Standard Operating Procedures

CardiovascuLar Effects of Aerosols in Residences (CLEAR) Study

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Preface

This document describes standard operating procedures (SOP's) for exposure and health outcome measures of the CardiovascuLar Effects of Aerosols in Residences study (CLEAR). The SOP's that are included result from multiple revisions including input from numerous study technicians and students over the course of several years. The lead authors acknowledge the valuable contributions of this large group of individuals. The CLEAR study was supported by the Canadian Institutes of Health Research (funding reference number 111042).

Study Protocol I - Home Visits

Home visits are labeled as X, 1, & 2

- X for the initial visit (blood only and all equipment set-up)
- 1 for the first visit with blood draw and endoPAT performed
- 2 for the second visit with blood draw and endoPAT performed

Afternoon / Evening before Visit X

Environmental technician to call participants to remind them of next day's visit, of procedures, of the need to fast but to have water in the evening and in the morning (but no tea, coffee, juice). Also confirm time – noting the expected time that visit X will take. Contact other team members if time is changed from that on calendar.

Visit X – Initial set-up

1) Introduce yourselves and thank participants for taking part in the study;

2) Both Techs: explain what you are going to be doing at this visit and where:

   a) **Environmental technician**: explain that you have some preliminary work to get the instruments up and running and where you would like to do this set-up. That, once ready, you will be placing the instruments in their living room and outdoors – ensuring that the placing of instruments in both locations is okay with subjects.

      i. Equipment set-up:

      Indoors – bedroom:
      - Air cleaner (+/- HEPA filter) – toss coin to decide, and record

      Indoors – main room:
      - Air cleaner (+/- HEPA filter, same as bedroom)
      - Nephelometer – ensure it is logging data
      - Harvard Impactor
      - Pump – calibrate pump flow before starting
      - UFP
      - Field blank filter (if scheduled)
      - HOBO temp and RH monitor
      - Langan CO monitor

      Outdoors:
      - Nephelometer – ensure it is logging data and heater is on
      - UFP
      - Harvard Impactor
      - Pump – calibrate pump flow before starting

      ii. Get GPS reading for home.

b) **Health Technician**: explain that you have some paperwork to fill out with them starting with the consent form after which you will answer any questions they may have. Ensure the participants read the consent form, making special note of the last page's bulleted items. Quickly review consent form once signed to make sure completed correctly. If there are two participants, each must sign a separate form. Let participants know that they will have a copy of the consent left in the booklet which is left in their home for the duration of sampling

i) Once the consents are completed, you can let the people know that you have a few short questionnaires to complete with them and a few other administrative tasks, but that you could do the blood draws first and then do the questionnaires (if they are anxious for a cup of tea, coffee, or something to eat)

ii) Perform the blood draw on each participant (1 – 10ml gold SST and 1 – 6ml lavender EDTA tube per participant). Find a suitable place to draw the blood, use a blood bloc pad underneath the participant's arm. Ensure all necessary items are within arm's reach. Ensure the correct label is placed on the tube (subject A / subject B). Fill in the date on the tube(s) labels and the date on the biohazard bag label. Place the blood tubes into the biohazard bag with the bag label and place in cooler.

iii) Complete the Dwelling Info form with participant(s)

iv) Go over the other forms that are a part of the booklet:

- Study Contacts Sheet: if there are any problems/questions/concerns ask them to give us a call
- Yellow (health log) sheets: point out that these sheets are for you, the Health Tech, to use at the next 2 visits
- Pink (weekly meds & activities) sheets: will be filled out by them but you would like them to fill them out with you at the next and last visit as they pertain to the week prior to the endoPAT tests (ask them to have a look at them so they know what will be asked)
- Diary sheets: to be filled out each day noting the "subject A" and "subject B" sections and the "Location" key

3) Both Technicians: Make sure everything is cleaned up (all garbage generated by all tests/procedures to be brought out of the home).

4) Environmental Technician: Ensure that the placing of instruments (indoor and outdoor) is going to work for the subjects. Also ensure that they know what to do if something stops running (call us), falls over, needs to be moved or is impacting on them too much (call us). Reiterate that the instruments will/should run continuously until we return the next week. That we do not want them to move the instruments and to call us if they do need to be moved. And lastly, they do not need to do anything in the interim unless they notice something is too quiet or something else seems wrong.

5) Lastly: Thank them for their participation and confirm that you will see them at the <u>same time</u> next week. (You may want to take the opportunity to remind what tests will be done at the next visit and how long you will be there for next week)

Day 7 – Reminder phone call

Phone participant(s) to confirm visit the next morning. Remind them about fasting, but drinking water (helps with blood draw), that endoPAT measurement(s) will be done with each participant and expected duration of visit.

Visit 1 – Sampler change and health sample measurements

Health Tech: (complete in this order)

1) Remind participants of tests to be done
2) Decide which participant is to go first
3) Do blood pressure measurement with participant(s)
4) Do endoPAT measurement with participant(s)
5) Collect blood sample from participant(s)
6) Complete Health Symptom log with participants - Irritant page to be completed by participant(s)
7) Review activity log and ask for clarifications as needed
8) Confirm visit at same time next week – remind about fasting, blood draws and endoPAT

Environmental Tech:

1. Equipment:

    Indoors – bedroom (once EndoPAT measurements are complete!):

    - Air cleaner – add or remove HEPA filter
    - Check UFP (alcohol)

    Indoors – main room:

    - Air cleaner – add or remove HEPA filter
    - Nephelometer – download data and restart, ensure it is logging correctly
    - HI – change filter
    - Pump – record time and volume, check flow rate with BIOS, download history, re-calibrate and start
    - Field blank filter (if scheduled)
    - HOBO – download data, relaunch
    - Langan CO – download data, relaunch

    Outdoors:

    - Nephelometer – download data and restart, ensure it is logging and heater is on
    - HI – change filter
    - Pump – record time and volume, check flow rate with BIOS, download history, re-calibrate and start

Day 14 – Reminder phone call

Phone participant(s) to confirm visit the next morning. Remind them about fasting, but drinking water (helps with blood draw), that endoPAT measurement(s) will be done with each participant and expected duration of visit. Confirm time to arrive.

Day 15 – Equipment removal and health sample data collection

Health Tech: (complete in this order)

1. Remind participants of tests to be done and order of the procedures
2. (Start with participant that went first the week before)
3. Collect blood pressure measurement
4. Do endoPAT measurement
5. Collect blood sample
6. Complete Health Symptom log with participants - Irritant page to be completed by participant(s)
7. Review activity log and ask for clarifications as needed
8. Confirm that all questionnaires are completed and legible; asking for clarifications where necessary.

Environmental Tech:

1. Confirm with participants that there were no problems with instruments during the week.
2. Remove all equipment and samplers:

   Indoors:

   - 2 Air cleaners (bedroom/living room)
   - Nephelometer – download data
   - HI – remove filter
   - Pump – record time and volume, check flow rate with BIOS, download history
   - UFP
   - HOBO – download data
   - Langan CO – download data
   - Other miscellaneous instruments (or cords, etc.) taken into the home

   Outdoors:

   - Nephelometer – download data
   - HI – remove filter
   - Pump – record time and volume, check flow with BIOS, download history
3. Take blood to iCAPTURE Lab – St. Paul's Hospital. See instructions on page 6.

Before leaving:

1. Explain when approximately (1-1.5 weeks) and how (by cheque in the mail) they will receive the honorarium and their summary of study results (and that it will be at the earliest October 2012).
2. ***Thank all residents and participants and ask if they know of anyone else living nearby who may be interested in participating*.**

Checklist for Visit X:

Forms (all should be in the house booklet):

- Blank Consent forms (2 per subject)
- Environmental Data log sheets
- Health Log Sheet
- Dwelling Info form
- Blank Activity log (for 14 days)
- Contact form to leave with participant

Equipment and Samplers: Environmental Tech

- 2 Air cleaners, with HEPA filter (1 large and 1 small)
- 2 Nephelometers (for Radiance nephelometers: 2 fans, 1 heater, 2 AC adaptors)
- 2 clean Harvard Impactors, with clean impactor plate installed
- 2 appropriately-labeled filters for Harvard Impactor, loaded into yellow holders
- 1 field blank filter, if needed
- 2 Pumps, with AC adaptor – plus spare pump if possible
- 1 BIOS pump calibrator
- 1 HOBO, launched and ready
- 1 CO, launched and ready
- GPS unit
- Laptop with power cord

Supplies: Environmental Tech

- 1 Outdoor nephelometer case
- 1 Outdoor HI case and stand (tripod)
- 1 Indoor case (for pump noise reduction and as stand for instruments)
- 1 Outdoor extension cord
- 1 Indoor extension cord
- Tubing and adaptors for pump and HI

Supplies: Health Tech

- Blood block pads
- Blood collection tubes
- Sharp's container
- Alcohol wipes
- Gloves
- Garbage container
- Needles
- Cooler and cold packs for blood tubes
- Bio-hazard bags for blood tube transport
- CLEAR labels for bio-hazard bags

Checklist for Visit 1 and Visit 2

Forms (all should be in the home booklet):

- Health Log Sheet
- Symptoms Questionnaire (1 per subject)

Equipment and Samplers: Environmental Tech

- Laptop with power cord
- 2 HEPA filters (if not currently installed)
- 2 new appropriately-labeled filters for Harvard Impactor, loaded into yellow holders
- 1 field blank filter, if needed
- 1 BIOS pump calibrator

Supplies: Health Tech

- EndoPAT with laptop and accessories
- Unused EndoPAT probes
- Arm supports
- Blood pressure monitor
- Needles, Gloves, tourniquet
- Garbage container
- Labeled blood collection tubes (one 10ml gold SST and one 6ml lavender EDTA)
- Antiseptic wipes, Band-Aids, gauze
- Sharp's Container for used needles
- Cooler with frozen ice-packs & bio-hazard bag for tubes

Post-visit activities: Environmental Tech

1. Take blood to St. Paul's Hospital – drop at iCAPTURE Lab in fridge (clear door) and write on whiteboard that CLEAR Samples are in the fridge. If lab is not open, blood can be left at the iCAPTURE reception, but always try lab first. Sheets were made ½ way through the study to pass to the receptionist when blood was put in the fridge so that he/she would remember to call the lab about the blood.
2. Harvard Impactor filters:
   - Give to research coordinator to take to UBC.
3. Harvard Impactor:
   - Disassemble and clean all parts according to protocols, including impaction plate.
   - Reassemble HI with clean, oiled impaction plate.
4. Data files:
   - Back up all computer data files
   - Enter paper data into computer spreadsheets
   - Give all data to Ryan when data sheets updated (upload onto secure shared drive)
   - Store all paper records securely in locking file cabinet

Post-visit activities: Health Tech

1. Ensure adequate supplies for next visit
2. Supply memory stick to Environ Tech for data backup

Study Protocol III - Equipment Set-up

Sampling Equipment Set-up Overview

Indoor set-up

Air Sampling Instruments

- Locate in main living room of home.
- Within this room, instruments should be located as far away as possible from both the air cleaner and a wood stove, if present.
- Select a location in this room that is close to an electrical outlet and that is approximately 1 meter from walls, windows, air conditioners, and any other ventilation outlets/inlets. Try to satisfy as many conditions as possible, making sure that the sampler is away from normal traffic. **Note**: Avoid locating samplers in a corner because turbulence is likely to occur.
- You will need to use a power bar to plug in everything.
- The nephelometer case forms a platform for the equipment – place it horizontally on the floor
- The pump will be running inside the nephelometer case (to reduce the noise disturbance to residents). Tubing and power cord will run out of the nephelometer case hole.
- The nephelometer, Harvard Impactor, and HOBO monitor will be placed on top of the nephelometer case. Ensure the nephelometer and Harvard impactor are at opposite ends so that their airflows do not disturb each other.

Outdoor set-up

Air Sampling Instruments

- Locate in a secure area outside home – preferably backyard
- Select a location that is reasonably close to an electrical outlet and that is approximately ***1 meter from any walls***, trees, and any other large objects. Try to satisfy as many conditions as possible, making sure to place the sampler away from busy areas.
- The nephelometer case forms a protective case for the nephelometer (with fan and heater) and pump. Place it horizontally, preferably on top of two milk crates or other platform (so that it is not in snow).
- A power cord, the pump tubing, and flexible tubing for the nephelometer will come out of the U-shaped inlet in the case.
- For the nephelometer, the flexible tubing should be attached to copper tubing, so that the inlet height is approximately 1 m off the ground, and so that it is pointing down (i.e. so rain and snow will not enter the tubing).
- The Harvard Impactor will be placed upside-down (i.e. inlet down) in the grey Pelican case, attached to the tripod. Ensure tripod is stable and will not be tipped. It may be necessary to secure tripod legs with heavy rocks or other means.

Study protocol IV - Pre- and Post-Weighing of Filters

All preparation and post tests/analyses of filters are being performed at/by the SPPH OEH Lab, UBC

To start: New filters and filters returned from the field must be stored in the lab's weighing room for a minimum of 48 hours to allow them to equilibrate to temperature and humidity before any pre-weighing or post-weighing. For the CLEAR Study, all filters will need to undergo the following in the order listed:

1. Equilibration – 48 hour minimum
2. Pre-weighing
3. (Sent into the field for sampling with the exception of lab blanks)
4. Equilibration – 48 hour minimum
5. Post-weighing (field blanks included)
6. Reflectance
7. Levoglucosan and Hopane analysis

Equipment:

- Teflo® w/ring 37mm membrane – Pall Part# 22PJO37 – CA28139-109 (50/pack)
- Clean petri dishes
- Forceps and tweezers
- Labels (or appropriate tape to make labels) and non-smudge pen (labels need to peel off and re-adhere easily)
- Filter weighing log sheets
- Control Filters (on shelf in weighing room) – for CLEAR we are using the Ministry of Environment Q.C. Filters – numbers 4, 5, & 6.

Procedure:

Preparation:

- Store filters (new or those returned after sampling) a minimum of 48 hours in the lab's Environment Room before weighing (room needs to be within appropriate temperature and relative humidity range)
- For each filter to be weighed, put 3 Labels with identical filter ID on each petri dish
- For new filters, hold each up to light to inspect for holes, damage or defects prior to weighing. If there is any damage to filter, discard and select a new filter for use.
- Weigh 3 gravimetric control filters *before* and re-weigh at least 1 filter *after* each weighing session – Use the same control filters for pre and post weight (for CLEAR use the Ministry of Environment Q.C. filters – numbers 4, 5, 6).
    - Record weights in Control QC spreadsheet
    - Check that control filter weights are within 2 standard deviations of the mean of all prior measurements (for each filter). If not, start procedure again.

Weighing:

- Record date, temperature, RH and your name on filter weighing log sheet (ensure room is in compliance – see door)
- Tare balance
- Using tweezers, pass filter between electrostatic reduction plates a few times
- Smoothly open the balance lid, carefully place filter in centre of pan and close lid – ensuring that it is completely closed
- Once stability circle appears on balance display, record weight on sheet
- Remove filter and allow balance to return to zero. Tare if necessary.
- Weigh each filter three times (ensuring balance is at zero between weights and filter is passed between electrostatic reduction plates).
- Record 3 weights on filter weighing log sheet
- If repeat weights differ by more than 0.01, restart.
- For example, 102.112 to 102.122 ok
  102.112 to 102.130 not ok
  This must be true among all three weights
- Place weighed filter in pre-labeled Petri dish.
- Record temperature and RH again at the end of the weighing session.
- Store UNUSED (blank) pre-weighed filters in their Petri dish on appropriately labeled shelf (CLEAR Study) in weighing room.
- Store post-weighed (USED) filters in their Petri dish on shelf after sampling, prior to reflectance measurements.

Study Protocol V - Harvard Impactor Sampling

The order of steps at a visit is dependent on whether the visit is the initial visit, the second visit or the final visit.

1. **Steps for initial visit – Visit X:**
   a) Install new filter into HI
   b) Perform pre-calibration
   c) Deploy
2. **Steps for visit 1 – after first 7-day sampling session:**
   a) Perform post-calibration
   b) Remove used filter
   c) Download and save pump data
   d) Perform field blank if scheduled
   e) Install new filter into HI
   f) Perform pre-calibration
   g) Deploy
3. **Steps for final visit – end of second 7-day sampling session:**
   a) Perform post calibration
   b) Remove used filter
   c) Download and save pump data

Pre-session prep at the lab – prior to Visit X

Loading new filters into filter cassettes

1. Ensure that all equipment and surfaces are clean. See CLEANING – Appendix 1
2. Carefully open the Petri dish and using forceps remove the filter by grasping only the outer support ring. It's a good idea to have a quick look at the filter to ensure there are not defects. If you suspect a defect replace the filter and do not use it. If the filter passes your scrutiny, insert the filter into the bottom "female" or recessed half of a clean yellow cassette. The PMP Support Ring (shiny, thick ring surrounding the Teflon filter material) should be facing up (**Fig. 1**). Place the top "male" piece snugly over filter and bottom piece.
3. Place cassette with filter into the filter's labeled Petri dish and seal with tape, indicting it is ready for sampling.
4. A former practice was to use coloured tape to indicate whether a filter had been used or not. I'm not convinced this was always done correctly. If you find this helpful, please use different coloured tape around the Petri dish after the filter has been used.

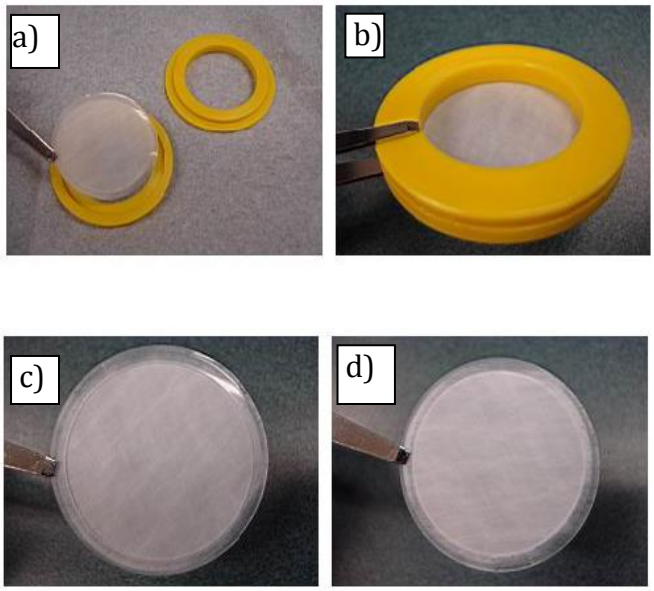


Figure 1: Proper filter placement into an Anderson filter holder. a) Proper placement of filter (use forceps as shown ensuring to grasp filter by only the outer ring). Also ensure that the shiny side of the PMP ring on the filter is facing upwards (refer to Figure B1-2c) and is placed in the recessed half of the cassette. b) Properly loaded Anderson filter holder. c) Photo showing the shiny side of the PMP ring. This side should be facing towards you when filter is placed into the recessed side of the Anderson filter holder. d) Photo showing the dull side (bottom) of the PMP ring.

A. Upon Initial Arrival at the Home

Prepare indoor work surface by laying out a large Kimwipe. Prepare impaction plate if not done ahead of time: Take a clean impaction plate and add one drop of impaction plate oil onto the sintered metal surface and let it sit while you proceed with the steps below.

Record the Date, home, Technician and the Leland Legacy pump ID on the *Sampling Log Sheet*. Proceed with loading the filter into the HI, reassembling, and calibrating.

Pump Calibration using the BIOS DryCal DC-Lite Flow Calibrator (Must be completed OUTDOORS for the OUTDOOR Sampling – Indoors for the Indoor sampling). Prior to Calibration, a sample filter must be installed. Prior to a sample filter being installed for calibration, a Field Blank must be performed if one is scheduled.

B. Installing a New Filter – Field Blank or Sample Filter

** The sample filter must be installed in the Harvard Impactor to calibrate and to perform post calibration.

B.1 If a <u>Field Blank</u> is scheduled, follow these instructions. If no Field Blank for this session, proceed to B.2

1. If a filter field blank is scheduled for the coming session, prepare that filter in the lab the same as a sample filter (i.e. load into clean yellow filter cassette and place into its Petri dish).
2. Always process the field blank **before** the sample filter:

   ✯ Install field blank filter or sample filter (in its cassette) into the cleaned and assembled Harvard Impactor:

   i. Remove the base of the Harvard Impactor by flipping the clamps at the base open;

   ii. Place the loaded filter cassette into the recessed hole containing a rubber gasket located in the base of the Impactor (**Fig. 2**). Cassette should be placed in orientation as in the picture to the right;

   iii. Clamp body of the Impactor and its base back together;

   iv. Remove one of the filter's 3 ID labels from the Petri dish cover and stick it onto the sampling log sheet being careful to place it in the correct spot:

   ✯ i.e., FIELD BLANK ID label needs to be placed in the field blank sticker spot on the field sheet;

   Note: Field blanks in their Petri Dishes will be returned to UBC with 2 ID labels whereas sample filters will return with only 1 ID sticker on their Petri Dish.

   v. Close the Harvard Impactor and then wait about 30 seconds;

   vi. After the 30 seconds, open the Harvard Impactor, remove the filter and its yellow cassette and place back into its Petri dish. Treat as a regular filter from now on.

3. Proceed with installing the sample filter and continuing with equipment set up and session start.

B.2 Inserting a New Sample Filter into the Harvard Impactor (MUST BE COMPLETED INDOORS)

1. Remove the new sample filter (pre-loaded in yellow Anderson filter holder cassette) from its labelled Petri dish.
2. Place the filter in the recessed hole containing a rubber gasket located in the base of the Harvard Impactor (be careful not to touch the filter) and clamp the body of the Impactor and its base back together (Figure 2 below). You can check to make sure the filter is aligned by removing the sampler inlet and looking down into the sampler. You should see a white circle (no edges) filling the hole.
3. Remove one of the three Filter ID stickers from the Petri dish and stick it onto the Harvard Impactor housing.
4. Remove the second Filter ID sticker from the Petri dish and stick it onto the sampling log sheet under FILTER IN – FILTER ID.
5. Place a Ziploc bag over the sample inlet and secure with elastic band if unit not deployed directly.

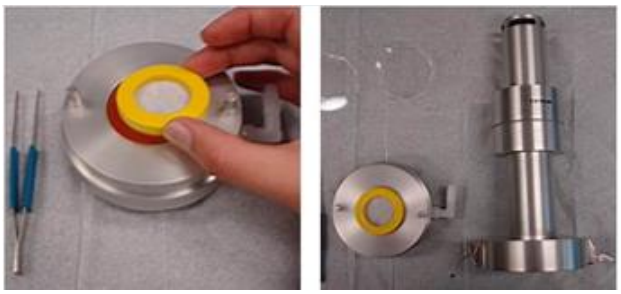


Figure 2 – Proper placement of filter into Harvard Impactor

C. Calibration

1. Bring Harvard Impactor, Leland pump and BIOS DryCal outside or remain inside to perform. Make sure the DryCal DC-Lite flow calibrator is placed on a flat, level surface.
2. Turn the Leland Legacy pump on (press the '▲▼' buttons simultaneously) and let run for at least 5 minutes (to warm up for calibration procedure).
3. After the pump has warmed up for at least 5 minutes, turn it off and reconnect to the HI. Ensure that you push the tubing firmly down onto the pump and twist a few times to secure adequately.
4. Remove the inlet (uppermost part) of the Harvard Impactor, and replace with the calibration cap (Figure 3:(a). Attach calibration cap tubing to the TOP outlet of the BIOS

(labelled *SUCTION* on the BIOS Defender 510 and *INLET* on the BIOS DC-Lite). Refer to setup in (b) Figure 3.

Figure 3 a) calibration cap [with tubing, inlet cap beside], b) calibration cap on HI attached to BIOS, c) HI, BIOS and Pump assembly for calibration

5. Connect the Leland Legacy pump to the Harvard Impactor base with tubing (Figure 3: (c) above).
6. Ensure the BIOS DryCal is placed on a level surface (if not, the measurement will be affected). Turn the BIOS DryCal on.
7. Turn the pump ON by pressing '*' on the keypad and begin flowing air through the pump by pressing the two ▲▼ keys simultaneously.
8. To begin calibration you must be in 'SETUP'. To get into SETUP press 4 keys in the sequence of: *▲▼*. This will bring 'SETUP' up on the LCD screen. It can be a tricky with some pumps to get into SETUP; just keep trying.
9. Once in 'SETUP' ensure the pump flow is set at 10.00 L/min. If necessary, set the pump flow rate using the ▲ or ▼ keys.
10. Once the pump is set at 10.00 L/min press the '*' key until you see 'ADJ FLOW' written diagonally across the top of the screen (the 'ADJ FLOW' will not flash, but the 'SET' and the numbers will).
11. On the Dry Cal highlight 'MEAS' on the screen; press enter. Highlight 'CONT', press enter. This will get a continuous flow reading (i.e. every 2 seconds or so) in units of litres per minute (L min-1). Press the 'READ' button & hold it down until it performs a continuous (i.e., every 2 seconds or so) flow reading to two decimal places, in units of litres per minute (L/min).
12. Adjust the pump flow rate using the ▲ or ▼ key until the flow calibrator screen shows an averaged reading as close to 10.00 L/min as possible.
13. Once pump is calibrated, allow the DryCal to run through three 10-reading cycles, and record the third average value. If the pump has strayed from 10.00 L/min, re-calibrate (steps 4-12).
14. Once calibration is complete, press '*' until you see 'Clr' on the LCD; then press the '▲▼' keys simultaneously to clear the pump's memory.
15. Press '*' key again, until the LCD screen reads 'End' (first it will read ESC then End). When it says 'End' press the '▲▼' keys simultaneously. This will exit the Setup session

and return to the LCD screen display mode where the pump should read 'HOLD' (blinking diagonally on the top centre) and 0.0 mins.

16. Remove the calibration cap and reattach the inlet. Pump and HI are now ready to deploy.

D. Downloading the Pump Calibration

1. Connect the Datatrac cable to the Leland pump and download the calibration data.
2. In the Leland Legacy pump software, from the menu select 'View' then 'Pump History'. Records of stored pump history data will appear in tabular form on the screen.
3. In the SKC Pump History window, click on 'File' then 'Save as Comma Separated Text', and then type the file name and save it to its assigned folder. Use the following file naming convention (red indicates where specific information needs to be inserted:

   CLEAR_HOMEID_PumpSerial#_FilterID_date_CAL.txt

   For example if the home is 05, the pump ID is LLP04, the filter number is W02 and the date is Dec 07/12, the file name would be:

   CLEAR_04_FixedSitePumpData_LLP04_ W02_Dec07_12_CAL.txt
4. Verify that the data has been saved properly by opening the Comma Delimited File in Excel. Look at the dates and data values to determine if the data seems reasonable or if any errors occurred.

E. On return to home after each 7-day sampling session - Record Pump Flow Rates

1. Put the pump on hold by pressing the '▲▼ ' buttons simultaneously. Remove Harvard Impactor from case and take with pump to where post calibration will be performed. Do not remove the sample filter yet.
2. Remove the Harvard Impactor sampling inlet and attach the calibration cap and tube. (Refer to Figure 2 photos, page 2). Attach calibration cap tubing to the TOP outlet of the BIOS (labelled *SUCTION* on the Bios Defender 510 and *INLET* on the Bios DC-Lite). Turn on the BIOS DryCal and allow it to warm up a bit (should already be warm, but no harm making sure it's nice and warm).
3. On the BIOS DryCal, highlight 'MEAS' then press enter. Highlight 'CONT', then press enter. The BIOS DryCal should start to take continuous measurements (every 2 seconds or so). Make sure the BIOS DryCal is level otherwise measurement will be affected.
4. Turn on the Leland Legacy pump by pressing the '▲▼ ' buttons simultaneously.
5. Let the BIOS DryCal run for three sets of 10 measurements. Press the 'STOP' button on the 10th measurement of the third set.
6. Press the '▲▼ ' buttons on the Leland pump simultaneously to stop the pump.
7. Record the 3rd 10-measurement average on the log sheet under POST FLOW RATE.

8. Record the total time elapsed and the air volume sampled for the Leland pump on the field sheet under **TOTAL TIME** (minutes) and **AIR VOLUME SAMPLED** (litres) respectively on the log sheet. To obtain this information, use the '*' key on the Leland pump to advance through the information screens. It is imperative that the information be recorded to correspond to the appropriate FILTER.
9. Remove the calibration cap from the Harvard Impactor and replace with the inlet cap.

F. Download Pump Data (at completion of sampling session)

1. After determining post flow rate, download pump data.
2. Connect DataTrac cable to the top of the pump (next to the port for the power charger), and connect the other end (RS-232 connector) to the back of the laptop.
3. Click on the 'Leland Legacy' Icon on the desktop. You will need to see the little handshake which confirms that the software is communicating with the pump.
4. Once in the LL pump software, go to menu bar in the upper-left hand side corner of the screen, and click on "Pump History". Records of stored pump history data will appear in tabular form on the screen.

   Click on "**Save as Comma Delimited File**", and then type in a destination. Save as:

   C:\CLEAR_HomeID_PumpData_[pumpID]_[date:from_to]

5. If you get an error message from the LLP software reporting that communication with the pump has failed, click on the "Retry" button, and communication will be re-established with the pump.
6. Turn the pump off by pressing the '*' button & holding it down until you see the pump do a shut-off countdown "OFF 3, 2, 1".
7. Verify that the data has been properly saved by opening the Comma Delimited File in Excel. Look at the dates and data values to determine if the data seems reasonable or if any errors occurred. Save a second copy of the file on a USB key.

- *Note 1: It is important to save the data on a USB key at this point. This protects the data in case of a computer software or hardware malfunction.*
- *Note 2: Only after the Pump's Volume, Time and Post Flow have been recorded can you prepare the pump for the next sampling session. (You can download the pump data a little later prior to redeploying the pump if necessary.)*

G. Removing the Loaded Filter and Cleaning the Harvard Impactor

****MUST BE COMPLETED INDOORS – ensure POST CALIBRATION is performed prior to filter being removed from Harvard Impactor**

1. Open the base of the Harvard Impactor by flipping the clamps at the base open.
2. Carefully remove the filter from the Harvard Impactor and gently place it [without removing it from its yellow cassette] into its corresponding labelled petri dish. Be careful not to touch the filter itself.

3. Remove the filter label on the housing of the Harvard Impactor and adhere it to the sampling sheet under FILTER OUT – FILTER ID (there should now be 1 label on the petri dish and 2 labels for this particular filter on the field sheet).
4. Secure the petri dish closed with tape. Place the petri dish into a Ziploc or other bag and place into the foam-padded box for transport back to the lab.

   ***Note:** It is important that the filters, which contain samples, are not jostled. Ensure that they are carefully handled and placed securely in the foam-padded box for transport back to the lab.

5. Disconnect the tubing connecting the pump and HI at the pump end. This step can involve some jarring of the HI so it is important to remove the filter before disconnecting the tubing.
6. Remove the impaction plate by twisting the widest cross-section of the Harvard Impactor body apart from one another (Figure 3, page 14). Handle these plates by their edges, being careful to avoid touching the sintered metal centre.
7. Place the dirty impaction plate in its own Ziploc bag or wrap it in a Kimwipe to protect the surfaces and place it in a bag of dirty plates.
8. Completely disassemble the Harvard Impactor and clean all parts of the inside and outside with Kimwipes and alcohol (Figure 4, page 14). Use a separate small Kimwipe for each part.

H. Preparing the Pump and Impactor for the next sampling session

1. Ensure that the Impactor is dry before reassembling.
2. Check the impaction plate - if the oil has been absorbed, add more oil in increments of one drop until the oil no longer absorbs. Wipe off any excess carefully with a Kimwipe.
3. Install the clean & oiled impaction plate. If not deploying Impactor directly, cover inlet with a plastic bag and secure with elastic band.

I. On Return to SFU Lab

Remove Filter from Cassette:

a. Carefully open Petri dish and remove filter in yellow cassette;
b. Carefully open the cassette and using clean forceps remove filter from cassette and place filter into its same Petri dish;
c. Reseal Petri dish with tape. It should have at least one ID label;
d. Store the used filters in a protected space (with a steady temperature) until the filter is taken back to UBC.

J. Verifying data and looking for problems:

1. Pump will run continuously for each of the two 7-day periods (fingers crossed);

2. BATT indicates a battery problem (may occur if power supply is disrupted);
3. FAULT indicates a disruption to the flow. Pump will automatically shut off if flow rate is impeded for a few seconds. It will then start up again. After 10 tries, the pump will shut off and not start again;
4. Calculate expected total elapsed time (min) from start and stop times recorded on log sheet. Calculate the expected total volume by multiplying total elapsed time by 10 L/min;
5. Elapsed time and total volume displayed by pump should equal those calculated. If the pump stopped at any time due to a power problem or flow disruption, then the actual values recorded by the pump will be less. Values recorded by the pump are the data to be used.

K. Field Blank Schedule:

Please do a field blank at the first home and then every second home visit after that (i.e., home 1, 3, 5, etc.).

L. SAMPLING ANOMALIES

1. **TIME CHANGES**: (daylight savings and standard time) If sampling overlaps a shift between daylight savings time and standard time, adjust **both** the pumps **and** computer to the new time. Continue to sample from 5 p.m. on the start date to 4 p.m. on the end date.
2. **FILTER TEAR OR CONTAMINATION** (even if only suspected): If a Teflon filter is contaminated in any way or damaged, use any other filter. Be sure to write the ID for the filter used on the Log Sheet. Return the damaged, torn, contaminated filter in its petri dish with other used filters when a shipment occurs clearly labelling why the filter was not used.
3. **Replacing O-Ring in Harvard Impactor:** Sometimes the O-rings in the Harvard Impactors crack or dry out. If the BIOS DryCal is not responding to flow rate changes (on the pump) and the filter is not damaged/faulty, the Harvard Impactor 1 or more of the o-rings may need replacing. In the very unlikely event that an o-ring needs to be replaced, record the replacement in the 'Notes' column on the *Log Sheet.*
4. **Harvard Impactor Disassembly:** If you are having difficulty accessing the impaction plates, use the strap wrenches to help. Once open, apply a small amount of silicone spray. If this is not successful apply a **VERY** thin coating of Vaseline to the o-rings. Be sure to wipe off excess Vaseline. Because we are sampling for HOPANES we did not ever use the Vaseline, only the Silicone spray so as not to contaminate samples.

M. Filter Shipments back to UBC Lab

The Study Coordinator will be responsible for delivery of the new pre-weighed filters to the tech at Simon Fraser University and also return of the used filters to UBC.

Study Protocol VII - Cleaning Equipment & Supplies

A. Cleaning Impaction Plates

1. Use the ultrasonic cleaner to clean used impaction plates: Mix a drop of soap into a beaker of distilled water, place impactor plates in beaker and place in tap-water-filled sonicator. The water level in the sonicator should measure slightly higher than the water level in the beaker. Run for a minimum of 15 minutes.
2. If the plates do not appear clean, repeat step 1. It may be necessary to first wait a few minutes to prevent sonicator from over-heating.
3. When plates are sufficiently clean, rinse thoroughly with distilled water (3 times).
4. Let plates air-dry overnight, on a Kimwipe-lined tray, covered with another Kimwipe.
5. Store dry plates in clean, labeled Ziploc bag.

Figure 4 : a) Assemble Harvard Impactor, b) Section of the Harvard Impactor that must be taken apart to access impaction plate.

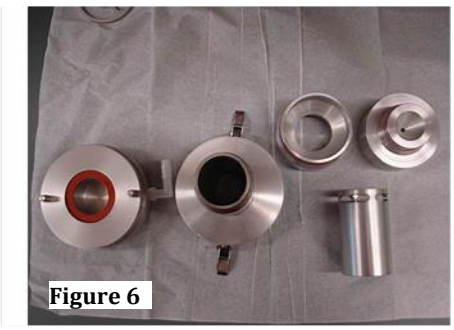


Figure 5 A completely disassembled Harvard Impactor. All parts must be cleaned every filter change.

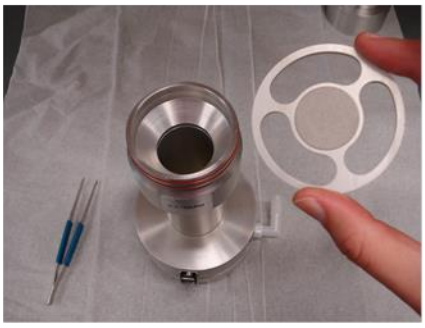


Figure 5: Installation of a clean and oiled impaction plate.

B. Cleaning Filter Cassettes

1. Thoroughly wipe all surfaces of each yellow filter holder cassette with a Kimwipe and alcohol.
2. Set them out to dry on a clean Kimwipe, covered by another Kimwipe.
3. When dry, put them in a clean and labeled Ziploc bag for their next filter loading.

C. Cleaning Harvard Impactors

1. Take the Impactor apart by twisting it. If it does not come apart easily it may be necessary to use a bit more silicone when putting it back together. Remove the

impaction plate by twisting the widest cross-section of the Harvard Impactor body apart from one another (**Fig. 4**). Handle these plates by their edges, being careful to avoid touching the sintered metal centre.

2. Completely disassemble the Harvard Impactor and clean the inside and outside of all parts with a Kimwipe and alcohol (**Fig. 5**).
3. Ensure that all parts are completely dry before reassembling.
4. Once all parts of the Harvard Impactor are dry, take a clean impaction plate and add one drop of impaction plate oil onto the sintered metal surface. If it absorbs, add more oil in increments of one drop until the oil no longer absorbs. Wipe off any excess with a Kimwipe.
5. Install the clean oiled impaction plate (**Fig. 6**) and reassemble the Harvard Impactor.
6. *Make sure that the impaction plate is installed with the sintered disk facing upward, toward the tapered inlet of the impactor nozzle.*
7. Install a slotted inlet over the impactor nozzle if it is not already in place. To protect the sampler during transport from the field lab to the sampling site, cover the inlet of the impactor stage with a ziplock bag and elastic to hold the bag in place.

Study Protocol VIII - EndoPAT Procedure

Protocol for EndoPAT2000

A. General Conditions

1. Ask if the subject is comfortable doing the test lying in their bed – this is the ideal place.
2. If not, try either lying down on a couch or sitting (semi-reclined) on a comfy chair
3. There should be no disturbances in the room: e.g. other people, music, radio or TV, phone that could ring
4. Room should be a comfortable temperature, with no breeze or draft. If the subject is cold, cover them with a blanket.
5. Lighting should be comfortable – not dark and not bright or harsh
6. Subject should be wearing comfortable, loose clothing – a T-shirt is best, so that you can apply the cuff and there is no constriction on the arms.
7. Ask whether the subject has anything to eat or drink this morning (other than water).

Note: Plug and turn on the Endo-PAT at least 20 minutes before test. Do this first, as subject gets comfortable for the blood pressure measurement.

B. Blood pressure measurement – done on dominant arm, at least 10 minutes before EndoPAT test

1. This is the first thing to do when you arrive at subject's home. If subject is active, busy or agitated, ask them to sit or lie down and relax for 5-10 minutes before taking reading.
2. Use either the large or small cuff, depending on size of subject's arm. Fit the cuff on their **dominant arm**, so that the tube is nearer their hand. The white arrow on the cuff should be directly over the inside elbow. Cuff should be closed snugly.
3. Plug tubing into left-hand side of monitor ("cuff" icon).
4. Lay arm palm upward so that it is a heart-level. Use the EndoPAT arm support, if helpful.
5. Ensure that the monitor is in MAM mode (icon displayed in screen). To enter MAM mode, hold time the left button 🕒 for several seconds. The MAM icon will flash ON or OFF. Set to ON, by pressing the right (M) button. Then press the 🕒 button to confirm – you will then be in the mode for setting the date and time. Press the 🕒 button repeatedly to bypass these until nothing on screen is flashing. You should see MAM icon displayed.
6. Ask the subject not to move or speak during measurement – tell them there will be three readings done in succession and you will let them know when it is finished.
7. To begin measurement, press the large middle (power) button.

8. The cuff will inflate and deflate 3 times to take 3 readings. When the readings are complete, the average value will be displayed – record this value on your log sheet.
9. Press the middle power button for several seconds to shut off power. If no buttons are pressed for several minutes, the monitor will shut off by itself.
10. Determine the pressure to which you will inflate cuff for the EndoPAT test and record on log sheet. This will be a minimum of 200mmHg, at least 60mmHg above average systolic pressure, to a maximum of 300mmHg.

    For example, if blood pressure is 120/80, you would pump the pressure up to 200mmHg; if blood pressure is 145/85, you would pump to 205mmHg.

C. EndoPAT Set-up

1. Set the EndoPAT on a flat, stable surface. Set up laptop so that screen is not visible to subject.
2. Connect the EndoPAT to the laptop and open the EndoPAT software. Ensure they are communicating – the PAT box in lower right hand side of screen should be green and the "PAT state" should read "IDLE".
3. Connect the probe tubes to the EndoPAT, ensure screws are snugly turned. Ensure that tubes are tidy and will not be pinched or stepped on.
4. Attach new probes to the Endo-PAT connectors, pressing firmly to ensure that there is a tight seal.
5. Deflate the probes either by pressing the deflate button on the ENDO-Pat unit or by clicking the deflate button on the computer screen.
6. Make sure that participant's nails are trimmed on the two fingers that will be used for testing. Nails cannot extend more than 0.5 cm above fingertip. You must use the same finger on each hand (preferably, index on both). If subject has large fingers, you may need to try a smaller finger. Avoid using the thumb.
7. Check that participant is comfortable and that their hands are at heart level. Using the arm supports will help, depending on the situation.

D. Pre-test

1. Select New Participant on the Endo-PAT Screen, and enter the following participant information:
    - Subject code = subject ID and session number (e.g. SUB_08_02)
    - Blood pressure (remember that the systolic and diastolic are reversed on EndoPAT software)
    - PATographer (your name).
2. Remember to ask participant if they:
    a. Need to go to the restroom (since the test lasts 15-20 mins.)
    b. Have turned off any cell phones or noisy alarms.
    c. Have removed all jewelry and watches on both arms/hands.

d. Have any allergies to latex – if so, what was the allergy?

3. Place the blood pressure cuff on the test arm (non-dominant arm). It should be about an inch above the elbow, with the tubing coming out towards the hand inside the elbow.
4. Place probes in the arm supports (if using) and place participant's fingers in the probes. Have the participant feel for a metal bar at the end – this should be at the tip of their finger.

   **To stay consistent, place probe 1 on the test finger (arm with cuff) and probe 2 on the control finger.*
5. Inflate the probes. Remove fingers with probes from the arm support and rest hands flat on arm support.
6. Place the blue foam anchor pads on finger(s) next to the testing finger, and make a loop between the anchor and probe – this should not touch the participant's hand.
7. Use medical tape to loosely tape the connector tube to the anchor finger.
8. Have the participant rest both hands over the edge of the arm supports, palm side down, dangling freely. Ensure that nothing is touching the probes (other fingers, thumb, tubing, arm support, foam anchor) – you may need to use some tissues or additional tape.

E. Signal check

1. Select Stand-by on the Endo-Pat screen (yellow button), and check to make sure that everything is ready to go: the participant is comfortable, the probes are properly placed and not touching anything, the control and testing probes are registering a signal, and that there are no ongoing leaks.
2. Adjust the scale and scope of the screen so that you can clearly see the readings from both fingers (generally, 1 minute time base and 500 gain) .
3. If leaks are detected you will hear the EndoPAT re-inflating and one of the buttons on bottom right hand side of screen will flash red. Re-secure all tubing connections. If leaks persist, see "troubleshooting".
4. Tap on finger of arm with cuff, to confirm that this is probe 1.
5. Stay in standby mode for 1-2 minutes.

Note: if for any reason you need to stop the test up until this point the same probes can be used. Once you press "Go" and start recording, the probes cannot be reused.

F. Testing

Test times: Baseline at least 5 mins; Occlusion <u>exactly</u> 5 mins; Post-Occlusion at least 5 mins

1. Set timer to 5 mins, so that it is ready for timing occlusion. Do not start stopwatch yet.

2. Select the green button on the computer screen to begin baseline recording
3. About 15-20 seconds before the Baseline testing is done, increase the scale of the testing probe (probe 1) to 20,000 and adjust time base to 15 seconds.
4. When at least 5 minutes of Baseline data has been collected (see time below signal on screen), you will begin occlusion. Tell subject "I'm going to pump up the cuff now".
5. Quickly pump up the blood pressure cuff to the predetermined level and then start the timer. During the occlusion period you need to check:
   - That occlusion is complete by ensuring that no rhythm is evident on probe 1.
   - That pressure is maintained on the cuff - you may need to increase the pressure.
   - The timer, so that you see when it reaches zero – the timer will not make a sound.
6. If you see some rhythm on probe 1 (repeating signal, rather than just noise or just one signal peak), increase pressure on the cuff.
7. As the timer approaches zero, tell subject "I will now let out the pressure from the cuff. Remember there are still 5 minutes left for the test so remain still."
8. When the timer reaches precisely zero, quickly release the blood pressure cuff. On the computer, decrease the testing probe's scale to match the control probe.
9. Restart stopwatch. The Post-Occlusion phase should last at least 5 minutes.
10. Selecting the red hand icon on the Endo-PAT screen to stop the test. This should also deflate the probes. Wrap tape around the used probes and discard them right away.
11. All data is automatically saved to the hard drive.

Note: If you must interrupt test during the baseline measurement, you will need to new probes to begin again. Once the occlusion has begun, the test cannot be restarted – do your best to complete the test, even if there is a problem. Record anything unusual.

G. Data Analysis

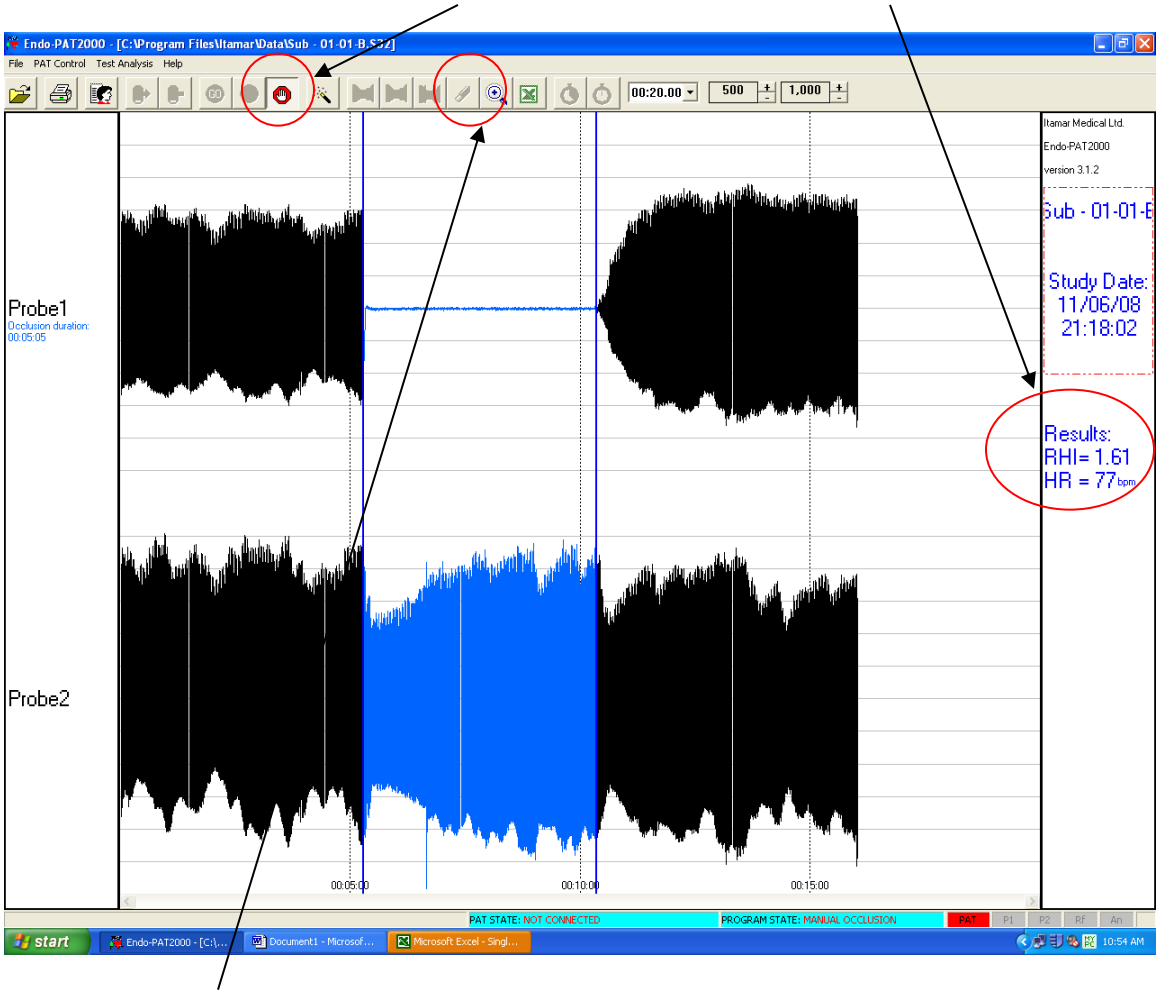
1. On the Endo-PAT screen, select the open file icon, and select the file just completed.
2. Select the "magic wand" icon; the computer will calculate the test values and display them.
3. Select the Excel file icon to open data file. The session just completed will be the data line displayed.
4. Check column I (yellow) for any warnings or errors. If there is a warning or error, record it on data log. You will not be able to repeat the test today on this subject. Take steps to resolve any problems before you go to test the next subject.

H. Data backup

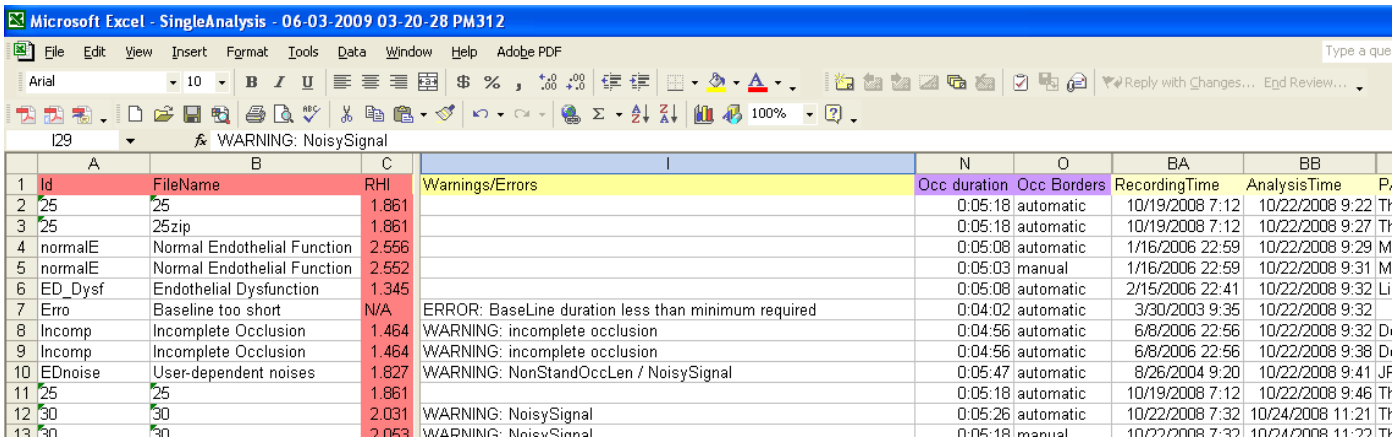
1. After each session, ensure that data files are backed up.
2. Copy both data files (.m32 and .s32) files onto a memory key.
3. Give memory key to Paul weekly for a full backup

Study Protocol IX - Cleaning EndoPAT data

After completing test, click on the “magic wand” button to obtain RHI and AI values.



Click on the Excel button on the menu bar to ensure that these values are logged onto the “Single Analysis Worksheet”. All activities (e.g. tests, manual changes) are logged onto this spreadsheet. If the activity does not appear at the bottom of the list, close the spreadsheet, click on the magic wand and Excel button again to reopen the sheet. The latest record will be at the bottom of the spreadsheet.



Microsoft Excel - SingleAnalysis - 06-03-2009 03-20-28 PM312

I29 WARNING: NoisySignal

	A	B	C	I	N	O	BA	BB
1	Id	FileName	RHI	Warnings/Errors	Occ duration	Occ Borders	RecordingTime	AnalysisTime
2	25	25	1.861		0:05:18	automatic	10/19/2008 7:12	10/22/2008 9:22
3	25	25zip	1.861		0:05:18	automatic	10/19/2008 7:12	10/22/2008 9:27
4	normalE	Normal Endothelial Function	2.556		0:05:08	automatic	1/16/2006 22:59	10/22/2008 9:29
5	normalE	Normal Endothelial Function	2.552		0:05:03	manual	1/16/2006 22:59	10/22/2008 9:31
6	ED_Dysf	Endothelial Dysfunction	1.345		0:05:08	automatic	2/15/2006 22:41	10/22/2008 9:32
7	Erro	Baseline too short	N/A	ERROR: BaseLine duration less than minimum required	0:04:02	automatic	3/30/2003 9:35	10/22/2008 9:32
8	Incomp	Incomplete Occlusion	1.464	WARNING: incomplete occlusion	0:04:56	automatic	6/8/2006 22:56	10/22/2008 9:32
9	Incomp	Incomplete Occlusion	1.464	WARNING: incomplete occlusion	0:04:56	automatic	6/8/2006 22:56	10/22/2008 9:38
10	EDnoise	User-dependent noises	1.827	WARNING: NonStandOccLen / NoisySignal	0:05:47	automatic	8/26/2004 9:20	10/22/2008 9:41
11	25	25	1.861		0:05:18	automatic	10/19/2008 7:12	10/22/2008 9:46
12	30	30	2.031	WARNING: NoisySignal	0:05:26	automatic	10/22/2008 7:32	10/24/2008 11:21

A regular test without any manual manipulation will display “automatic” under the “Occ Borders” column. If there are any changes to the occlusion boundaries, “manual” will be displayed. The recording and analysis dates are also recorded.

For a PAT index to be generated, there must be

- **At least** 5 minutes of baseline (pre-occlusion) data. If there is less, no value will be generated
- **Exactly** 5 minutes of occlusion. Note: the machine cannot detect exactly when the PATographer stops and starts the occlusion. Rather, it examines the wave amplitude and estimates the occlusion. The machine will allow estimates between 4:30- 5:30 minutes. This is where errors may occur if a subject’s post-occlusion amplitude takes a long time to return to normal.
- **At least** 5 minutes of post-occlusion

The computer algorithm calculates the ratio of the average amplitude in the last 2.5 minutes of baseline to the average amplitude for 1 minute, 90s post-occlusion. (These are default values and can be changed if necessary). Therefore, occlusion length plays an important role in determining the PAT index.

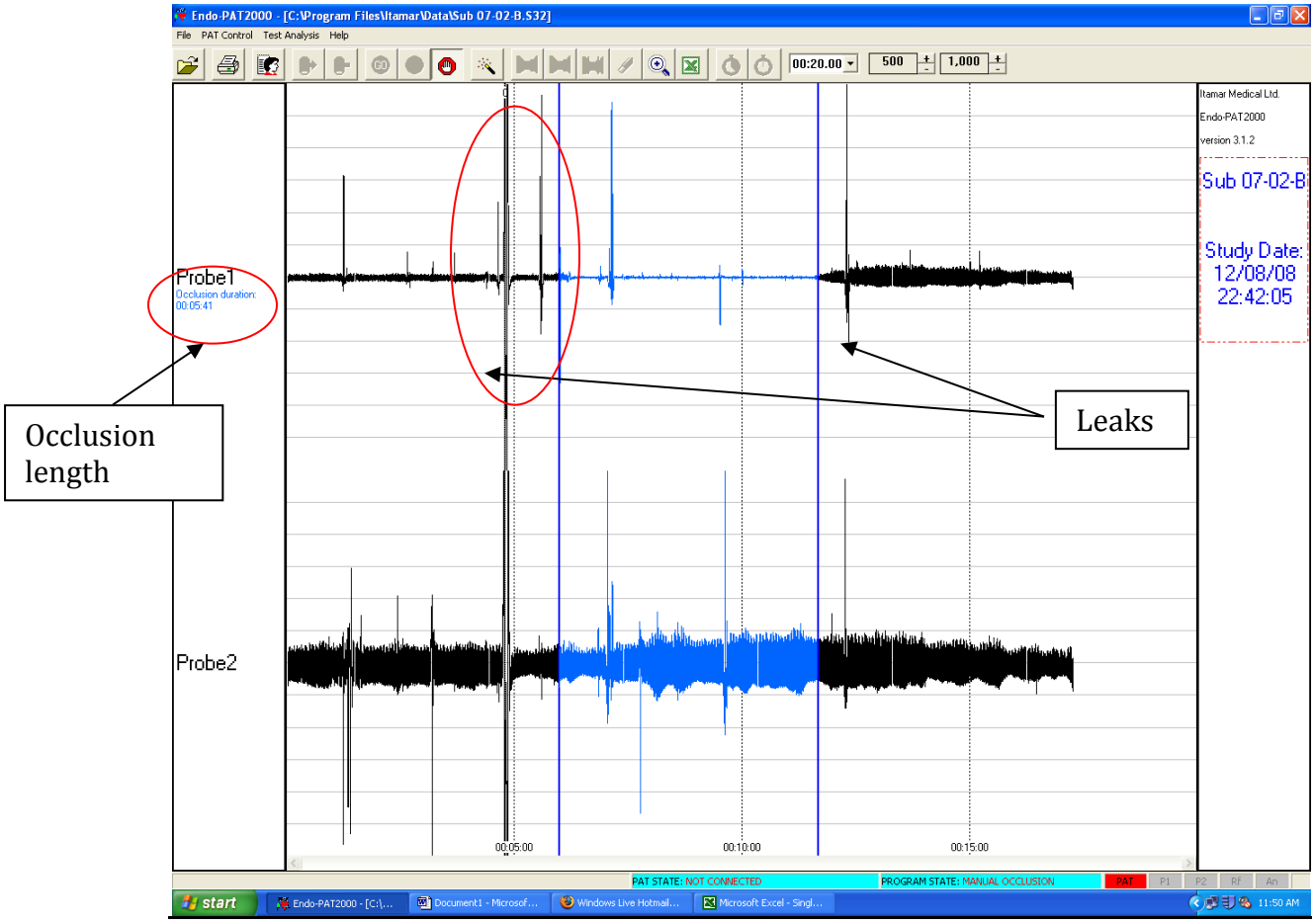
To check if there are any errors with the endoPAT test, look under the Warnings/Errors columns. Generally, you will find these types of errors:

- Incomplete occlusion
- Noisy Signal
- Baseline duration less than minimum required
- Non standard occlusion length
- Or a combination of any of these

If the error is incomplete occlusion or noisy signal, there is no way of manually changing the data. A note should be made with respect to these data during analyses. If the baseline duration is less than 5 minutes, a PAT index cannot be calculated.

If there is a non-standard occlusion length, you can manually change the occlusion borders.

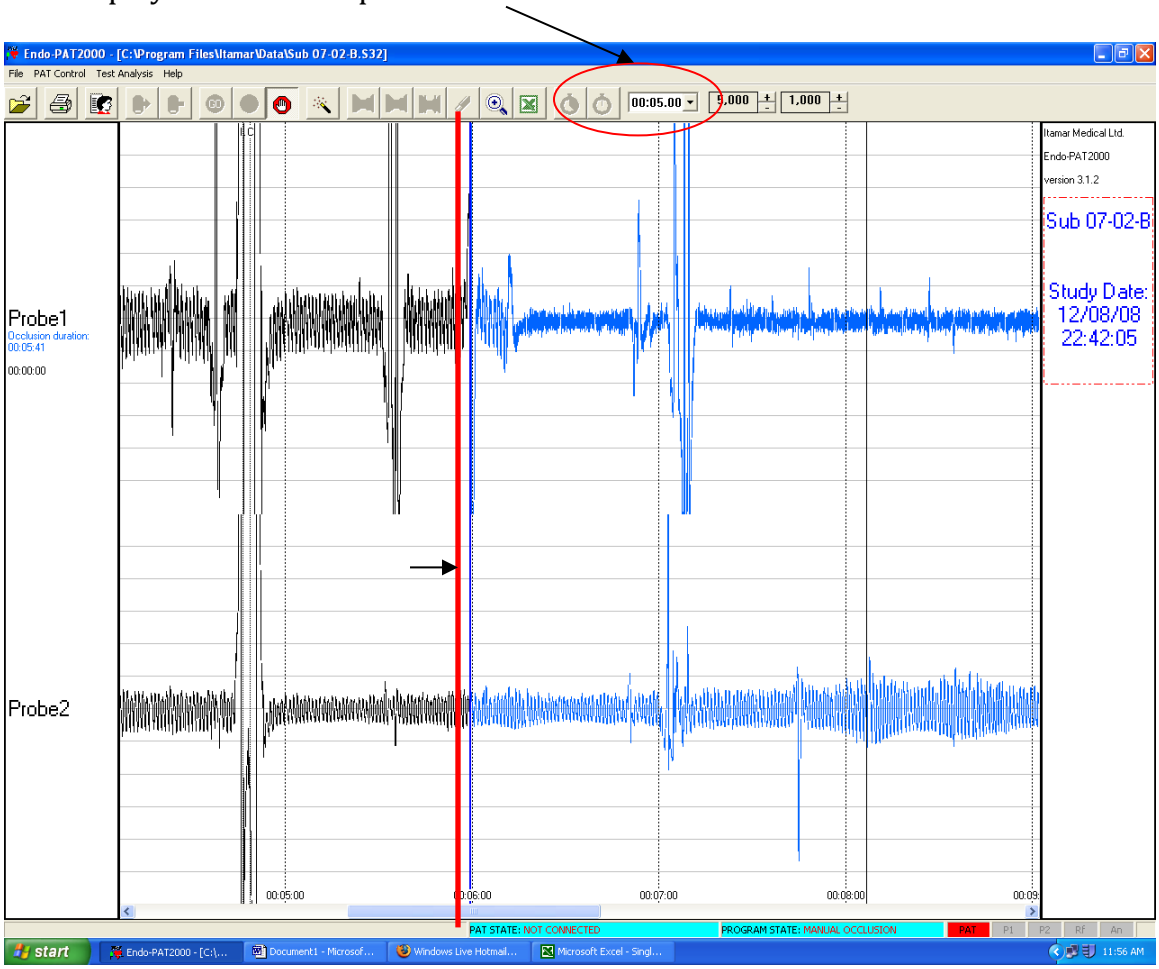
Example of changing occlusion borders



This is a weak signal and the program has estimated an occlusion length that is too long. Notice there are several leaks during the time periods endoPAT uses to calculate the PAT ratio – this may affect index values. When we check the error messages on the Excel spreadsheet, we find that there is a non-standard occlusion length (>5:30) and that there is an incomplete occlusion.

We first examine the signal to determine if the occlusion is complete – in this case, although it is weak, it is obviously occluded. However, we will need to manually change the occlusion borders to ensure a proper occlusion length.

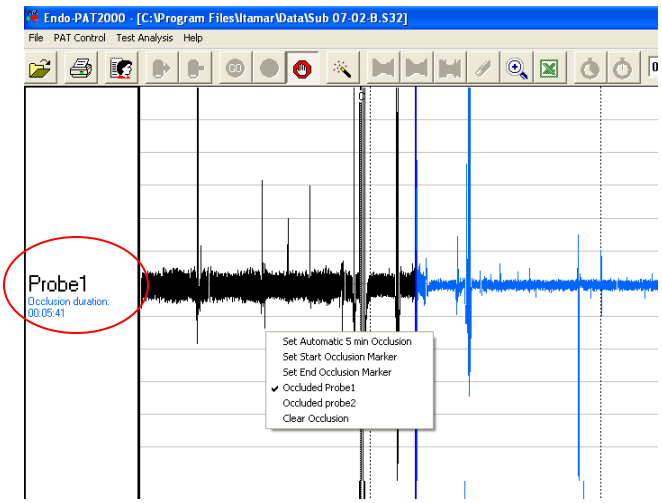
To choose the beginning of the occlusion, we first magnify the view by changing the time interval displayed and the amplitude scale.



To manually change the occlusion borders, we can move the blue borders just by clicking and dragging the mouse. We choose the beginning of occlusion where the amplitude shows a clear decrease in signal (red line).

The new occlusion length will be displayed on the left menu under the Probe label. If it is under 5:30, we can run a new analysis by clicking on the magic wand.

You can also change the occlusion borders by right clicking anywhere in the signal window. This will open a popup menu with several different options:



The new analysis will show up on the Excel spreadsheet on the last row. The occlusion borders will be listed as “manual” and the new RHI value will be listed.

Study Protocol X – Smoke Stain Reflectometer

Original: 5/26/2005
Updated: 10/24/2011

Definitions:

SSR:	Smoke Stain Reflectometer
Mask:	A round plate onto which the measuring head is placed during measurements
White standard:	white area (circle) on the standard plate
Grey standard:	grey area (circle) on the standard plate
Control filters:	A clean, non-exposed filter; must be similar to those used in sampling (taken from the same lot/batch of filters as the sampling filters)
Field Blank:	A control filter, not exposed to sampling air flow but otherwise handled like a regular sample filter

Equipment and Materials:

Equipment

- Smoke Stain Reflectometer: Diffusion Systems Ltd. Model 43 (M43D) or other comparable instrument
- Standard Plate (White/grey): supplied with the instrument
- Pair of tweezers

Materials

- Five (5) control filters
- CLEAR Study sample filters
- CLEAR Study field blank filters

IMPORTANT COMMENTS PRIOR TO MEASURING REFLECTANCE:

1. Take the reflectance measurements in as dark a room as possible so as to eliminate the effects of sun and other light sources on the measurements;
2. Do not point the measuring head toward any light source, as this may damage the instrument;
3. To prevent contamination of the filters while performing measurements, make sure that the instruments and working environment are clean;
4. All field blanks can be analyzed according to the reflectance measurement methods described in this SOP.

Procedure:

A. Preparation of the SSR for measurement (linearity check):

1. Switch on the SSR instrument and let it warm for at least 15 minutes.
2. Before attaching the measuring head, adjust the reading on the SSR to 0.0 by using the *zero* knob on the front panel of the SSR.
3. Clean the measuring head, mask, and standard plate with some alcohol, or other suitable solvent, using a lint-free cloth of Kimwipe.
4. Insert the measuring head tightly into the mask.
5. Attach the measuring head to the SSR central unit by plugging the connector into the SSR port.
6. Place the measuring head over the white standard and adjust the reflectance reading to 100.0 by using the *coarse* and *fine knobs* on the front panel.
7. Move the measuring head over the grey standard; the reading should be within the limits given for the standard plate in the manufacturer's manual (34.0 ±0.5).

B. Calibration

1. If linearity check performed in the SSR preparation steps described above yields acceptable reflectance values within the specified limits, place one of the 5 control filters (taken from the same lot/batch of filters are those used for sampling) centrally over the white standard, measure reflectance from the centre of the filter and adjust the reading to 100.0. Record this value on the data form.
2. Repeat the reflectance measurement 4 additional times, being sure to locate the measuring head in a different location than the centre each time (see Figure 1; 'Five Point Method'). Record all data on the data form.
3. Without re-adjusting the reflectance reading, measure the reflectance for the other 4 control filters using the 5-point method, and record these readings on the data form.
4. Calculate the arithmetic mean of all reflectance values for each control filter; the filter having the ***median mean*** of reflectance values is selected as the ***primary control filter*** that is used for recalibration of the SSR during the measurement of sample filters.
5. If the 5 values measured from the primary control filter have standard deviation > 0.5 units, disqualify the filter, pick a new clean filter from the batch, and redo the selection process until a suitable primary control filter is found.

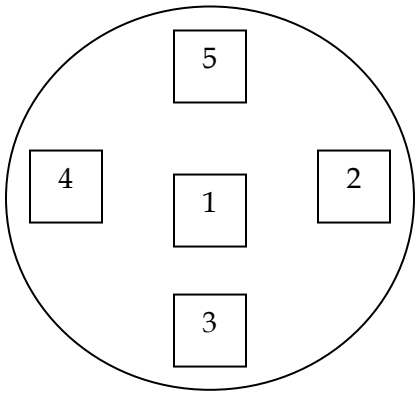


Figure 1. Five Point Method of Measuring Reflectance on Filters

Table 1. Example: Control Filter Reflectance Data Set with Averages

	Filter 1	Filter 2	Filter 3	Filter 4	Filter 5
Measurement 1	Adjust 100.0	**100.1**	99.7	100.1	100.4
Measurement 2	100.1	**100.1**	99.9	100.3	100.2
Measurement 3	100.1	**100.1**	100.1	100.2	100.4
Measurement 4	99.9	**100.2**	100.0	99.9	100.3
Measurement 5	100.1	**100.0**	100.0	100.4	100.1
Avg reflectance	100.03	**100.10**	99.94	100.18	100.28
Standard deviation	0.08	**0.06**	0.14	0.17	0.12
Median		**100.10**			

Table 1 contains example control filter reflectance data for the purposes of explaining the selection process for the primary control filter. Inspection of this data shows that the primary control filter would be **Filter 2**, as it has the median standard deviation and a standard deviation < 0.5 units.

Once you have chosen the primary control filter:

- Recalibrate the SSR to 100.0 using the selected primary control filter, and measuring reflectance at its midpoint.
- Repeat calibration using the primary control filter after measuring every series of 25 filters. Record the reflectance reading of this control filter on the data form before re-adjusting the reading to 100.0 once again.

C. Measurement of Reflectance

1. Calibrate the SSR as described above in section 'B' of the procedure.
2. Clean the measuring head, mask, standard plate and tweezers with alcohol using a non-lint cloth or Kimwipe.
3. Ensure that the measuring head is tightly attached to the mask.
4. Remove a sample filter from its Petri dish using tweezers and locate it centrally on the white standard.
5. Locate the measuring head with utmost caution over the sample filter and record the reflectance reading on the data form.
6. Make 4 additional reflectance measurements per sample filter using the 5-point method and record these values on the data form.
7. Clean the mask, standard plate, and tweezers after having measured each series of 25 sample filters (do this at the same time as the primary filter recalibration).

D. Quality Assurance

At the end of each measurement session, measure reflectances again for at least 10% of the total number of filters weighed (5 times per filter, using the 5-point method). If the average reflectance of the duplicate deviates more than 3% from the original results, all

of the filters measured during the measuring session will need to be re-measured (hope that this does not happen!).

Data Records and the Data Form

The following data should be recorded from the absorption coefficient measurements in the *Data Form* and computer files:

1. Date & place of measurements
2. Instrument operator ID data (name)
3. Relative humidity in the location where measurements were taken
4. Filter lot/batch # (printed on the filter package – can be taken from the weighing sheet)
5. Instrument data (manufacturer name, model name/number)
6. Filter ID codes
7. Reflectance readings from all control filters and all calibrations (specify filter type in the filter code column of the data form)
8. Reflectance readings and average readings from the sample filters and field blanks

Reflectance Measurements DATA FORM

Date/Place:								Filter Batch #:		
Analyst Initials:										
Instrument Data:										
Lights on or off:										
Filter Code		Measurement #					Average Reflectance	Sample Vol (m^3)	Absorption Coefficient	Notes
Primary =	X	1	2	3	4	5				
Control 1										
Control 2										
Control 3										
Control 4										
Control 5										

Study Protocol XI - Determination of Hopane by GC/MS

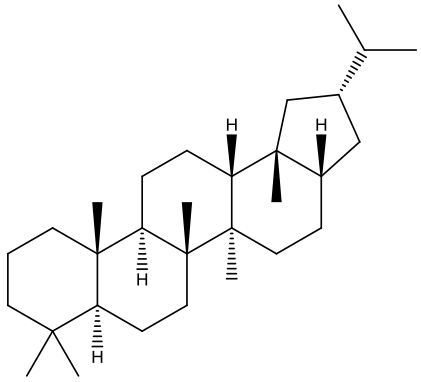
Creation Date: 08/30/12 Method Version: SPPH-SOP# AA.00.24

Introduction

Particulate Matter (PM) containing organic compounds is a common air pollutant in urban areas. PM is emitted by an array of sources, such as motor vehicle exhaust, wood combustion, and various industrial processes. The source of PM emission can be determined by identifying and quantifying certain tracer compounds, which are specific to different sources. Hopane (**Figure 1**) is a triterpene that is used as a tracer compound for motor vehicle emissions.

Extraction of hopane from 37 mm or 41 mm Teflon filters with iso-octane, followed by GC/MS analysis, is a sensitive and selective method.

Figure 1: Hopane
17α(H),21β(H)-Hopane (CAS#: 13849-96-2)



$C_{30}H_{52}$ M.W. = 412.7 g mol^{-1}

Apparatus and Chemicals

A. Apparatus

Analytical Instrument:
GC/MS System - Agilent Technologies 5973 GC/MSD

Centrifuge:
Hitachi HiMac centrifuge (CT5DL model)
Sampling Medium:
Gelman Teflo™ W/Ring – PTFE Membrane W/PMP Ring: 2.0 um 37 mm P/N R2PJ037

Filter Cutting Tool:
[Method 1] Teflon filter cutting tool (see Figure 2)
[Method 2] Scissors, scalpel, forceps and Petri dish.

Ultrasonicator
Branson 2200 Untrasonic Cleaner

B. Chemicals – Supplier Details

17α(H),21β(H)-Hopane solution (0.1 mg/mL in isooctane, analytical standard) – Sigma-Aldrich P/N 90656 – 1 mL (≥97.0% purity)

- n-Dotriacontane – Sigma-Aldrich P/N 44253 – 1 g (≥99.0% purity)
- Pyrene – Sigma-Aldrich (multiple purities available, use minimum 99%)
- Iso-octane – Fisher analytical grade

C. Chemicals – Preparation of Stock Solutions

Preparation of Hopane stock solution

With a digital micropipette, transfer 0.500 mL of hopane solution (analytical standard) to a 100 mL volumetric flask. Top up with iso-octane. Mix vigorously. The stock solution can be stored at -80 ^{o}C. Record the concentration (500 pg/µL) and the date of preparation.

Preparation of n-Dotriacontane stock solution

Weigh about 7.5 to 12.5 mg of n-dotriacontane into a weigh boat and record precisely the final weight. Transfer to a 100 mL volumetric flask and top up with iso-octane. Mix vigorously to dissolve all the crystals and to aid solubilizing, ultrasonication can assist in this process. Make sure no solid crystals are undissolved. The stock solution can be stored at -80 ^{o}C. Calculate the final concentration in nanograms per microliter (ng/uL) and record the date of preparation.

Preparation of Pyrene stock solution

Weigh about 5 to 7.5 mg of pyrene into a weigh boat and record precisely the final weight. Transfer to a 25 mL volumetric flask and top up with iso-octane. Mix vigorously to dissolve all the crystals and to aid solubilizing, ultrasonication can assist in this process. Make sure no solid crystals are undissolved. Dilute by a factor of 20 to achieve the appropriate concentration. Calculate the final concentration in nanograms per microliter (ng/uL) and record the date of preparation.

Procedure

A. Removal of Teflon portion of the filter

Note: Each Teflon filter has an outside plastic ring that maintains the Teflon filter's round shape. Removing the Telfon filter material requires a special tool (**Figure 2**) designed to position and cut out the Teflon portion.

If the entire filter is to be analyzed for hopane, Method 1 should be used. If the filter is to be cut in half (e.g. to analyze one half for hopane and one half for a different analyte), Method 2 should be used.

Method 1

1. For 37 mm Teflon filters place the filter inside a GPM cassette holder and install the support ring.
2. Snug down the support ring to prevent the filter from rotating during the cutting step.
3. Insert the cutting tube and rotate with a downward force. This will cut out the Teflon portion of the filter.
4. Using clean forceps transfer the filter to a 4 mL extraction vessel.
5. Prior to the extraction procedure, spike 50 uL of the stock 7-dehydrocholesterol to each vessel (surrogate standard).

Method 2

1. If the filter is to be cut in half, this must be done BEFORE removing the outer plastic ring. Using the forceps, grip the filter by the outer plastic ring and hold it above a Petri dish.
2. Use clean scissors to cut the filter in half as accurately as possible.
3. Place the two filter halves into separate Petri dishes.
4. Using the forceps to brace the outer ring, use the scalpel to carefully cut the filter material away from the ring.
5. Transfer the filter to a 4 mL extraction vessel.
6. Prior to the extraction procedure, spike 40 uL of the stock 7-dehydrocholesterol to each vessel (surrogate standard).

B. Extraction

1. Transfer 1 mL of isooctane into the extraction vessel and ultrasonicate for 20 mins.
2. Centrifuge only if the samples have high suspended particulate matter.

3. Spike all samples with 50 uL of pyrene stock solution (internal standard).
4. Transfer 500 uL (or as much as possible) of each sample solution to GC vials for analysis.

References

Cass, Glen R. (1998) Organic molecular tracers for particulate air pollution sources. *Trends in Analytical Chemistry, 17(6)*, 356-366.

Method Revisions

Revision Number	Author	Date	Description
SPPH-SOP# AA.00.24	Jeff Nichol	01/09/13	1st Versio

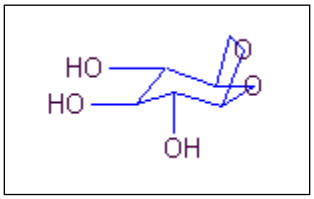
Study Protocol XII - Determination of Levoglucosan in Atmospheric Fine Particulate Matter by GC/MS

Creation Date: 07/14/05 Method Version: SOEH-SOP# A.00.18
Last Update: 01/09/13

Introduction

Levoglucosan (Figure 1) is a sugar anhydride and is used as a molecular marker for the detection and evaluation for the presence of wood smoke in air. The components detected in wood smoke are numerous: PAH'S, aldehydes, free radicals and methoxylated phenols, but the detection of levoglucosan has proven to be a reliable indicator for wood combustion from residential fireplaces or forest fires.
Solvent extraction of 37 mm or 41 mm teflon filters with ethyl acetate, derivatization of levoglucosan and subsequent GC/MS analysis is a very selective and sensitive quantitative method.

Figure 1: Levoglucosan
1,6-Anhydro-beta-D-glucopyranose (CAS #: 498-07-7)



$C_6H_{10}O_5$ M.W. = 162.142

Apparatus and Chemicals

A. Apparatus:

Analytical Instrument:
GC/MS System - Agilent Technologies 5973 GC/MSD

Centrifuge:
Hitachi HiMac centrifuge (CT5DL model)

Sampling Medium:
Gelman Teflo™ W/Ring – PTFE Membrane W/PMP Ring: 2.0 um 37 mm
P/N R2PJ037

Filter Cutting Tool:

[Method 1] Teflon filter cutting tool (see **Figure 2**)
[Method 2] Scissors, scalpel, forceps and Petri dish.

Figure 2: Teflon filter cutting tool

B. Chemicals – Supplier Details

1,6-Anhydro-beta-beta-D-glucopyranose (Levoglucosan) – Sigma-Aldrich P/N 316555-1G (99.9% purity)

1,3,5-Tri-isopropylbenzene (Internal Standard) – Fluka P/N 92075 (97% purity)

7-Dehydrocholesterol (Surrogate) - Sigma-Aldrich

Ethyl Acetate – Fisher analytical grade

MSTFA + 1% TMCS (N-Methyl-N-trimethylsilyltrifluoroacetamide + 1% Trimethylchlorosilane 10 x 1 mL ampules - Pierce Chemicals P/N 48915

Pyridine (ACS grade) – Fluka # 82702 - 99.8% purity

C. Chemicals – Preparation of Stock Solutions

Preparation of Levoglucosan Stock Solution:
Weigh about 0.010 to 0.030 grams an amount of levoglucosan into an aluminium boat and record precisely the final weight. Transfer to a 25 mL volumetric flask and top up with ethyl acetate (depending on sample matrix, this dilution can be altered). Mix vigorously to dissolve all the crystals and to aid solubilization, ultrasonication can assist in this process. Make sure no solid crystals are undissolved. The stock solution can be stored at -80 ^{o}C. Calculate the final concentration in nanograms per microliter (ng/uL) and record the date of preparation.

Preparation of 7-Dehydrocholesterol Surrogate Stock Solution:

Weigh about 0.1 grams an amount of 7-dehydrocholesterol into an aluminium boat and record precisely the final weight. Transfer to a 50 mL volumetric flask and top up with HPLC grade ethyl acetate. Mix vigorously to dissolve all the crystals and to aid solubilizing, ultrasonication can assist in this process. Make sure no solid crystals are undissolved. The stock solution can be stored at -80 ^{o}C. Calculate the final concentration in nanograms per microliter (ng/uL) and record the date of preparation.

Preparation of Tri-isopropylbenzene Internal Standard:
Transfer 5 uL of tri-isopropylbenzene into 25 mL volumetric flask and top up with ethyl acetate. Dilute to an intermediate stock at an appropriate level (5-20 times). Spike 10 uL of this solution into each GC vial after derivatization has been completed.

Sample Preparation Procedure

A. Removal of Teflon portion of the filter

If the entire filter is to be analyzed for levoglucosan, Method 1 should be used. If the filter is to be cut in half (e.g. to analyze one half for levoglucosan and one half for a different analyte), Method 2 should be used.

Method 1

Each teflon filter has an outer plastic ring that maintains the teflon filter's round shape.

Removing the telfon filter material is conducted with a special tool (***Figure 2***) designed to position and cut out the teflon portion and remove the outer plastic ring.

For 37 mm teflon filters place the filter inside a GPM cassette holder and install the support ring.

Snug down the support ring to prevent the filter from rotating during the cutting step.

Insert the cutting tube and rotate with a downward force. This will cut out the teflon portion of the filter.

Using clean forceps transfer the filter to a 4 mL extraction vessel.

Prior to the extraction and derivatization procedures spike each sample with 20 uL of the stock 7-dehydrocholesterol standard (surrogate).

Method 2

If the filter is to be cut in half, this must be done BEFORE removing the outer plastic ring. Using the forceps, grip the filter by the outer plastic ring and hold it above a Petri dish. Use clean scissors to cut the filter in half as accurately as possible.

Place the two filter halves into separate Petri dishes. Using the forceps to brace the outer ring, use the scalpel to carefully cut the filter material away from the ring.

Transfer the filter to a 4 mL extraction vessel.

Prior to the extraction and derivatization procedures spike each sample with 20 uL of the stock 7-dehydrocholesterol standard (surrogate).

Extraction and Derivatization

Levoglucosan is light sensitive so take precautions to not expose the sample vials to intense direct light.

Transfer 2 mL of ethyl acetate into the extraction vessel and ultrasonicate for 30 mins.

Centrifuge only if the samples have high suspended particulate matter.

Transfer exactly 100 uL of the final extract into GC vials that have 300 uL inserts installed. Try not to re-suspend the particulates.

Add 15 uL of pyridine and 30 uL of MSTFA + 1% TMCS solution.

Vortex for 10-20 secs and place the samples in a dark location for a minimum of 6 hours to complete the derivatization.

Prior to GC/MS analysis spike 10 uL of tri-isopropyl benzene internal standard into each vial.

GC/MS Conditions

- DB-5 (5% phenyl) capillary column 30 meters x 0.25 mm I.D. with 0.25 um film thickness
- Temperature Program: 65 ^{o}C (1 min hold) to 310 ^{o}C @ 25 ^{o}C (5 min hold)
- Run time (mins): 15 mins
- Injection Port Temperature: 290 ^{o}C
- Injection Vol (uL): 1 uL
- Splitless Injection Time (min): 0.50 min
- Inlet Pressure (psi): 10 psi with constant flow
- Initial Flow (mL/min): 1.1 mL/min

TABLE 1: Single Ion Monitoring (S.I.M.) for Levoglucosan, Internal Standard and Surrogate

Component	Retention Time (min)	Quan Mass (Q1)	Quan Mass (Q2)	Quan Mass (Q3)	Dwell Time (msec)
Istd	5.681	161.00	189.00	204.00	50
Levoglucosan	7.527	204.00	217.00	333.00	50
Surrogate	13.522	325.00	351.00	456.00	50

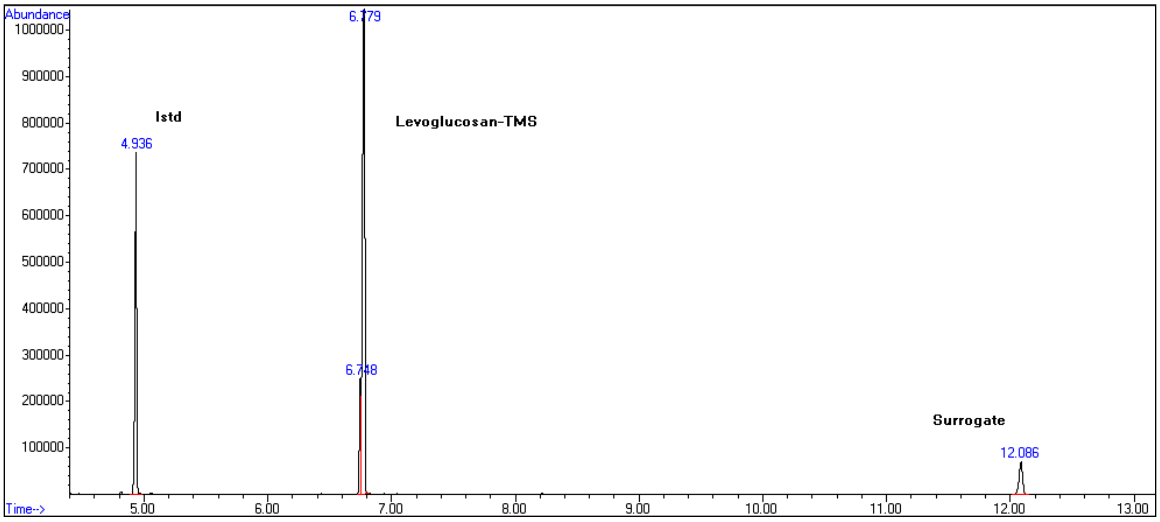


Figure 3: S.I.M. Chromatogram of Levoglucosan-TMS, Tri-isopropyl benzene (Istd) and 7-Dehydrocholesterol (Surrogate)

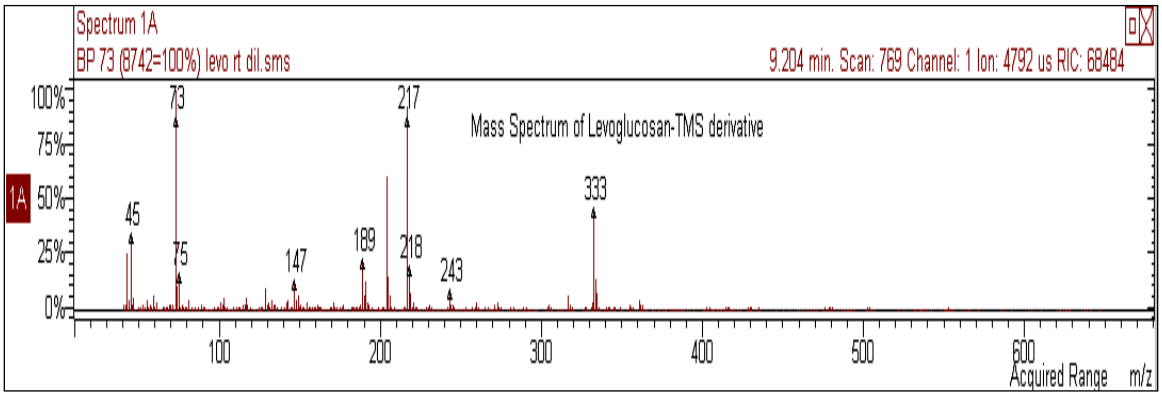


Figure 4: Full Scan Mass Spectrum of the Trimethylsilyl derivative of Levoglucosan

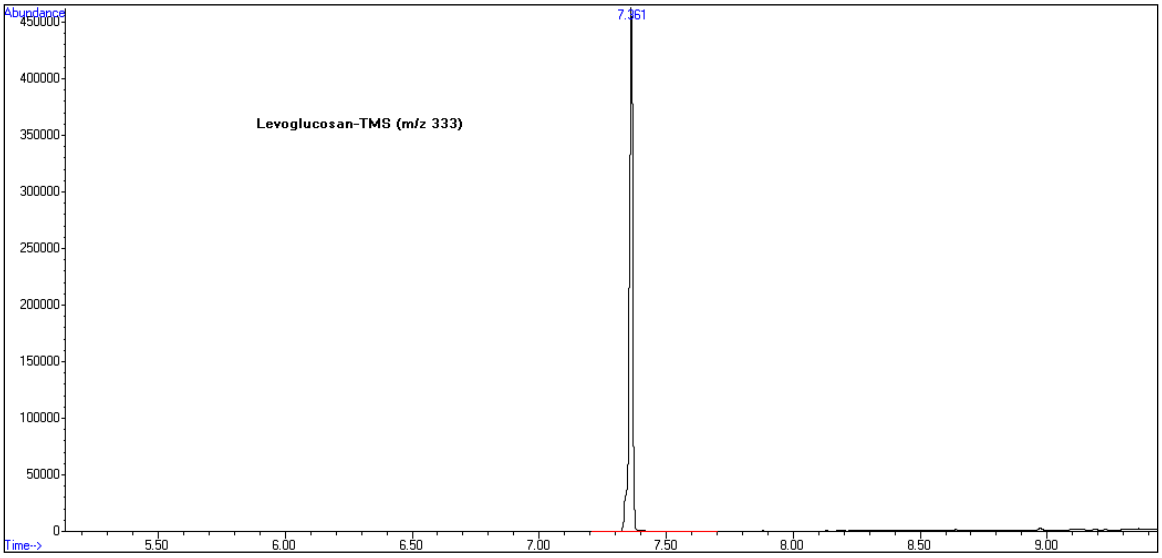


Figure 5: Limited Mass Chromatogram of Quan Ion of Levoglucosan-TMS (m/z 333)

A. References

Determination of Levoglucosan in Atmospheric Fine Particulate Matter – Christopher Simpson, Russell L.Dills, Bethany S. Katz, and David A. Kalman, Dept of Environmental and Occupational Health Sciences, University of Washington, Technical paper ISSN 1047-3289 J. Air and Waste Management Association 54:689-694

B. Method Revisions

Revision Number	Author	Date	Description
SOEH-SOP# A.00.16	Timothy Ma	07/14/05	1st Version
SOEH-SOP# A.00.17	Timothy Ma	09/24/08	2nd Version
SOEH-SOP# A.00.18	Jeff Nichol	01/08/13	3rd Version

Study Protocol XIV – Blood Collection and Processing

The iCAPTURE Lab, St. Paul's Hospital processed all blood samples for the CLEAR Study

Protocol for Obtaining Blood Specimens

A. Blood sample COLLECTION – at subject's home

- **Always complete endoPAT measurement before blood collection**

1. Wash your hands;
2. Ensure collection area is appropriate and all supplies are readily at hand;
3. Select appropriate pre-labeled tubes:
    a. 1 x (10ml gold SST tube)
    b. 1 x (6ml lavender EDTA tube);
4. Ensure participant is sufficiently warm and comfortable;
5. Locate a suitable vein in the antecubital area;
6. Once a suitable vein is located, proceed with donning gloves, cleaning the site (alcohol) and applying the tourniquet. The tourniquet should not be left on the arm for more than 1 minute. Leaving the tourniquet on for over 1 minute increases the risk that the blood in the draw site will become hemoconcentrated (if this happens the lab can report higher hemoglobins, red cell counts, and a multitude of other falsely elevated results). Whenever finding and accessing a vein takes longer than one minute, the tourniquet should be released and two minutes should be allowed to pass before reapplying it so that the blood can return to a basal state;
7. once the vein has been accessed, collect blood samples in order: gold tube then lavender tub (order of draw is important as the EDTA tube contains an additive which you don't want in the SST tube). Remember to gently invert tubes when full;
8. when 2nd blood tube is ½ full remove tourniquet, once this 2nd blood tube is full (allow both tubes to fill until vacuum is exhausted) remove tube from needle assembly, quickly remove needle from arm having gauze handy to place over site. Have participant apply gentle pressure to gauze for a few minutes. Ask participant to NOT bend their arm as this can increase chance of hematoma;
9. Immediately label tubes (pre-printed labels are taped into booklets – please ensure you just fill in the date);
10. Gently invert tubes 8-10 time and place in cooler with ice packs for transport to the lab.

Study Protocol XV - Processing Blood Specimens

B. Blood sample PROCESSING – immediately after collection, at lab (within 4-6 hrs max. after collection)

The iCAPTURE Lab, St. Paul's Hospital processed all blood samples for the CLEAR Study

1. Fill out appropriate labels with date, sample type and study name & ID. Place labels on eppendorf (0.6 ml) tubes – approximately 10 will be needed. (Actual lab procedure was to do the labeling last when it was known how many tubes were needed for each sample type. The lab tech would write on the caps and then attach the labels at the end.)

2. Place 6ml EDTA blood tube on tube rocker for 5 minutes. Run CBC. Print CBC report.

3. Make 2 blood smears of EDTA blood, air dry for 15 min then fix in Methanol for 3 seconds. Wright stain.

4. Centrifuge all blood tubes at 2565 rpm for 15 min.

5. Using a transfer pipette remove the SST serum and place in a 12 x 75mm tube. Withdraw and expel the serum 3 times to ensure sample is well mixed. Aliquot (500ul) into pre-labeled eppendorf tubes.

6. Remove the EDTA plasma and place into separate 12 x 75mm tubes. Withdraw and expel the plasma 3 times to ensure sample is well mixed. Aliquot (500ul) into pre-labeled eppendorf tubes.

7. Remove Buffy coat (thin layer just above the RBC's and some of the RBCs) from EDTA tube place into separate 12 x 75mm tubes. Withdraw and expel the buffy coat 3 times to ensure sample is well mixed. Divide equally into 2 pre-labeled eppendorf tubes.

8. Place aliquots in freezer boxes. Place in freezer noting where in freezer samples are located.

9. Record sample numbers in log book with collection details. (Actual lab procedure was to keep a freezer box log as well as the log book. The freezer box log is handy when/if specific samples need to be pulled. It is done by making a 10x10 grid in excel to represent all locations in a freezer box.)

Study Protocol XVI – Blood Sample Analysis

All blood samples for the CLEAR Study were analyzed by The UBC James Hogg Research Centre,
St. Paul's Hospital under the direction of Dr. Stephan van Eeden (with the exception of the CBC, which was performed at the iCapture Lab, St. Paul's Hospital as samples were processed)

Blood Sample Analysis

All samples were kept frozen at -80° until analyses performed. Samples were analyzed for C-Reactive Protein, IL-6, MIP-1 and Band Cell counts (& a CBC was performed on all blood samples at processing. There are no electronic data for the CBC – only hard copy data. Neutrophil data is the only measurement that was manually entered for selected analysis.)

1. **C-Reactive Protein:**

   The James Hogg Research Centre Lab used the following manufacturer's protocol for CRP: http://www.rndsystems.com/pdf/lucb000.pdf with the following notes/additions/modifications:

   - CLEAR plasma was used in the assay (serum was also available)
   - Samples were diluted 200-fold (manufacturer's protocol suggests 100-fold)

   After thawing the plasma aliquots and prior to their use in the assay, samples were vortexed (to mix the aliquots) and then centrifuged at 10,000x g for 1 minute to remove viscous/particulate material that could clog the filter plate and machine probe.

   All measurements performed by a Luminex 100 analyzer.

2. **Interleukin-6 (IL-6):**
   The James Hogg Research Centre Lab used the following manufacturer's protocol for IL-6:
   http://www.millipore.com/userguides.nsf/a73664f9f981af8c852569b9005b4eee/cf6732d9b9125cc98525756e005610f1/$FILE/HSCYTO-60SK.pdf with the following notes/additions/modifications:

   - undiluted CLEAR plasma was used in the assay

   After thawing the plasma aliquots and prior to their use in the assay, samples were vortexed (to mix the aliquots) and then centrifuged at 10,000 x g for 1 min to remove viscous/particulate material that could clog the filter plate and machine probe.

   All measurements were done by a Luminex 100 analyzer.

3. **Macrophage Inflammatory Protein (MIP-1alpha)**
   The James Hogg Research Centre Lab used the following manufacturer's protocol for MIP-1alpha:
   http://www.rndsystems.com/pdf/luh000.pdf with the following notes/additions/modifications:

   - CLEAR plasma used in the assay was diluted 4-fold as suggested by the protocol

   After thawing the plasma aliquots and prior to their use in the assay, samples were vortexed (to mix the aliquots) and then centrifuged at 10,000 x g for 1 min to remove viscous/particulate material that could clog the filter plate and machine probe.

   All measurements were done by a Luminex 100 analyzer.

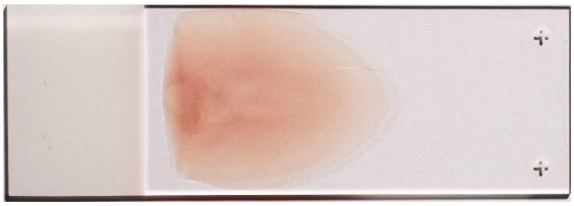
4. **Band Cell Counts – see separate Protocol for Band Cell Counts**

Study Protocol XVII – Band Cell Counts

Blood smears prepared by staff of the iCAPTURE Lab, St. Paul's Hospital. Band Cell Counts performed by David Ngan, The UBC James Hogg Research Centre under Dr. S. van Eeden;

1. Blood Processing:

Prepare 2 blood smears. Air dry for at least 15 min. Dip in methanol and air dry. Place slides in slide storage box. This initial step performed by the iCAPTURE Lab, St. Paul's Hospital Lab.

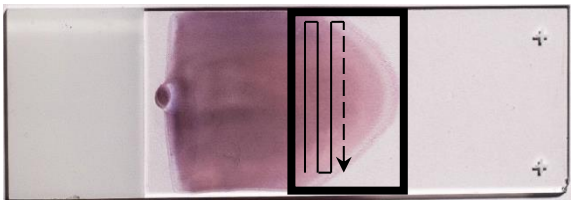


2. Wright-Giemsa Stain:

Load slides into continuous-feed automatic slide stainer (St. Paul's Hospital Laboratory; Bayer HEMA-TEK 2000 Slide Stainer with Hematek Stain Pak – Wright-Giemsa Stain). Dip in May-Grünwald stain, wash in water, and air dry. Return slides to slide storage box.

3. White Blood Cell Count and Differential (for band neutrophils):

Under the 40× objective of a light microscope, count the number of band neutrophils per 100 granulocytes (neutrophils / eosinophils / basophils) at the feathered edge section of the blood smear. Begin counting at the first appearance of a thin, single-cell layer of red blood cells. Count in a movement pattern of continuous adjacent microscopic fields perpendicular to the direction of the blood smear and gradually moving away from the blood smear origin (systematic randomization).



↑ feathered edge

Band neutrophils are immature neutrophils characterized by the absence of nuclear lobes. Depending on orientation, the nucleus appears in a C-shape, U-shape, or straight band but with no distinguishable lobulation.