) demonstrated in the other wall (typically, it is easier to image a double line on the far wall than the near wall).
Over 1 centimeter of clearly measureable intimal walls showing that the vessel has been transected by the ultrasound beam at the widest diameter portion of the artery
Artifacts interfere minimally with intimal definition
Minimum of 3-4 QRS complexes recorded as demonstrated by the ECG
Vessel may be slanted but walls are clear enough for measurement

Level 3 – Poor

Intimal and medial layers not clearly defined on near or far wall (i.e. no double lines on near or far wall)
Slanted or irregularly shaped vessel
Vessel not transected by the ultrasound beam at widest diameter portion of artery
Not a full centimeter of demonstrated walls to measure
Artifacts interfering with wall determination
Image taken in inconsistent locations based on imaging landmarks
An arrhythmia is present with variable cardiac cycle lengths and no periods of regular sinus rhythm to analyze

3.5) In addition to quantifying the image quality, a phantom study is conducted by each center twice a year to simulate known responses from each ultrasound machine to ensure equipment quality (see Appendix 2. Phantom Protocol for Quality Assurance of Brachial Artery Ultrasound in HEI MOSES Study in Appendix 2.) The same phantom is used by each site and for each phantom scan. Sites submit the HEI MOSES Phantom Scan Form for Quality Assurance (Appendix 3) and a CD of images made with the phantom device. Against the baseline phantom scans produced by each site, the core lab compares subsequent scans. If variation is observed from baseline scans in axial or lateral resolution, horizontal or vertical measurements or in image uniformity (grey scale) or vessel simulation, sites are immediately notified by email and are responsible to schedule any required repairs immediately upon notification. Otherwise, an email with feedback is sent to each site confirming the receipt of an acceptable phantom scan and document. Comments confirming or rejecting the images are made on the bottom section of the HEI MOSES Phantom Scan Form for Quality Assurance submitted by each site and stored at the core lab. Consistency in image quality and measurements can be assured with such phantom scanning procedures.

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Appendices

Appendix 1. Brachial Artery Ultrasound Measurement

Appendix 2. Phantom Protocol for Quality Assurance of Brachial Artery Ultrasound in HEI MOSES Study

Appendix 3. HEI MOSES Phantom Scan Form for Quality Assurance

MOSES Study

Brachial Artery Ultrasound Measurements

A1. Subject ID Label: A2. Visit: ☐ PRE1 ☐ EXP1 A4: Date of Study: __ __-__ __ __-__ __ __ __

A3. Initials of Sonographer: __ __ __ ☐ PRE2 ☐ EXP2 A5: Date Read: __ __- __ __ __ -__ __ __ __

A6: Initials of Reader: __ __ __ ☐ PRE3 ☐ EXP3

B1. DOB: __ __ - __ __ __ - __ __ __ __ B2. Gender: ☐Male ☐Female

B3. Arrival Time to FMD: __ __ __ __ B4. Tape/CD #_____

Baseline (BSL)

Image Rating	Diameter # in mm	**1st**	**2nd**	**3rd**	**Avg BSL in mm**
	1	__ .__ __	__. __ __	__ .__ __	__ .__ __
	2	__. __ __	__ .__ __	__ .__ __	__ .__ __

Avg Diam in mm of both BSL Measurements __ . __ __
BSL BP (mm/Hg) __ __ __ / __ __ __ Heart Rate (bpm) __ __ __

BSL ___ or RE BSL ___ Flow **VTI** 1st Cardiac Cycle (cm) __ __ . __ 2nd __ __ . __ 3rd __ __ . __

BSL Avg Flow **VTI** of 1st three Cardiac Cycles (cm) __ __ . __ HR at VTI __ __ __

Reactive Hyperemia Reaction (RH)

RH- Flow VTI 1st Cardiac Cycle (cm) __ __ __ . __ 2nd__ __ __.__ 3rd __ __ __ . __

RH Avg Flow **VTI** of 1st three Cardiac Cycles (cm) __ __ __ . __ HR at VTI __ __ __

ImageRating	Time	RH-Diam.mm	RH-Diam mm	RH-Diam mm	Average of 3	FMD Chg(%)
	@30 sec	__ . __ __	__ . __ __	__ . __ __	__ . __ __	__ __ . __ __
	@45 sec	__ .__ __	__ .__ __	__ .__ __	__ .__ __	__ __ . __ __
	@60 sec	__ . __ __	__ . __ __	__ . __ __	__ . __ __	__ __ . __ __
	@75 sec	__ . __ __	__ . __ __	__ . __ __	__ . __ __	__ __ . __ __
	@90 sec	__ . __ __	__ . __ __	__ . __ __	__ . __ __	__ __ . __ __
	@105 sec	__. __ __	__ . __ __	__. __ __	__. __ __	__ __ . __ __
	@120 sec	__. __ __	__. __ __	__. __ __	__. __ __	__ __ . __ __

Post RH BP (mm/Hg) __ __ __ / __ __ __ Heart Rate (bpm) __ __ __
Comments:

Phantom Protocol for Quality Assurance of Brachial Artery Ultrasounds in HEI MOSES Study

A quality assurance program for the ultrasounds in the HEI MOSES study will provide assurance of consistency of data for the Flow Mediated Dilation measurements. Phantom measuring of the ultrasound machines every 6 months is necessary to monitor machine drift or degrading of imaging quality. Phantoms will be performed with the same settings each time. The BAU preset should be used for all phantom scans.

The unchangeable settings are:

Power
Frequency
Frame Rate
Dynamic Range
Gain
Time Gain Compensation (set at Manufacturer's Zero, for most manufacturers the pods are positioned in the middle with negative to the left and positive to the right.)
Image Depth
Focal Zone Depth
Number of Focal Zones

Image Quality Indicators to be evaluated with each phantom scan will be the ultrasound machines':

Axial Resolution (3 images)
Lateral Resolution (3 images)
Distance Accuracy in both Horizontal and Vertical directions, (3 images for each direction)
Cylinder Diameter (3 images)
Image Uniformity (1 image)

Pixels will also be evaluated to assure uniformity from the first phantom scan submitted through the last a study site submits.

A total of 16 images will be submitted. Image quality indicators with measurements using calipers will be submitted with and without the calipers on the image. The quality indicators utilizing calipers are the Axial and Lateral Resolution, the Distance Accuracy in both directions and the Cylinder Diameter images. The Image Uniformity image will be without calipers as it is to examine the grey scale consistency and to examine for banding or vertical breaks in the image which can occur due to hardware issues such as broken transducer crystals or issues with electrical contacts or processing circuitry.

Lateral Resolution will be examined to ensure structures perpendicular to the ultrasound beam are imaged properly by the machine. Pins will be measured from side to side (ie. left or right) to ensure consistency of the machine's imaging of structures positioned perpendicular to the beam. If problems exist with lateral resolution, the transducer or the system's beam-forming circuits may need to be examined by a technician.

Axial Resolution will be examined by measuring pin structures from top to bottom to analyze the consistency in how structures that are positioned directly in the beams axis are imaged by the machine. Axial resolution is affected by the spatial pulse length or pulse duration.

Distance Accuracy is self explanatory as is Cylinder Diameter measuring. Vertical distances examine distances measured within the beam's axis whereas distances measured horizontally examine accuracy in distances perpendicular to the beam's axis. Pins will be measured from leading edge to leading edge for vertical distance and from center of pin to center of pin for horizontal distance. Determining diameters of the brachial artery is the main focus of this ultrasound study, therefore, consistency in distance accuracy and cylinder measurement, which simulates a vessel, is essential to the study's endpoints. See sample images for caliper placement and other guidance.

Phantom scan information is to be entered for Patient Info as:

Last name: 802262-4020-6 (Phantom serial number)
First name: UNC MCC (site name and initials of sonographer, UR with SJ or CP, UCSF with CM)
Patient ID: MOSES Phantom 1 (or subsequent correlating numbers, 2, 3, 4)
Birthdate: date of scan

If images do not reflect the information from the Patient Information page when compressed, this information is to be typed onto each image across the top of the image in a way that does not interfere with the image pins or cylinder.

All images are to be labeled with the ***type of image quality indicator to be evaluated*** typed onto the bottom left corner of the image or at the bottom someplace where it will certainly be included on submitted images even if/when compressed.

Apply ECG leads to yourself in the normal positions or on your legs (one on right, two on left) to demonstrate timing of cine-loops which will be 3 full heart beats.

Images to be submitted (example images are provided):

1) **Axial Resolution** – Save a clip store (cine loop) of 3 heartbeats focusing on leading top pin while making all pins in focus.
2) **Axial Resolution –**
   a) Open up the previously saved clip store labeled Axial Resolution

b) Select the best image of the top pin with overall good quality and freeze. Save this image.

3) Axial Resolution –

a) Open up the saved still image and apply calipers to measure the size of the top pin. Measure the top pin with calipers from leading edge to far edge of the pin. (should be 0.5 mm)

b) Save this image. *All three images for Axial Resolution should be labeled Axial Resolution.*

Measurement requirement of pin of 0.5 mm as set by phantom manufacturer. Actionable measurement for a service: if measurement varies from the baseline phantom scan by more than 2 pixels

4) Lateral Resolution - Save a clip store of 3 heartbeats focusing on leading top pin while making all pins in focus.

5) Lateral Resolution -

a) Open up the previously saved clip store labeled Lateral Resolution

b) Select the best image of the top pin and freeze. Save this image.

6) Lateral Resolution -

a) Open up the still image labeled Lateral Resolution and apply calipers to the still image and measure the top pin from left to right with calipers. (should be 0.5 mm)

b) Save this image. *All three images for Lateral Resolution should be labeled Lateral Resolution.*

Measurement requirement of 0.5 mm as set by phantom manufacturer. Actionable measurement for a service: if measurement varies from the baseline phantom scan by more than 2 pixels.

7) Horizontal Distance – Save a clip store of 3 heartbeats focusing on holding 3 cross pins horizontally while making all other pins in focus. Horizontal pins have 10.0 mm distances.

8) Horizontal Distance –

a) Open up the previously saved clip store labeled Horizontal Distance

b) Select the best image of the 3 cross pins horizontal and freeze. Save this image.

9) Horizontal Distance –

a) Open up the still image labeled Horizontal Distance and apply the caliper to the middle of the pin that is positioned just left of the center pin. Place the next caliper in the middle of the pin that is positioned just right of the center pin. (distance should be 20.0 mm)

c) Save this image. *All three images for Horizontal Distance should be labeled Horizontal Distance.*

Measurement requirement between horizontal pins of 10.0 mm as set by manufacturer. Measurement must be within 2 pixels of baseline phantom scan.

10) Vertical Distance – Save a clip store of 3 heartbeats while holding top 2 pins descending vertically in focus. Vertical pins have 5 mm distances.

11) Vertical Distance –

a) Open up the previously saved clip store labeled Vertical Distance

b) Select the best image and freeze. Save this image.

12) Vertical Distance –

a) Open up the still image labeled Vertical Distance. Apply the first caliper at leading edge of top pin and place the second caliper at leading edge of next pin directly below it. (should be 5.00mm)

c) Save this image. *All three images for Vertical Distance should be labeled Vertical Distance.* Measurement requirement between vertical pins of 5.0 mm as set by manufacturer.

13) Cylinder Diameter – Save a clip store of 3 heartbeats with near and far wall in focus with walls extending to transverse edges of image.

14) Cylinder Diameter –

a) Open up the previously saved clip store.

b) Select the best image of the cylinder and freeze this image. Save this image.

15) Cylinder Diameter –

a) Open up the still image labeled Vertical Distance. Measure with calipers the diameter of the cylinder from far edge of near wall to near edge of far wall. (should be 3.5 mm)

b) Save this image. *All images for Cylinder Diameter should be labeled Cylinder Diameter.* Phantom distance from near to far cylinder wall is 3.5 mm as set by manufacturer.

16) Image Uniformity – still image with cyst structures and pins for grey scale reproducibility. Evaluate for any banding or vertical breaking in image. Submit images without reverberation artifacts. Match subsequent phantom scans with baseline scan for consistency in the grey scale and texture disbursement throughout the image. Match sample images provided for location on phantom to scan.

Care of the phantom device

The phantom must be stored upright, out of direct sunlight, and always within it's zip enclosed plastic bag to prevent drying and desiccation. Always attach the scanning surface cover when not in use. Do not allow it to freeze or be exposed to heat. Store between 35 and 105 degrees F. The phantom should be moved about carefully, never dropped or banged into. It is delicately calibrated and rough treatment is to be avoided. Only use ultrasound gel when scanning. **Do not press the transducer deeply into the surface as this can damage the phantom.** At the core lab, the phantom should be weighed when received and every six months thereafter.

Scanning with the Phantom

Always scan on a level surface that is stable. Do not peel off any surface material from the phantom's scanning surface. Use a **large** quantity of gel. Use only Ultrasound gel for scanning and always clean the surface immediately after use with soft cloth or paper towels. Soap and water may be used when needed. **Pressing into the phantom's surface (when cleaning or scanning) will shorten its lifespan.**

Paperwork and Shipping

Complete the one page HEI MOSES Phantom Scan Form for Quality Assurance. Maintain a file at the site of all phantom scan paperwork. Ship Phantom Scan Form with CD of Ultrasound Scan to:

Peter Ganz, MD, Chief, Division of Cardiology
1001 Potrero Avenue, Room 5G1 Cardiology
San Francisco General Hospital
San Francisco, CA 94110
(415-206-3503)

HEI MOSES Phantom Scan Form for Quality Assurance

Site: _____ Tech Initials: _____

Phantom: Gammex 404GS LE Grey Scale and Small Parts Phantom 0.7dB/cm/MHz

Date of QA Scan: _____ Scan#: _____

Phantom Serial Number: _____

Machine:_____

Machine Serial Number:_____

Transducer Model Number: _____

Transducer Serial Number:_____

The unchangeable settings to be same from baseline scan through all subsequent scans:

Power:	Frequency:	Frame Rate:	Dynamic Range:	Gain:	TGC:	Image Depth	Focal Zone Depth:	#Focal Zones
_____dBs	_____MHz	_____Hz	_____dBs	_____dBs	aligned at Zero	4.0 cm	2.0 cm	One
					not adjustable	***not adjustable***	***not adjustable***	

Match supplied images for pins and cylinders to measure. Use same selected frame saved from 3 heartbeat clip store once with calipers and once without.

Axial Resolution

_____mm leading edge of pin to its far edge

Lateral Resolution

_____mm left edge of pin to its right edge

Hornizontal Distance Accuracy

_____mm middle of pin left of central pin to middle of pin to the right of center

Vertical Distance Accuracy

_____mm leading edge of central pin to leading edge of pin below it

Transverse Vessel Simutation using 3.5mm cylinder with calipers at far edge of near wall to near edge of far wall: _____mm

Near & far vessel walls fully visible from left side of screen to right: Yes____ No____

For Completion by Core Lab:

Images same as BASELINE: YES____ NO ____

Settings same as BASELINE: YES____ NO ____

Image Uniformity (Low 1, High 10) _____

Pixels _____mm/pixel Same pixel size as BASELINE YES____ NO____

MOSES Standard Operating Procedure (SOP)

SOP # 41 PES – PES Assembling, Cleaning, And Storing

TITLE: Personal Exposure Sampler Assembling, Cleaning, and Storing

Version: **1.0** Directory/file[a]: **//**

Author: MOSES investigators Signature: Date: March 23, 2012

Revised by: New version[b]: Date:

Revisions/additions:

Signature:

Revised by: New version[b]: Date:

Revisions/additions:

Signature:

ANNUAL REVIEW[C]

Signature	**Date**

SOP UPDATED TO VERSION [d]

Explanations: [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!

[c] Annual review does not change the version of the SOP.

[d] Change the version # as necessary.

PERSONAL EXPOSURE SAMPLER ASSEMBLING, CLEANING, AND STORING

1. SCOPE and APPLICATION

The purpose of this SOP is provide instruction for cleaning the Personal Exposure Sampler (PES) components, storing the PES after assembly, disassembling it after use by the subjects, and shipping the exposed filters for analyses. This procedure applies to the PES used to measure the exposures to ozone and nitrogen dioxide (NO_2) of the subjects prior to the chamber exposure. The PES should be worn by subjects for three days prior to the pre-exposure day starting at 12:00 noon of the third day.

2. PREREQUISITES

No equipment is needed. The supplies needed are described below. No specific training is required.

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATION

Because the shelf-life of the filters used to collect the pollutants is limited, care must be taken to keep track of the date the filters were received, the date the sampler was assembled, and the date it was given to a subject.

4. PROCEDURES

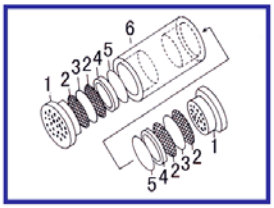
4.1 Description

The Ogawa sampler to be used in MOSES consists of 1) a small plastic reusable cylinder (PES body) with diffusion end-caps 2) a glass fiber filter coated with nitrite-based solution for collecting ozone, 3) a cellulose fiber filter coated with triethanolamine for collecting NO_2, and 4) a pin-clip holder. When the sampler is used for personal air sampling, it requires a 10 x 10 cm polystyrene backing plate clipped to the shirt of the subject to prevent absorption of ozone and NO_2 by the subject's clothing.

<u>Components of each badge kit</u> (see also Figure 1)

- 1 sampler body (with 2 spacer disks & 2 rings)
- 2 diffusion end-caps (1 per end)
- 4 stainless steel screens (2 screens per end)
- Two filters, one for ozone and one for NO_2 (these are not part of the sampler kit)
- 1 pin-clip holder
- 1 re-sealable plastic bag
- 1 storage brown vial (amber polystyrene)

Figure 1 Components of the sampler body



Other items needed

- Sampler identification (ID) labels (2 per sampler) provided by NERI
- 1 or 2 non-serrated blunt forceps for filter handling
- 1 plastic squeeze bottle with Milli-Q water
- Plastic re-sealable bags (gallon and quart size)
- 2 shipping vials provided by Ogawa
- 1 Gram Indicating Silica Gel Packets from www.silicagelpackets.com.
- Ice packs
- Insulated Styrofoam container

4.2 Storage of filters before use

Pre-coated filters are supplied by Ogawa & Co., USA, Inc. (www.ogawausa.com) in vials labeled with a batch number. Each vial contains 40 filters.

1. Record on PES Data Log Sheet the date of receipt of the filters and the batch number.
2. The pre-coated filters must be stored in their original containers in the refrigerator. If stored under these conditions they can be used up to a year. As a coated ozone filter ages, a slow conversion of nitrite to nitrate occurs.
3. All filters used for a designated batch (and corresponding field blanks) must be removed from the refrigerated vial and prepared at the same time.

4.3 Cleaning the sampler's components

All sampler components should be carefully rinsed with pure water (Milli-Q or equivalent purity) and dried for several days before assembly following the instructions below. Lay out the parts to dry on Kimwipes and cover them with Kimwipes to keep out dust.

All components can be washed ahead of time and stored in separate containers until assembly.

1. Initial cleaning:
    1. Disassemble the sampler into its main components: a) separate the cylinder body with end-caps from the pin-clip; b) separate the end-caps and stainless steel screens from the body. Each of the individual parts must be thoroughly rinsed in separate groups with Milli-Q water.
    2. After cleaning, lay the parts on Kimwipes to dry for several days. While drying, cover with one large Kimwipe to prevent dust/dirt from settling on the clean parts.

These can be kept in closed containers as long as it is necessary before loading the coated pads.

2. Routine cleaning:

    1. Disassemble the samplers and set aside the cylinder bodies (with spacer disks and rings still inside) to be cleaned separately. In old samplers sometimes the rings come out. When this happens, the plate should also be taken out, and the rings and plates cleaned separate from the bodies.

    2. Rinse the end-caps with Milli-Q water, then set the parts on Kimwipes to dry. It may be necessary to tap the water out of the holes in the end-caps in order for them to dry completely.

    3. Wipe the cylindrical bodies clean with Kimwipes moistened with Milli-Q water. Then use a dry Kimwipe to wipe off excess water.

    4. Place the stainless screens in a beaker, then rinse them several times with Milli-Q water. Fill the beaker again with Milli-Q and place it in a sonication bath. Sonicate the screens for *5* minutes, then rotate the beaker a quarter turn; this procedure must be continued for a total of 15 minutes. Be careful not to damage or lose the wire mesh screens during handling.

    5. All parts of the sampler must be clean and completely dry before assembling the sampler. After cleaning, lay the parts on Kimwipes for several days to dry completely. While drying, cover with one large Kimwipe to prevent dust/dirt from settling on the clean parts. These can be kept in closed containers as long as it is necessary before loading the coated pads.

    6. Inspect the pin-clips for obvious dust, dirt, etc. If necessary, rinse with Milli-Q water and lay on Kimwipes to dry.

4.4 Cleaning the filters' storage vials

The new vials (and caps) for storing the exposed filters must be rinsed several times with high purity water, and allowed to dry at room temperature covered by large Kim wipes for at least two days before use (after they dry, put on the caps to keep them clean before use.) They can be cleaned ahead of time and kept in a closed container as long as it is necessary before use.

4.5 PES Assembly

Note that two filters (one for ozone and one for NO2) must be placed into each sampler, one at either end of the sampler body. All components must remain completely dry during the assembly. This requires that after forceps are wiped clean with a moist Kimwipe, **they must be completely dried before handling the filters and screens.** Use the blunt forceps whenever possible to prevent filter damage. Use the following assembly steps for each sampler (see also figure 2).

1. You should have two identical labels with the **Sampler ID** (site #MOSES 1, site #MOSES 2, etc.) from NERI for each sampler that you assemble. Place <u>one of the two labels</u> on the back of the pin-clip holder.

2. Have all the parts needed to assemble the sampler near you. Wear gloves.

3. Place a fresh large Kimwipe on the lab bench; place a cylindrical sampler body upright on a clean Kimwipe, or alternatively hold it between 2 fingers. **Avoid touching the inside of the ends of the body with fingers.**

4. Using the forceps (which should be clean and dry), place a Teflon disk in the inner most part of the cylinder and then a Teflon ring on top of the disk. Add a stainless screen on the disk. Be careful not to bend or damage the screen, and make sure that it sits flat on the Teflon spacer ring.

5. <u>NOTE</u>: It is better to have in advance the Teflon disks and rings already inserted into the bodies for all the samplers that are to be assembled. The first of the two stainless steel screens should also be inserted before opening the vial of coated filters This minimizes the time that the filters may be exposed (either inside a glove box or not).

6. Take the vials with the ozone and NO_2 filters out of the refrigerator and place them on the lab bench. Open the vial containing each set of filters. Use the blunt forceps to gently grip one of the filters by its edge.

7. Place the ozone filter in the end of the cylinder. Again, be careful not to damage the filter and watch that it sits flat on the stainless screen. If a filter is dropped, discard it and get another from the glass vial. Be careful not to contaminate the unused filters. Mark the end of the barrel (not the end cap) with the ozone filter with a red marker.

8. Place a second stainless screen over the filter, taking the same precautions as before.

9. Pick up a diffusion end-cap by its edge. Avoid touching its flat sides. Securely place an end-cap into the end of the body. If the end-cap is slightly loose, the retaining ridge on the pin-clip will hold it in place.

   <u>NOTE</u>: Sometime the end-cap fits quite tightly to the body. To get it to go all the in, it is sometimes necessary to put a small Kimwipe on the outside and push hard until it clicks in.

10. Next, turn the body of the badge over and repeat directions 2 through *5*, to insert a screen, a NO_2 filter, another screen, and the end-cap into the opposite end of the cylinder. **Use different forceps for handling this filter or make sure that the forceps are thoroughly cleaned before handling the NO_2 filters because contamination from the large amount of nitrite coating on the ozone filters will make a large positive artifact for NO_2 measurement.**

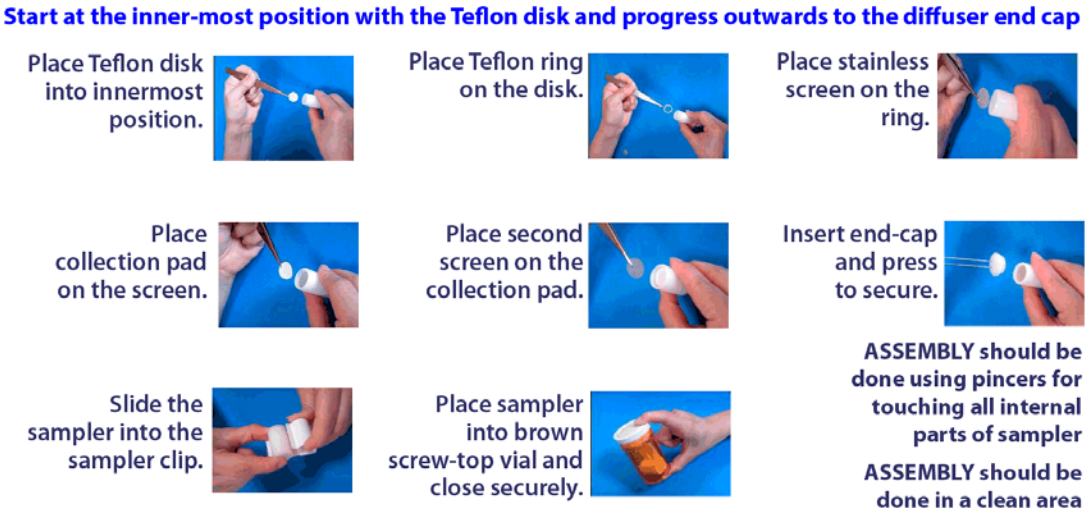
11. Snap the assembled body into a pin-clip backing (which had a sampler ID label attached before placing in the glove box), **without touching the flat surface of the end-caps. If you inadvertently touch the end cap, after attaching it to the pin-clip holder wipe it off with a moist Kimwipe.**

12. Place the completed sampler into a re-sealable bag. Add a gel packet to absorb any moisture. Expel the air from the bag and seal it.

13. **Place the sealed bag inside the airtight brown vial and firmly seat cap on vial. <u>Place the second sampler ID label on the side of the vial</u>.** After all the samplers have been prepared, put them in the refrigerator.

Figure 2. Assembly of PES

The body of the sampler is comprised of two identical chambers. Each one is assembled as described below. Repeat the first 6 steps (from left to right) to assemble the second chamber.



IMPORTANT: The figure shows the fingers touching the end caps. The end caps should not be touched. Also the figure leaves out the re-sealable plastic bag!!

4.6 Storage of assembled badge (with coated filters)

The assembled badge inside a resealable bag placed in the brown vial can be stored in the refrigerator <u>until given to the subject</u>. Filters must be exposed by subject and sent for analysis within 1 year of the time they were received from the manufacturer. It is not necessary to bring the badge to room temperature before using it because it will quickly adjust to the room temperature.

4.7 To prepare subjects to measure personal exposure

1. Take a brown storage vial containing an assembled sampler from the refrigerator. Put it in a quart-size re-sealable plastic bag along with a frozen ice pack.

2. Prepare a gallon-size re-sealable plastic bag to send home with the subject at the end of the Training Visit or a Post-Exposure Visit. This large (gallon) plastic bag should contain:

- Small plastic bag (quart) containing the brown storage vial and frozen ice pack.
- Backing plate
- Subject Instructions for Use of Personal Ozone Sampler
- Time-Activity Diary (4 copies)
- Subject Instruction for Filling the Time Activity Diary
- General Subject Instructions

3. Instruct subjects to put the small plastic bag into the refrigerator when they get home (or to work). It is important that they think about where they might be at noon three days before their Pre-Exposure Visit so that the PES is close by when it is time for them to put it on. If a subject is traveling during this period, they can take the quart bag with them in the car/train/plane if they make sure it stays cold (in an insulated cooler with an ice pack) until the time they are scheduled to put it on.

4. Explain the use of the PES. Instruct the subject to start wearing the sampler at noon on the third day before the Pre-exposure visit. It is important that subjects to record the time that they actually put the sampler on in the activity diary.

NOTE about the plastic backing. There have been issues occasionally in the past about the clip on the backing sheet coming off while the sampler is worn by the subject. The solution to this problem is the following: have Scotch (3M) brand double coated foam tape available. [It comes in either squares or rolls. The cover layer is a green and white plaid (i.e. "Scottish").] Cut a piece the size of the pad on the clip and stick it on the backing, then press firmly together.

4.8 To end measuring personal exposure (when the subject arrives to the clinic)

1. Remove the sampler from the subject's clothing. Record the time removed on the Pre-Exposure Visit checklist, as well as the time the subject put on the sampler (taken from activity diary). ADEPT will provide the total exposure time in minutes. This information is needed by RTI to calculate the pollutants' concentrations.

   NOTE: The subject should bring the original resealable bag and the vial back to the clinic. If this does not happen, use another resealable bag and disassemble the sampler as described in section 5 as soon as possible.

2. Place the sampler in the resealable bag, then in the storage bottle, securing the screw-on cap.
3. Make notes regarding unusual circumstances, or appearance of the sampler.
4. Keep the storage bottle refrigerated until the sampler is disassembled and the filters are unloaded.

4.9 Disassembling the sampler after exposure

1. As soon as it is convenient, disassemble the sampler by unloading the filters and put each one into a 8 ml polyethylene vial, with self-sealing cap, provided by Ogawa USA.
2. Have two clean, completely dry shipping vials ready.

3. Remove the sampler ID labels from the PES storage vial and the pin-clip holder and put each one on a filter shipping vial. Save the gel packet for future use.
4. Hold the sampler body over the clean Petri dish, with one end pointing up. Remove the top end-cap of the sampler body (use clean flat forceps as a wedge, if necessary) and place the end-cap in the beaker for used end-caps.
5. Still holding the body above the Petri dish, tilt it to allow the filter and the two stainless steel screens to fall onto the dish. Use forceps, if necessary, to get the filter and screens out of the body, being **careful not to damage the screens.**
6. Separate the filter from the screens using the different clean forceps. **Remember that the end of the badge with the ozone filter should have a red mark**
7. Place the filter into the shipping vial, if the filter does not insert easily, fold the filter using the two forceps together. Then cap the shipping vial securely. **Write OZ or NO2 on the filter label ID.**
8. Repeat steps **4-7** for the other filter.
9. Put the vials in the refrigerator. Rinse the brown sampler storage vials and store it for future use. Complete the MOSES PES Data Log Sheet in ADEPT.

4.10 Blanks

Blank samplers should be prepared together with the field samplers and kept, as much as possible, at the same temperature as the field samplers. At least 10% of the samplers from each batch must be used as field blanks.

For this study, you should have approximately one blank per month for each batch of samplers. This brown storage vial containing a sampler to be used as blank must be kept closed and should be left out of the refrigerator in parallel with an exposure sampling that occurs approximately in the middle of the month.

<u>Blanks start-up and end</u>

1. Take the bottles out of the refrigerator at the time as the field samples and keep them at room temperature. Log sampler ID number and date on PES log sheet.
2. When the exposed sampler is returned, put both blank and exposed sampler (in their respective storage vials) in the refrigerator at the same time and process them in parallel. The total time out of the refrigerator (at room temperature, more or less) should be the same as that for every group of field samples. Then all the blanks will be comparable and can all be pooled for the analysis. The blanks for NO2 are stable for at least 6 months, even at room temp (but with badges containing both filters kept cold, they are probably stable a lot longer.) The ozone blanks are stable only when kept refrigerated.

4.11 Storage and shipping of exposed filters and blanks

The exposed and blank filters can be kept in the vial refrigerated for up to three months before shipping (less if their expiration date relative to the date of receipt precedes three months).

A group of exposed filters (and blanks) that are intended to be analyzed together must also be shipped together, by the fastest shipping available. Include a Shipping Log listing, each filter included in the shipment:

- shipping date,
- filter ID number
- exposure start date and end date
- PES exposure duration (in minutes)
- Contact information

Since it is would be difficult to determine the average temperature and relative humidity while the PES was exposed, RTI will use 70 % RH and 20 ^{o}C as the default. **The shipping date needs to be entered also in the PES Data Log Sheet.**

NOTE:

- Use insulated coolers with frozen ice-packs for shipping.
- Ship with priority overnight service.
- DO NOT ship out on a Thursday or Friday (just in case the package is not delivered the next day for any reason.)
- DO NOT ship the sampler with the filters because the samplers are needed for another subject.

SHIP TO:

Eva D. Hardison
Manager, Environmental Chemistry Department
RTI International
3040 Cornwallis Road, Bldg 6
RTP, NC 27709

Phone: 919-541-5926
Fax: 919-541-8778
e-mail: eva rti.org

Inform the RTI manager by phone or e-mail about the shipment.

4.12 Chemical analysis

The chemical analyses of the filters are contracted through the Research Triangle Institute in North Carolina. For ozone, the filter is extracted with ultra-pure (Milli-Q) water and the extract is analyzed by ion chromatography (IC) to determine the nitrate ion concentration, which is used to calculate the total amount of ozone collected. For NO2, the filter is extracted with ultra-pure (Milli-Q) water and then a color-producing reagent is added. The amount of colored derivative is determined by IC.

4.13 Recordkeeping

The PES Log sheet will be filled out directly in the ADEPT system. The spreadsheet from RTI with the results of the filters' analyses will be provided to the individual centers as well as to the Data Management Center. The concentrations of ozone and NO2 will be reported in ppb and will be blank-corrected and will be entered in the ADEPT system.

5. QUALITY CONTROL

The ADEPT System will provide QC checks of the data entered in the log sheets.

6. QUALITY ASSURANCE

HEI may request copies of the PES log sheets.

7. REFERENCES

Ward Brown K, Sarnat JA, Suh HH, Coull BA, Koutrakis P. 2009. Factors influencing relationships between personal and ambient concentrations of gaseous and particulate pollutants. Sci Total Environ 407:3754–3765.

Koutrakis P,Wolfson JM, Bunyaviroch A, Froehlich SE, Hirano K, Mulik JD. Measurement of ambient ozone using a nitrite-coated filter. Anal Chem 1993;65:209–14.

Liu L.-JS, Koutrakis P, Suh HH, Mulik JD, Burton RB. 1993. Use of personal measurements for ozone exposure assessment: A pilot study. Environ Health Persp 101, 318-.

Liu L.-JS, Olson III, MP, Allen GA, Koutrakis P, McDonnell WF, Gerrity TR. 1994. Evaluation of the Harvard ozone passive sampler on human subjects indoors. Environ Sci Technol 28: 915.

Liu L.-J.S, Koutrakis P. 1994. Assessment of Ozone Exposures in the Greater Metropolitan Toronto Area. J Air Waste Manage Assoc 45:223-234.

8. APPENDICES

1. Subject instruction for using the PES
2. Filter and PES Data Log Sheet (in ADEPT)
3. PES Filters Shipping Log

MOSES

Subject instructions for use of the personal exposure sampler (PES)

You will be given a zip-lock bag containing:

(1) a plastic backing with a clip,

(2) a plastic vial that contains a resealable bag and the PES (and a small gel packet) inside of it. The PES consists of a pin-clip holder that holds a barrel (or body of the sampler).

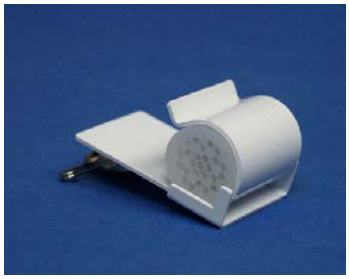
(3) Time Activity Diary daily forms (see separate instruction sheet).

Put the zip-lock bag (with its content) in the refrigerator as soon as possible after leaving the clinic and leave it there until the 3rd day before your exposure visit. You will receive a call to remind you.

START WEARING THE PES at 12:00 noon of the 3rd day before the exposure visit

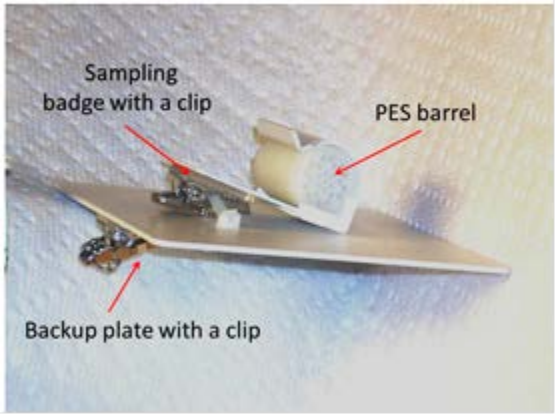
1. When it is time for you to start wearing the PES, open the zip lock bag and empty its content.
2. Open the vial and remove the PES from the resealable bag. Do not touch the ends of the PES barrel. Only touch the pin-clip holder.

Brown vial with PES inside resealable bag



PES consisting of a badge that holds the barrel

3. Replace the re-sealable bag (with the gel packet still inside) in the brown storage vial and tighten cap securely.
4. Put the vial in the zip lock bag. Keep the bag in a safe place as you will need to bring it to the clinic.
5. Secure the PES to the plastic backing as follows (see picture below): clip the pin-clip holder onto the wire tie loop of the backing so that the PES barrel (with little round holes on two ends) is in the middle of the backing. The air diffuses through the holes to the coated filters inside, so placing the PES barrel in the center of the backing (as shown in the picture below) minimizes losses on clothing.
6. Attach the backing to your clothes: If there is a pocket on the clothing at the height of your chest, use the clip to attach the backing. If not, use the safety pin on the clip to attach it.



7. On the Time Activity Diary, indicate the date and time you start wearing the PES; check off each hour you wear it in the Monitor column. If you do not wear it for a time period, please record where the device was and why you did not wear it in the NOTES column. Keep the diary with you or near you at all times.
8. You should not wear the PES, or bring it in the bathroom, if you take a shower (or bath). You should not wear the PES while in bed (i.e., at night), taking a nap, or participating in an activity where the monitor could break. When not worn, place the backing plate with the sampler facing up on a table outside the bathroom or near the bed, or close to where you are doing the activity.
9. Do not allow the PES to get wet outside in the rain, snow or heavy fog. Also do not allow the PES to be at temperatures below freezing. In all these instances wear the PES under a jacket or a coat.
10. Continue wearing the PES until you arrive at the clinic for the pre-exposure visit. Bring (1) the Activity Diary forms and (2) the plastic bag with the brown vial with the resealable bag inside to the clinic. It will be needed to store the PES until it is sent to the lab for analysis.

Filter and PES Data Log Sheet - SOP #41 Appendix 2

Site	**Subject ID**	**Sampler/Container ID**	**Ozone Filter Lot #** (stable for 1 year)	**NO2 Filter Lot #** (stable for 1 year)	**Date Received**	**Date Pads Expire**	**Date Samplers Assembled** (stable for 90 days at 5°C)	**Date Sampler given to Subject**	**Date Sampler Returned by Subject**	**Date Collection Pads sent for Analysis**

RTI Chain of Custody Form

3040 East Cornwallis Road, Durham NC
Building 6

Project Name: MOSES

Date of form: July 2, 2013

Person/Company/Client Name:
Address:
Phone:
E-mail:
Date Shipped:

For RTI Use Only
Date Received at RTI:
Person Logging in Samples:

						Place 'X' in cell for each species needed							
Sample ID	Use As Blank	Start Date	Start Time	Stop Date	Stop Time	NO2	NOx (+ NO2)	NH3	SO2	O3	Average Temperature (°C)	Average Relative Humidity (%)	Comments

Appendix 3

MOSES Standard Operating Procedure (SOP)

SOP # 42 – OZ DIARY

TITLE: Technician Instruction for filling out the Time Activity Diary (TAD)

Version: 1.0 **Directory/file[a]:** //

Author: MOSES Investigators **Signature:** **Date: March 23, 2012**

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

Revised by: **New version[b]:** **Date:**

Revisions/additions:

Signature:

ANNUAL REVIEW[C]

Signature	**Date**

SOP UPDATED TO VERSION [d]

Explanations: [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. <u>If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!</u>

[c] Annual review does not change the version of the SOP.

[d] Change the version # as necessary.

INSTRUCTIONS FOR FILLING OUT THE TIME ACTIVITY DIARY (TAD)

1. SCOPE and APPLICATION

The purpose of this SOP is provide instruction for the study technician for filling out the time activity dairy This procedure applies to recording the subject's activities during the 3 days preceding the exposure while wearing the Personal Exposure Sampler.

The purpose of the time activity diary (TAD) is to determine how and where our participants spend their time while they are wearing the Personal Exposure Monitor. We are particularly interested in whether they were traveling (car, bike, walking, etc.), where they were (home, work, other place), activities that may increase their exposure to particles (cooking, vacuuming), and whether they were exposed to a heating or cooling system. The technician's job is to help participants keep accurate diaries and ensure that they are complete.

This SOP contains an explanation of the components of the TAD, some examples of categories of activities, and a sample TAD. It is important to try to get as much detailed information as possible.

2. PREREQUISITES

2.1 Equipment and Supplies

None

2.2 Training Requirements

Familiarity with the TAD is recommended

3. CAUTIONARY NOTES OR SPECIAL CONSIDERATION

None

4. PROCEDURES

4.1 TAD Components and Instructions for Completing the TAD

The TAD will be given to the subject at the end of the Training visit or at the end of the first and second Post-Exposure visits. At the end of any of these visits, after you have scheduled the date of the pre-exposure day visit, and have explained the use of the personal exposure sampler, show the participant the diary. You should give him/her four copies of the diary after affixing a label with the Subject ID, and the four dates for the four days he/she will wear the monitor; the subject should bring the TAD back to the clinic at the next visit. The first day starts at 12:00 noon.

Explain the various parts of the diary.

1. The **time column** shows the time breakdown into 1 hour increments. The first day starts at 12:00 noon (the time the subjects should start wearing the Personal Exposure Sampler.) For the other days the diary starts at 6 AM.

2. The columns to the right of the time markers should indicate **whether the subject was wearing the monitor** during that hour. If the subject was wearing it for any portion of that hour, he/she should check the box for that hour. Be sure to inform the participant that they should be careful to explain where the sampler is at a given time if it is not being worn, in the NOTES section all the way to the right of the Diary.

3. Next to the right are columns asking about **the subject's location** during each hour.

   3.1 First, the subject should say **whether he/she was traveling**, and if so, whether it was by foot, bicycle/bike, motorcycle, car/taxi, bus, or train/subway. This should include time spent waiting for a train or bus. If the subject was traveling by a method (e.g. foot, bike, etc.) any portion of that hour, he/she should check the box for that hour. Thus, one could check off multiple "traveling by" boxes for that hour.

   3.2 Second, the subject should indicate the **location where he/she was** during that hour, and whether they were indoors or outdoors. If the subject was inside or outside work, home, or another place for any portion of that hour, he/she should check that box. Thus, one could check off multiple "I am currently at" boxes (as well as multiple "I am traveling by" boxes) for the same hour.

   a) "Home - Indoors " should be checked whenever the subject is anywhere inside the home, including the garage or basement. If the subject's home is an apartment, this would denote anywhere inside of the apartment building, including the laundry room.

   b) "Outside" should be checked whenever the subject is outside of his/her own home ("Home – Outside") or a neighbor's home ("Other place – Outside"). This does not include going into a neighbor's home, which would instead be ""Other place – Inside". This column would also refer to time spent on the participant's or a neighbor's porch, patio, or balcony. Please note, if a subject traveled to a neighbor's house and walked outside to do so, then "traveled, by foot" should also be checked.

   c) "Other place" refers to the time spent in other "indoor" or "outdoor" locations (a friend's house, a park, a restaurant, a shopping mall, a church, a grocery store, a gym, a movie theater, a stadium, a rink, a church.

   d) "Outside" should be checked whenever you are outside of your own home ("Home – Outside") or a neighbor's home ("Other place – Outside"). This column would also refer to time spent on a porch or balcony. If you travel to a neighbor's house and walk outside to do so, then "traveled, by foot" should also be checked.

4. Next to the right, are **Activity** columns asking about **what the subject was doing** in a location during each hour.

4.1 Explain that the "Activity" column must be filled out noting the participant's activities in the same 1 hour increments. If the subject begins an activity during one of the 1 hour time slots, they should record the activity in this space. There is enough room in this column to record more than one activity. Examples of which activities should be noted include sleeping, eating, watching TV, exercising, sweeping/dusting/vacuuming, painting/welding, and cooking. Painting and welding also include sanding and sawing. If the subject was doing that activity for any portion of that hour, he/she should check the box for that hour. As with other section, one can check off multiple "activities" per hour.

4.2 Inform the subject that detailed information about their cooking and cleaning activities is strongly desired. We are particularly interested in the type of cooking, such as frying, grilling, broiling, toasting, barbecuing, etc., which should be noted in the NOTES column.

4.3 Ask the subject to identify the person doing the cooking or cleaning activity for each entry in the Notes column.

5. Next, ask the subject to indicate **whether someone was smoking** in the room with them during that hour. NOTE: if the subject has filled out that box, you should find out more about the duration of the exposure and discuss with the PI whether the visit should be rescheduled.

6. Last, ask the subject to record if the room they were in **a room heated** by a kerosene heater or a wood burning stove or fireplace, or if the room **was cooled** by air conditioning. .

7. Conclude by saying that we would like the subjects to carry this diary with him/her during the day and to jot down the times when they change from one of these locations to another. Remind the subject that if he/she goes to more than one place, he/she should check all that apply for that 1 hour time period. If there is any problem in completing the diary, the participant should note the time and record the problem. He/she should try to be as specific as possible.

8. Tell the subject to start filling out the diary at the time he/she starts wearing the personal monitor (12:00 noon) and write the date and time on each page of the TAD. Ask the subject to note the exact date/time when he/she first wears the personal exposure sampler, if different from the one you wrote.

4.2 Sample TAD

Show the subject a sample TAD that has already been completed. This will give him/her an idea of what a completed TAD should look like.

4.3 Recordkeeping

The data will be entered in the ADEPT database.

5. QUALITY CONTROL – Review of the TAD

When the subject returns the TAD upon arrival for the pre-exposure visit, go over the diaries with the participant and ask for clarification of any incomplete items. Each day should start and end at 6:00 AM (except the first one), and there should be no gaps in the time. There should also be three meals each day. In addition, if the participant reports a change from an indoor or outdoor location, there should be some transit time in between. This would not apply for "Home – inside" to "Home - outside". However, it would apply for movement from "Home - inside" to "Other place-inside" or "Work-Inside" away from home."

6. QUALITY ASSURANCE

As part of the QA, NERI or HEI may request copies of the TADs

7. REFERENCES

None

8. APPENDICES

TAD

Multicenter Ozone Study in Elderly Subjects
ACTIVITY DIARY

Subject ID: _____

Date of this report: _____

Date/Time **directed by Technician to start wearing personal sampler** __ __/__ __/__ __ (MM / DD / YY) __ __:__ __ (HH : MM) AM / PM Circle One

Date/Time **you First put on the personal sampler:** __ __/__ __/__ __ (MM / DD / YY) __ __:__ __ (HH : MM) AM / PM Circle One

Please check ***all*** the circles in the table that apply to you. If during any portion of the indicated time period, the item/activity occurred, please check the box.

	MONITOR	LOCATION												ACTIVITIES								HEATING		COOLING	
		I am travelling by…						I am currently at…						I am…											
	I am wearing my ***personal*** sampler*	foot	bike	motor-cycle	car/ taxi	bus	train or sub-way	home		work		other place		cooking or near cooking	sleeping	exercising, or other type of exertion	sweeping, dusting, vacuuming (at work or home)*	painting, welding, (at work or home)	watching TV, reading, sitting at desk	eating a meal or snack	**Someone is smoking** in the same room	I am in a room being heated by a kerosene heater	I am in a room being heated by a woodstove or fireplace	I am in a room being cooled by an air conditioner	
TIME								inside	outside	inside	outside	inside	outside												**NOTES**
AM 6:00-6:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
AM 7:00-7:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
AM 8:00-8:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
AM 9:00-9:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
AM 10:00-10:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
AM 11:00-11:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
PM 12:00-12:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
PM 1:00 - 1:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
PM 2:00 - 2:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
PM 3:00 - 3:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
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PM 6:00 - 6:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
PM 7:00 - 7:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
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PM 9:00 - 9:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
PM 10:00 - 10:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
PM 11:00 - 11:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
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AM 2:00-2:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
AM 3:00-3:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
AM 4:00-4:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	
AM 5:00-5:59	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	❍	

* If you are not wearing the sampler, please explain in the NOTES column where you put the sampler.

Explanatory notes:
"I am traveling" column - include time spent waiting for bus, etc.
"Home, inside" column - include garage, basement, laundry room
"Home, outside" column - include yard, garden, patio, porch
"Other place, inside" column - include shopping mall, supermarket, restaurant, neighbor's home, movie theater, indoor stadium, rink, church
"I am, cooking" column - include outside barbecue (BBQ)
"I am, exercising" column - include playing sports (baseball, soccer, etc.)
"I am, painting, welding" column - include working on a hobby, e.g., sawing wood, sanding, etc.

For more information see the Subject Instructions for Flling Out the Activity Diary

MOSES Standard Operating Procedure (SOP)

SOP #51– AD EVENT

TITLE: Process for reporting an adverse event

Version: 1.0 **Directory/file[a]:** //

Author: Patti Nash **Signature:** **Date: April 11, 2012**

Revised by: Patti Nash **New version[b]:2** **Date: September 6, 2012**

Revisions/additions: the text has been made consistent with the protocol; description of for stopping the study was simplified. The section on Subject Withdrawal From the Study was expanded

Signature:

Revised by: Maria Costantini **New version[b]:3** **Date: December 21, 2012**

Revisions/additions: the definition of Grade 2 Adverse Events has been clarified to indicate that "*minimal non-invasive intervention or therapy is allowed*" (Section 2). This new text replaced the former text "no medical intervention or therapy required".

Signature:

ANNUAL REVIEW[C]

Signature	**Date**

SOP UPDATED TO VERSION [d]

Explanations: [a] Incorporate the version of SOP into the file name, e.g., Ind_sputum_v10_sop.

[b] If the revision is minor the version number is increased by 0.1, i.e., to 1.1. <u>If the revision is major, do not overwrite the old document but create a new one with the updated file name and a new cover sheet with the new version number, i.e., 2.0. You must keep the old version on the server in SOPS directory and in your files!</u>

[c] Annual review does not change the version of the SOP.

[d] Change the version # as necessary.

PROCESS FOR REPORTING AN ADVERSE EVENT

1. SCOPE AND APPLICATIONS

The purpose of this SOP is to provide guidelines and instructions for evaluating and reporting an adverse event and for deciding when the study should be stopped and resumed. Text in italics is taken from the Common Protocol of March 23, 2012.

2. DEFINITION

The Principal Investigator should be notified and the determination of the event grade should be made according to the following scale:

Grade 1 Mild: transient mild discomfort; no limitation of activity; no medical intervention or therapy required.

Grade 2 Moderate: mild to moderate limitation in activity, some assistance may be needed; minimal noninvasive intervention or therapy (such as over-the-counter medication, application of ice, etc.) is allowed.~~no medical intervention or therapy required.~~

Grade 3 Severe: marked limitation in activity; some assistance required; medical intervention or therapy required, hospitalization possible.

Grade 4 Life-threatening: extreme limitation in activity, significant assistance required; significant medical intervention or therapy required; hospitalization probable.

3. REPORTING

3.1 *Subjects should be encouraged to contact the clinical staff if any adverse effects are noted after visits to the laboratories. They should be given a 24-hour contact and pager numbers to report any potential adverse events*

3.2 *All adverse events shall be reported to the center's IRB in accordance with the institutional guidelines* and entered into ADEPT according to the process described in section 3.2.

3.3 The Project Coordinator shall fill out the Adverse Event Report Form (attached) in the ADEPT database (managed by the Data Analysis Center). *Grade 3 and 4 events shall be reported within 24 hours of the occurrence (or as soon as the center is notified of the occurrence)* ; *grade 1 and 2 events within 5 working days of occurrence (or as soon as the Center is notified of the occurrence*

Every event, regardless of grade, shall be reported separately even they occur after the same event.

3.4 The ADEPT program at the Data Coordinating and Analysis Center automatically will send out an e-mail to the Steering Committee and the DMB, and upon the entry of a grade 3 or 4 event; the e-mail will state the cumulative number of adverse events that have occurred across sites. The e-mail will also alert all investigators in the event that the study needs to be stopped based on an adverse event.

If the adverse event is not resolved at the time Adverse Event Report Form is submitted, a follow up Adverse Event Reporting Form shall be submitted. All events should have a final status given to them prior to study end.

3.5 *The Data Coordinating and Analysis Center shall periodically generate a report of all adverse events and submit to the Steering Committee, the DMB, and HEI.*

4. SUBJECT WITHDWAL FROM THE STUDY

Subjects with 2 or more Grade 3 adverse events or one grade 4 adverse event shall be considered to have completed the study and should receive the full compensation. The participant will not be asked to participate in any remaining exercise, exposures or other data collection activities.

5. STOPPING THE STUDY

If the adverse event meets one of the following stopping rules, the entire study (at all participating centers) shall be stopped.

Rules:

1. *Any Grade 4 adverse events.*
2. *Any grade 3 adverse event* that requires hospitalization.
3. *Five subjects* (cumulatively across all sites) *with Grade 3 adverse events*.
4. In all these cases ADEPT will generate an alert and the NERI Data Manager will inform the MOSES Steering Committee, the DMB, and the HEI Project Manager and the HEI Director of Science, provide the Adverse Event Report(s) for said event(s), and state that the study has to be stopped.

6. RESUMING THE STUDY AFTER STOPPAGE

The study will not be resumed until all the adverse event(s) information has been discussed with the Steering Committee, the DMB, and the relevant IRBs and HEI have concurred with resumption of the study. HEI will send an official notification to the Principal Investigator after the determination of resuming the study has been made. For details about the process for resuming the study see Appendix B "MOSES Organizational Structure and Safety Monitoring Plan' of the Manual of Operation. Once the study has resumed the AE count should go back to zero.

7. RECORDKEEPING

All adverse events shall be reported to the center's IRB in accordance with the institutional guidelines and entered into ADEPT according to the process described in section 3.2.

8. ATTACHMENTS

Adverse Event Report Form

MOSES Study
Adverse Event Report Form

A1. Subject ID Label
A2. Date site became aware of the AE: __ __ - __ __ __ - __ __ __ __

General Adverse Event Information			
B1.	Is this an initial or follow-up adverse event report?	☐ Initial ☐ Follow Up	
B2.	AE Number (Populated from ADEPT)	__ __ __	
B3.	What is the adverse event term? USE MEDDRA TO POPULATE _____ _____ _____ _____ _____		
B4.	What is the date the adverse event started?	__ __ - __ __ __ - __ __ __ __	
B5.	Time when the adverse event started	__ __ - __ __	
B6.	Is the adverse event still ongoing?	☐ Yes ☐ No	
B7.	What date did the adverse event end?	__ __ - __ __ __ - __ __ __ __	
B8.	At what time did the adverse event end?	__ __ - __ __	
B9.	Enter descriptive narrative: _____ _____ _____ _____ _____ _____ _____ _____ _____ _____		

MOSES Study
Adverse Event Report Form

A1. Subject ID Label
A2. Date site became aware of the AE: __ __ - __ __ __ - __ __ __ __

Adverse Event Severity and Associations		
C1.	What was the severity of the adverse event? ☐ Mild (Grade 1) - transient mild discomfort; no limitation activity; no medical intervention or therapy required. ☐ Moderate (Grade 2) - mild to moderate limitation in activity, some assistance may be needed; minimal noninvasive intervention or therapy (such as over-the-counter medication, application of ice, etc.) is allowed. ☐ Severe (Grade 3) - marked limitation in activity; some assistance required; medical intervention or therapy required, hospitalization possible. ☐ Life threatening (Grade 4) - extreme limitation in activity, significant assistance required; significant medical intervention or therapy required; hospitalization probable.	
C2.	What was the relationship between the adverse event and the study treatment(s)	☐ Definitely related ☐ Probably related ☐ Possibly related ☐ Not related
C3.	Did the adverse event result in hospitalization?	☐ Yes ☐ No
C4.	Did the adverse event result in death?	☐ Yes ☐ No
AE Actions and Outcomes		
D1.	What action was taken with study treatment?	☐ Study treatment/procedure stopped ☐ Study procedure/treatment temporarily stopped and restarted ☐ No action taken ☐ Other
D2.	What other action was taken in response to this adverse event? _____ _____ _____	
D3.	What was the outcome of this adverse event?	☐ Recovered/resolved without sequelae ☐ Recovering/resolving ☐ Not recovered/not resolved ☐ Recovered/resolved with sequelae ☐ Fatal ☐ Unknown
D4.	Did the adverse event cause the subject to be discontinued from the study?	☐ Yes ☐ No