Exhaled Breath Sampling Technical Manual
Meso-ORIGINS

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1 Overview

The aim of this manual is to provide instructions for sampling of exhaled breath in the Meso-ORIGINS study. This guidance should be followed for all study participants.

2 Contact Information

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When contacting, please include the following information:

- Study name (Meso-ORIGINS)
- Your name, email address and telephone number
- Your centre details
- Participant study number (if applicable)

3 Related Documents

- Meso-ORIGINS Sample handling manual

4 Exhaled breath sampling procedures

4.1 Location

Breath sampling should be performed in a separate, clean room that is free from exposure to scented products and materials. Any room used for lung function activities is suitable.

4.2 Materials

- Hans Rudolph 2-way non rebreathing valve (2-WNRV)
- Teflon adaptor from 2-WNRV to A2-filter
- Teflon adaptor from 2-WNRV to mouthpiece
- Teflon adaptor from 2-WNRV to Tedlar bag
- A2-filter with black screw cap
- Mouthpiece
- Viral filter
- Tedlar bag
- Nose clip
- Sampling pump
- Tenax GR sorbent tubes (packed in silver paper in a glass jar)
- Teflon adaptor from Tedlar bag to Tenax tube
- Cleaning solutions
- Glass jar

4.3 Exhaled Breath Sampling training

Please view the exhaled breath sampling training videos in advance as these instructional videos support the instructions below. Only individuals who have completed this training should handle the equipment and take exhaled breath samples.

Videos available:

1) Assembling set
2) Placing inhalation filter
3) Handling and connecting the Tedlar bag
4) Setting the pump
5) Tube handling and transfer of breath sample
6) Obtaining a breath sample
7) Disconnection and cleaning

The instructions below will refer to relevant videos to support these instructions.

4.4 Assembly of sampling set

Assembly of the sampling set should be carried out while wearing gloves.

Assemble using the instructions below **(*see exhaled breath training video 1- Assembling set)***

Note: step 1 can be done in advance as long as pre-assembled kit is kept dry and clean, but step 2 must only be done before sampling.

1. Put the different parts together as displayed in diagram 1:

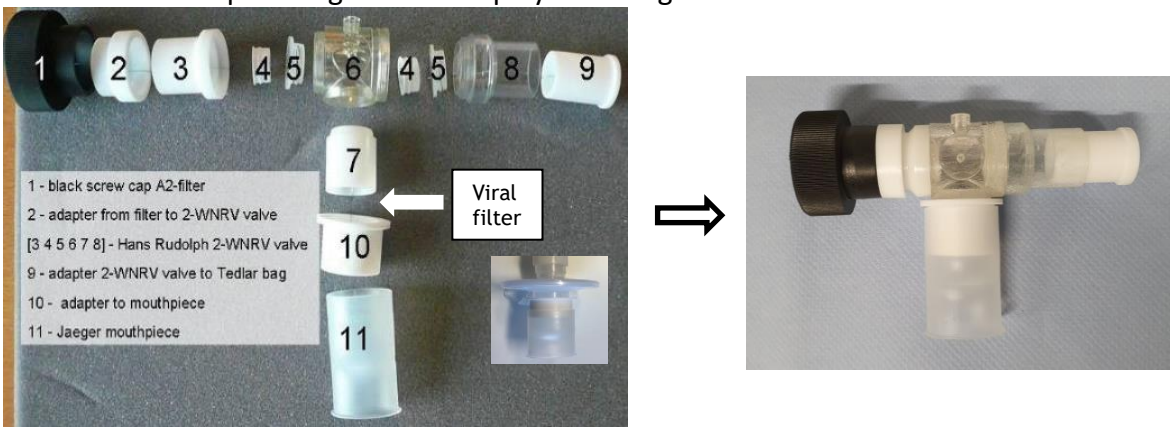

Diagram 1

- Assemble the valve system (2-WNRV) by placing the two valves (No. 4/5) inside the main casing (No. 6) and screwing the tree tubes (No. 3/7/8) in place. Make sure the valves are positioned in the right direction (inlet via No. 1, outlet via No. 9).
- Connect the adaptor (No. 9) for the Tedlar bag to the expiratory port of the 2-WNRV (No. 8).
- A viral filer is provided in the kit. This should be placed between the adaptor (No. 10) and the middle port of the 2-WNRV (No. 7). Connect the mouthpiece (No. 11) to the adaptor (No. 10). The resulting connection should look as follows (diagram 2):

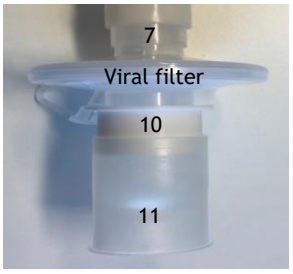

Diagram 2

- Connect the black screw cap (No. 1) to the adaptor (No. 2) and the inspiratory port of the 2-WNRV (No. 3).

2. Just before sampling, screw the A2- filter onto the black screw cap (No. 1) and open the filter by removing the plug- diagram 3. **(*See exhaled breath training video 2- placing inhalation filter*)**

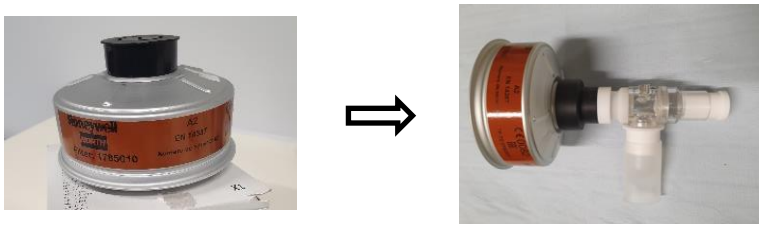

Diagram 3

Note: The A2-filter can be used for approximately 6 months.

<u>Always close the filter (by placing the plug back on) when not in use to prevent saturation with ambient VOCs.</u>

4.5 Taking a background sample

1. Assemble the set-up as per section 4.4, except for the mouthpiece (number 11 diagram 1)
2. Slide the Teflon adapter piece (see diagram 5) for the Tenax column into number 7 (diagram 1) with the connection for the column external.
3. Remove the first cap from the end of the Tenax tube, but leave the Swagelok connector around the tube, which should immediately be connected to the pump. The arrow on the tube should now point in the direction of the pump.
4. Unscrew the second cap at the other end of the Tenax tube and slide the tube into the Teflon adaptor piece. (The arrow on the tube should point away from the sampling set-up).
5. Start the pump (at a flow rate of 100 mL/min) and let it run for 5 minutes (until a total of 500 mL is sampled, as displayed on the screen of the pump).
6. After the 5 minutes, turn off the pump.
7. Immediately disconnect the Tenax tube from the sampling set-up and seal with a cap.
8. Disconnect the Tenax tube from the pump and seal with a cap.
9. Make sure both caps are screwed on tightly (use wrenches if necessary).
10. Wrap the Tenax tube in silver paper and store in a glass jar or desiccator until shipment (shielded from light and air).
11. See Meso-ORIGINS Sample handling manual for storage and shipping steps

4.6 Participant preparation

Before participation, please ensure you are confident in connecting the Tedlar bag to the sampling set for the exhalation step in section 3.6, step 5. ***(See exhaled breath training video 3- handling and connecting the Tedlar bag placing inhalation filter)***

1. Explain the procedure to the participant.
2. Ask the participant if they have eaten, drank, brushed their teeth or washed their hands in the last 2 hours, and if they are wearing perfume or make up (record in worksheet at the end of this manual)
3. Have the participant put on gloves.
4. Have the participant rinse his/her mouth with distilled water.
5. Have the participant put on a nose clip just before the start of the sampling.

4.7 Collection of exhaled breath in Tedlar bag

Supported by exhaled breath training video 3 - handling and connecting the Tedlar bag; and video 6 – obtaining a breath sample

1. Have the participant place the mouthpiece of the sampling set (with screwed on filter) in their mouth and have them tightly enclose it with their lips. ***(See exhaled breath training video 6- obtaining a breath sample)***
2. Let the participant breathe gently into the mouthpiece for 5 minutes (washout-period for removal of ambient VOCs from the pulmonary tract).
3. After the 5-minute washout-period, ask the participant to inhale as deeply as possible and to hold their breath.
4. Meanwhile, connect the Tedlar bag to the connection piece (No. 9 in diagram 1) of the sampling set (this may feel as a bit 'loose' however make sure it is pushed in all the way and does not fall out. See diagram 4 for image of complete set up of bag connected to sampling set) and open the bag by turning the valve counter clockwise 2 turns (turn the white ridged knob above the tip and not the black cap on top). ***(See exhaled breath training video 3- handling and connecting the Tedlar bag placing inhalation filter)***

Diagram 4

5. The participant should now exhale gently and fully into the mouthpiece connected to the Tedlar bag to fill the bag with exhaled breath.
6. Ask the patient to hold up a hand when they are close to a full exhale so you can prepare for step 7
7. When the participant has fully exhaled, close the valve of the Tedlar bag immediately.
8. The participant can now remove the mouthpiece and nose clip. Remove the bag from the setup.
9. Close the filter by inserting the plug.
10. Transfer of the exhaled breath sample inside the Tedlar bag to a Tenax tube must occur within 10 minutes after collection.

4.8 Exhaled breath transfer from Tedlar bag to Tenax tube

Supported by exhaled breath training video 4- Setting the pump; and video 5- Tube handling and transfer of breath sample

Never use any type of detergent when working with or near Tenax tubes (the absorbent material is very sensitive to residues when the tubes are opened).

1. Note the number of the Tenax tube used for the participant on the Meso-ORIGINS exhaled breath sample worksheet (this is provided in the Meso-ORIGINS sample handling manual)
2. Slide the Teflon adaptor piece over the Tedlar bag fitting. (see diagram 5)
3. Pay attention to the orientation of the column, the arrow (highlighted in diagram 5 by a red circle) should always face away from the bag and towards the pump. (The arrow donates direction of airflow) ***(See exhaled breath training video 5 - Tube handling and transfer of breath sample)***

Diagram 5

4. Connect the Tenax tube to the pump FIRST. Remove the cap from the end of the Tenax tube that the arrow points to (this is the end of the tube that should be connected to the pump as per diagram 5 above) but leave the Swagelok connector around the tube. Once you remove the cap immediately connect the tube to the pump using the connector on the pump (diagram 5). The arrow on the tube should now point in the direction of the pump. Diagram 5 illustrates how this should look

5. Unscrew the second cap at the other end of the Tenax tube and slide the tube into the Teflon adaptor piece (see diagram 6). The arrow on the tube should point away from the Tedlar bag.

Diagram 6

6. Open the Tedlar bag by turning the valve counterclockwise one turn. ***(see exhaled breath training video 3- handling and connecting the Tedlar bag)***
7. Start the pump (at a flow rate of 100 mL/min) and let it run for 5 minutes (until a total of 500 mL is sampled, as displayed on the screen of the pump). **(*see exhaled breath training video 4- Setting the pump)***
8. After the 5 minutes, turn off the pump and close the valve of the Tedlar bag.
9. Disconnect the Tenax tube from the Tedlar bag FIRST and seal with a cap.
10. Disconnect the Tenax tube from the pump and seal with a cap.
11. Make sure both caps are screwed on tightly (use wrenches if necessary).
12. Wrap the Tenax tube in silver foil and store in the glass jar until shipment (shielded from light and air).
13. See Meso-ORIGINS Sample handling manual for storage and shipping steps
14. Manually empty the bag by rolling it gently and then closing the valve ***(See exhaled breath training video 5 - Tube handling and transfer of breath sample)***

4.9 Cleaning of equipment

Cleaning should be performed in a separate room to prevent possible confounding by detergent smell during sampling. **(*See exhaled breath training video 7- Disconnection and cleaning*)**

1. Disconnect the filter and ensure capped. This does not need cleaning and should be stored for future uses.
2. Disassemble the different parts of the sampling set.
3. Let the parts and the tube connection piece soak in the provided cleaning solution (Clinell; dilute accordingly) for 10 minutes.
4. Remove from solution and rinse them with tap water to remove the detergent.
5. Then let all pieces soak in normal alcohol disinfectant that your department uses (as long as this is not pure ethanol; a 70% ethanol solution is provided) for 10 minutes.
6. Let them air dry overnight.
7. The materials can now be reused for sampling.

Bags are single use for sampling and then must be returned to prof Lamote at University of Antwerp for cleaning. Please empty the bag of excessive breath, roll or fold and place in an envelope and return to Prof Kevin Lamote (see section 2 for contact information).

5 Transport of Exhaled Breath

See Meso-ORIGINS Sample handling manual for storage and shipping steps.

6 Exhaled breath sampling worksheet

Meso-ORIGINS ID	
Date of sampling	
Eating < 2 hr ago	Y/N
Drinking < 2 hr ago	Y/N
Tooth brushing < 2 hr ago	Y/N
Hand hygiene < 2 hr ago	Y/N
Perfume	Y/N
Make up	Y/N